CHAPTER

9

Human IgG Subclasses

Theo Rispens[a] *and Gestur Vidarsson*[b]

[a]Department of Immunopathology, and [b]Department of Experimental Immunohematology, Sanquin Research, and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, The Netherlands

INTRODUCTION: IMMUNOGLOBULINS AND HUMORAL IMMUNITY

Immunoglobulins are a group of closely related glycoproteins composed of 82 to 96% protein and 4 to 18% carbohydrate. In humans, there are five classes of immunoglobulins, which differ in heavy-chain structure. Immunoglobulin G (IgG) is the major class of immunoglobulins in blood and can be further subdivided in subclasses. The four subclasses of IgG were discovered in the 1960s following extensive studies using specific rabbit antisera against human IgG myeloma proteins.[1] They are designated IgG_1, IgG_2, IgG_3, and IgG_4, in order of decreasing abundance. Several decades of research has revealed subtle but profound differences among the subclasses. Each subclass has a unique profile with respect to antigen binding, immune complex formation, complement activation, triggering of effector cells, and placental transport (Table 9.1). In addition, IgG antibody responses to different types of antigens or pathogens often lead to marked skewing toward one of the subclasses. On the other hand, selective subclass deficiencies are usually not detrimental to the individual but do sometimes lead to enhanced susceptibility toward specific classes of pathogens. All in all, the acquired variability within the Ig locus seems to have selected for beneficial changes during evolution for optimizing or fine-tuning the antibody-mediated immune response.

GENETICS

Immunoglobulins are encoded by three nonlinked clusters of autosomal genes, one cluster coding for heavy (H) chains, a second one for κ-light chains and a third one for λ-light chains. In humans, the H gene family is on chromosome 14 (Figure 9.1A), the κ gene family is on chromosome 2, and the λ gene family is on chromosome 22. A unique variable region is created within developing B cells early in their development by the random recombination of three distinct types of gene segments for the heavy chain—designated VH (variable), D (diversity), and JH (joining)—and two for the light chain (VL and JL). Each B cell only

TABLE 9.1 Properties of Human IgG Subclasses

	IgG1	IgG2	IgG3	IgG4
General				
Molecular mass (kD)	146	146	170	146
Amino acids in hinge region	15	12	62[a]	12
Inter-heavy chain disulfide bonds	2	4[b]	11[a]	2
Susceptibility to proteolytic enzymes	++	+/−	+++	+
Mean adult serum level (g/l)	6.98	3.8	0.51	0.56
Proportion of total IgG (%)	60	32	4	4
Half-life (days)	21	21	7/~21[a]	21
Placental transfer	++++	++	++	+++
Antibody response to:				
Proteins	++	+/−	++	++[e]
Polysaccharides	+	+++	+/−	+/−
Allergens	+	(−)	(−)	++
Complement activation				
C1q binding	++	+	+++	−
Fc receptors				
FcγRI	+++[c]	−	++++	++
	65[d]	−	61	34
$FcγRIIa_{H131}$	+++	++	++++	++
	5.2	0.45	0.89	0.17
$FcγRIIa_{R131}$	+++	+	++++	++
	3.5	0.10	0.91	0.21
FcγRIIb/c	+	−	++	+
	0.12	0.02	0.17	0.20
$FcγRIIIa_{F158}$	++	−	++++	−
	1.2	0.03	7.7	0.20
$FcγRIIIa_{V158}$	+++	+	++++	++
	2.0	0.07	9.8	0.25
FcγRIIIb	+++	−	++++	−
	0.2	−	1.1	−
FcRn (at pH<6.5)	+++	+++	++/+++[a]	+++

(*Continued*)

TABLE 9.1 (Continued)

	IgG1	IgG2	IgG3	IgG4
Prot A/G				
Protein A binding	+++	+++	–[a]	++
Protein G binding	+++	+++	+++	+++

Notes:
[a] *Depends on allotype*
[b] *For A2 isomer.*
[c] *Multivalent binding to transfected cells*
[d] *Association constants for monovalent binding as measured with Biacore (10^{-6} M^{-1})* [61]
[e] *After repeated encounters with protein antigens, often allergens*

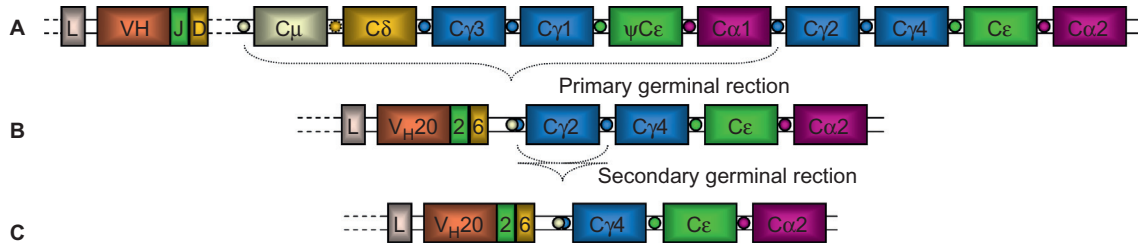

FIGURE 9.1 Heavy-chain locus on chromosome 14. (A) Pre- (VDJ)-arranged heavy chain locus of a naïve B cell. A functional RNA is transcribed from the promoter upstream of the V region, leading to a long transcript that can be spliced into mRNA encoding for either IgM or IgD. In naïve B cells, this mRNA encodes for Ig with transmembrane regions, giving rise to the B-cell receptor that forms the "sensing" part of the B cell. Upon recognition of its cognate antigen, the B cell becomes activated, proliferates, and initiates alternative splicing mechanisms, splicing out the transmembrane part of IgM, leading to secretion of IgM. (B) Upon further stimulus, the B cell may undergo class switching by excision of chromosomal DNA between that of the switch region (small circles upstream of H chain locuses) of IgM and downstream heavy-chain loci. This leads to loss of heavy-chain loci between IgM and the target heavy-chain locus. (C) Upon even further stimulus, subsequent class switching may occur. For protein antigens, a common result is class switching to IgG_4, the most 3′ of the IgG locuses.

expresses one allele of the recombined VDJ gene (the product of which is the VH segment) and one allele of either the κ or the λ locus (the product of which is the VL segment), ensuring the clonal specificity of the naïve B cell when it exits the bone marrow. These processes (i.e., VDJ recombination and clonal specificity through allelic exclusion) are extensively reviewed elsewhere.[2]

Early on, the B cell expresses its fully formed immunoglobulin using the V-gene proximal constant region—that is, IgM and IgD (through alternative splicing) as membrane-bound receptors (Figure 9.1). Once cross-linked by exogenous antigen, given the appropriate environment (e.g., stimulation through Toll-like receptors), the B cell may start secreting IgM by alternative splicing, resulting in the absence of the transmembrane region of IgM. If T cell help and other immune stimuli are applied (as for most protein antigens), class switching can occur, resulting in loss of the IgM and other intervening heavy-chain constant regions between IgM and the target constant (C) locus (Figure 9.1B). The choice of this locus is not random, as it is preceded by sterile transcription of

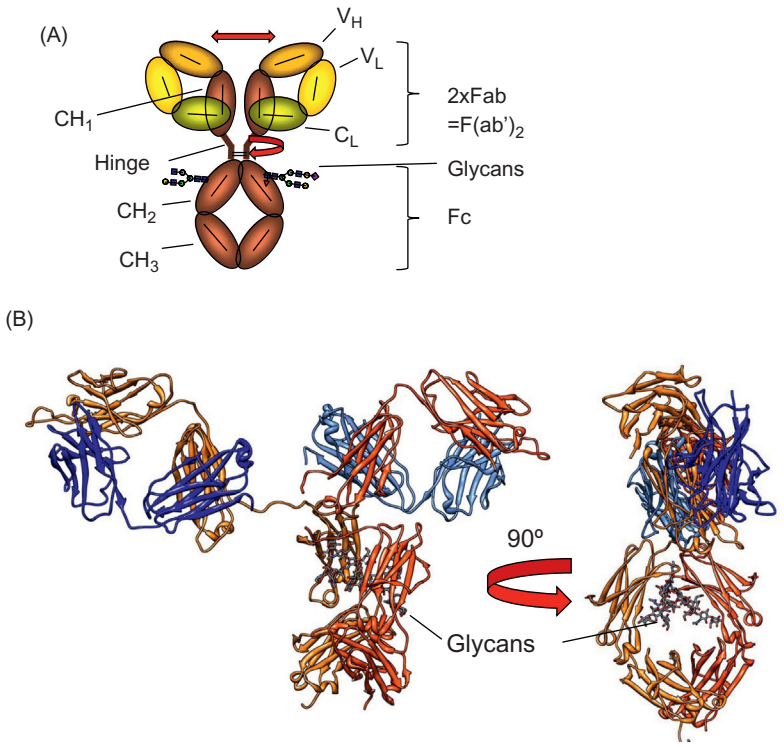

FIGURE 9.2 **Structure of IgG.** (A) Illustration of the structure of an IgG molecule with the different domains indicated. Inter- and intra-chain disulfide bonds are indicated with black lines within Ig-like domains or between the two heavy chains in the hinge region. (B) Crystal structure of an IgG molecule (1HZH) viewed from two different angles, demonstrating the flexibility of the two Fab fragments with respect to each other and the Fc tail. The location of the glycan attached to each of the heavy chains (N297) is also indicated.

the targeted locus.[3] The opening of those loci is guided by the cytokine environment and the cellular niche in which the B cell resides during its encounter with the corresponding antigen in the germinal reaction. This genetic excision reaction (class switching) has also been extensively reviewed elsewhere.[4]

STRUCTURE[5–7]

The basic immunoglobulin molecule has a four-chain structure, comprised of two identical γ heavy (H) chains and two identical κ or λ light (L) chains, linked together by inter-chain disulfide bonds. Each heavy chain consists of an N-terminal variable domain (VH) and three constant domains (CH1, CH2, CH3), with an additional hinge region between CH1 and CH2 (Figure 9.2). Light chains consist of an N-terminal variable domain (VL) and a constant domain (CL) and are associated with the VH and CH1 domains to form a Fab (fragment antigen-binding) arm. Functionally, the V regions are involved in antigen binding. Two heavy-chain/light-chain heterodimers (HL) are combined into a single antibody molecule (H_2L_2) via disulfide bonds in the hinge region and non-covalent interactions between the CH3 domains. The part of the antibody

formed by the lower hinge region and the CH2/CH3 domains is called fragment crystalline (*Fc*); it is formed after mild proteolysis using papain, which cleaves intact IgG molecules into two Fab fragments and an Fc fragment. The latter fragment crystallizes easily, hence its designation. The Fc part is responsible for effector functions of antibodies, as it contains a largly overlapping binding site for C1q (complement) and IgG–Fc receptors (FcγR) on effector cells of the innate immune system. It is also contains the binding site for the neonatal Fc receptor (FcRn) that is responsible for the prolonged half-life of IgG antibodies and placental passage. The highly conserved glycosylation site N297 is located at the interface between the two CH2/CH3, forming the Fc of an IgG molecule (Figure 9.2) that is responsible for subtle but important changes of quaternary structure of the Fc, allowing for a more exposed docking site for FcγR. As discussed in other chapters in this book, these glycans also directly participate in FcγR binding, but they can also modulate these interactions through highly specific modifications of the N297 glycan—changes that seem to be regulated during specific immune responses in humans.[8] Although this glycosylation site is considered to be the single glycosylation site in IgG, the V region of approximately 30% of all antibodies has also been found to be glycosylated, sometimes arising through VDJ recombination or somatic hypermutation, and this glycosylation has been reported to affect antibody binding characteristics.[9,10]

The global structures of the four human IgG subclasses are very similar but with important differences that affect the functionality of each subclass (Figure 9.3A). The four subclasses show over 90% homology in amino acid sequence, with differences that are not randomly distributed. Much variation is found in the hinge region and N-terminal CH2 domain, whereas fewer amino acid differences are found in the other domains. Of these, least is known about the functional consequences (if any) of structural variations found within the CH1 domain. On the other hand, structural differences in the CH2/CH3 domains, forming the Fc tail, are relatively well studied. The binding profiles of the subclasses to FcγR and C1q are quite different and have been investigated in detail. Differences in the primary sequences of the IgG subclasses (Fc and hinge) lead to variations in tertiary structural elements, thereby critically influencing the properties of each subclass, as discussed in greater detail below.

Structural Variation in the Hinge Region

The hinge region forms a flexible linker between the Fab arms and the Fc part. Length and flexibility of the hinge region vary extensively among the IgG subclasses.[7] This length variation has consequences for the possible conformations of the Fab arms relative to the Fc domain as well as to each other. The hinge region of IgG_1 encompasses 15 amino acids and is very flexible. IgG_2 has a shorter hinge than IgG_1, with 12 amino acid residues. The lower hinge region of IgG_2 (actually encoded by the CH2 region) also has a one amino acid deletion (G236) and contains a rigid polyproline double helix, stabilized by four extra inter-heavy-chain disulfide bridges (Figure 9.1). These properties restrict the flexibility of the IgG_2 molecule. IgG_3 has a much longer hinge region than any other IgG subclasses or Ig human isotype; it is about four times as long as the IgG_1 hinge, containing 62 amino acids (including 21 prolines and 11 cysteines) and forming a polyproline double helix with limited flexibility.[11,12] In IgG_3, the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. This long hinge of IgG_3 is a result of duplications of the hinge exon, encoded by one exon in IgG_1, IgG_2, and IgG_4 but up to four exons in IgG_3. One of those exons is common to all IgG_3 allotypes, but IgG_3 also has one

(A)

CH1

	131	133	137	138	189	192	193	196	199	203	214
IgG1	S	K	G	G	P	S	L	Q	I	N	K
IgG2	C	R	E	S	P	N	F	Q	T	D	T
IgG3	C	R	G	G	P	S	L	Q	T	N	R
IgG4	C	R	E	S	P	S	L	K	T	D	R

hinge

	*	217	219	220	221	222	223	224	225	228
IgG1		P	S	C	D	K	T	H	T	P
IgG2		R	C	C	.	.	V	.	E	P
IgG3	*	P	S	C	D	T	P	P	P	R
IgG4		S	.	.	.	Y	G	P	P	S
										fae

CH2

	233	234	235	236	268	274	276	282	291	292	296	300	309	327	330	331	339
IgG1	E	L	L	G	H	K	N	V	P	R	Y	Y	L	A	A	P	A
IgG2	P	V	A	.	H	Q	N	V	P	R	F	F	V	G	A	P	T
IgG3	E	L	L	G	H	Q	K	V	P	R	Y	F	L	A	A	P	T
IgG4	E	F	L	G	Q	Q	N	V	P	R	F	Y	L	G	S	S	A
	γ	γ	c/γ	γ					r				r	γ	γ	c	

CH3

	355	356	358	379	384	397	409	419	422	431	435	436	445
IgG1	R	D	L	V	N	V	K	Q	V	A	H	Y	P
IgG2	R	E	M	V	N	M	K	Q	V	A	H	Y	P
IgG3	R	E	M	V	S	M	K	Q	I	A	R	F	P
IgG4	Q	E	M	V	N	V	R	E	V	A	H	Y	L
							fae				r	r	

(B)

IgG1	CH1 214	CH3→ 356	358	431
z,a	K	D	L	A
z,a,x	K	D	L	G
f	R	E	M	A
f,a	R	D	L	A
	↓	↓	↓	↓
	z (K)/ f (R)		a	x

IgG2	CH1 189	CH2 282
n	T	M
..	P	V

IgG4	CH2 309	CH3 409
4a I	L	R
4a II	L	K
4b	V	R
	↓	
	4a (L)/ 4b (V)	

IgG3	CH2→ 291	292	CH3→ 379	384	397	409	419	422	435	436
b*	P	R	V	S	M	K	Q	I	R	F
c3*	P	R	V	S	M	K	E	I	R	F
c3c5*	P	R	V	S	V	R	E	V	R	F
s*	P	R	M	S	V	K	Q	I	H	Y
st*	P	W	M	S	V	K	Q	I	H	Y
g*	L	R	V	N	M	K	Q	I	R	Y
	↓	↓	↓				↓	↓	↓	↓
	g1	t	s				c3	c5	g5	b1

b* = b0,b1,b3,b4,b5,u,v
c3* = b0,b1,c3,b5,u,v
c3c5* = b0,b1,c3,u,c5
s* = b0,b3,b5,s,v
st* = b0,b3,b5,s,t,v
g* = g1,g5,u,v

FIGURE 9.3 **Variations in IgG between subclasses and individuals.** (A) Overview of the amino acid differences between the four IgG subclasses per domain. Residues also known to differ between individuals (allotypic residues) are indicated in green (IgG_1), magenta (IgG_2), blue (IgG_3), and red (IgG_4). Displayed allotypes are G1m(za), G2m(..), G3m(b*), and nGm4(a). The amino acid positions involved in differential binding of the subclasses to C1q, FcγR, and FcRn, are indicated below each sequence alignment with c, γ, and r, respectively. Residue positions marked with "fae" are involved in the Fab arm exchange of IgG_4. Hinge exons 1 to 3 for IgG_3 are not displayed. See text for more details. (B) Allotypes of the different IgG subclasses as classified by the alphabetical classification (genetic marker, Gm) system. For IgG_3, the allotypes indicated in the table represent a summary of the most frequently occurring alleles; the individual allotypes corresponding to these alleles are indicated below the table. The amino acids encoding for a particular allotype, providing the anti-allotype epitope, are indicated below each allotype with the corresponding alphabetical numbering.

to three copies of a homologous second type of IgG_3 hinge exon. The elongated hinge in IgG_3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG_4 is 12 amino acids long and thus shorter than that of IgG_1. Its flexibility is intermediate between that of IgG_1 and IgG_2.[13] The difference in hinge flexibility influences the relative orientation and movement of the Fab arms and Fc tail of the IgG antibody.

Binding sites for C1q and/or FcγR may be partially or completely shielded by Fab arms. Furthermore, the relative flexibility that both Fab arms may have with respect to each other

differs between subclasses as follows: IgG_3>IgG_1 >IgG_4>IgG_2.[13,14] This flexibility affects antigen-binding capacity, immune complex formation, and C1q and FcγR binding.

Inter-Chain Disulfide Bonds

The four IgG subclasses also differ with respect to the number of inter-heavy-chain disulfide bonds in the hinge region (Table 9.1). In addition, both IgG_2 and IgG_4 may be found as several isomers in which the hinge disulfide bonds are connected in different ways (see below). Another structural difference between the human IgG subclasses is the linkage of the heavy and light chain by a disulfide bond. This bond links the carboxy-terminal cysteine of the light chain to the cysteine at position 220 (in IgG_1) or at position 131 (in IgG_2, IgG_3, and IgG_4) in the CH1 domain. These two positions are spatially juxtaposed, and the essential structure and function of the molecule appears to be conserved between the two types of linkage between heavy and light chain.

Hinge Isomers in IgG_2 and IgG_4

In IgG_2, structural hinge isomers have been observed as a result of alternative formation of disulfide bonds between the cysteines in the hinge region of the heavy chains and those involved in the formation of disulfide bonds between light and heavy chains.[15,16] These isomers were found for IgG_2 antibodies with κ light chains but were absent for λ light chains. The major forms are the classical A form with four disulfide bridges between the two IgG_2 heavy chains and the B form in which hinge cysteine instead forms a disulfide bond with the light chain, although multiple other configurations exist.[16] These isoforms apparently form independently of each other, giving rise to A/A, B/B, and A/B isoforms. Fc receptor binding does not seem to be different for the different isomers.[17] IgG_2 has also been reported to form covalent dimers,[18] which might be regarded as an additional isomer.

Two isomers of IgG_4 differing in the disulfide bonding of hinge cysteines coexist. The core hinge of IgG is formed by a CXXC motif also found in redox-reactive proteins such as thioredoxins.[19] Compared to IgG_1, with a relatively rigid CPPC motif,[20] intra-chain disulfide bonds are more easily formed between cysteines 226 and 229 in IgG_4, which possesses a CPSC core hinge. The result is an observable amount of non-covalently linked half-molecules (HL, as opposed to H_2L_2), in addition to covalently linked inter-chain isomers.[21,22] An S228P mutant of IgG_4 does not form half-molecules, in agreement with the finding that this species does not occur in IgG_1. The process is reversible but depends on redox conditions. Formation of the intra-chain isomer (half-molecules) is an important step in Fab arm exchange (see below).

IgG_4 Fab Arm Exchange

In vivo, half-molecules of IgG_4 recombine randomly with half-molecules of other IgG_4, combining specificities from one IgG_4 with the specificities of another IgG_4 and effectively resulting in monovalent antibodies.[23,24] This process is controlled by redox conditions.[22] The unique S228 in the core hinge of IgG_4 allows formation of the intra-chain isomer, and R409 (rather than the equivalent lysine in IgG_1) results in weaker CH3–CH3 interactions.[25] Both determinants appear to be required to observe Fab arm exchange *in vivo*.[22]

Proteolysis

The structural differences between the IgG subclasses are also reflected in their susceptibility to proteolytic enzymes including papain,[26,27] plasmin,[28] trypsin,[29] and pepsin.[30] IgG_3 is more susceptible to cleavage by these enzymes, whereas IgG_2 is relatively resistant, and IgG_1 and IgG_4 generally exhibit an intermediary sensitivity. Because these proteolytic enzymes all cleave IgG molecules near or within the hinge region, it is likely that protease sensitivity is

related to hinge accessibility and conformational flexibility.[31] Because such proteolytic enzymes are utilized by numerous bacteria, the relatively resistant IgG_2 antibodies, often elicited to bacterial polysaccharides, may represent a host response to counteract the effects of these Fc-restricting enzymes.

Allotypes

In addition to isotypic variation, allelic variation is found among the subclasses (Figure 9.3). Allotypes are defined as the polymorphic epitopes of immunoglobulins[32] that were originally discovered on the basis of serological findings.[33] Immunogenic determinants were found on IgG from some individuals but not others. Subsequently, allotypic variations were genetically analyzed and a number of structural determinants identified.[34–37] A large number of polymorphisms were found for IgG, a finding made useful in, for example, paternity testing and forensic medicine before HLA typing became available.[33] Exposure of an individual to a non-self allotype can induce an anti-allotype response and may occur in transfused individuals.[33] However, not all variations in IgG amino acid sequence lead to determinants that are immunogenic because some determinants are found in other isotypes and are therefore called *isoallotypic* variants. The allotypes that were originally typed serologically, resulting originally in the alphabetical designation system (Gm, or genetic marker, including subclass designations such as G1m for IgG[33]), do not fully cover the structural variation among different allelic forms of IgG subclasses, particularly IgG_3, which shows extensive polymorphisms, often of isoallotypic origin (Figure 9.3B).[34] An example is the non-a or nG1m(a) determinant that can also be found on IgG_2, IgG_3, and IgG_4.[32]

The main allelic forms for IgG_1 (Figure 9.3B) are $G1m^{za}$, $G1m^{f}$, and $G1m^{fa}$.[32] The $G1m^{f}$ allele is only found in Caucasians, whereas the $G1m^{fa}$ allele is common in Asian populations. Two allelic forms of IgG_2 are known, whereas many allelic forms of IgG_3 are known; the most important ones are shown in Figure 9.3B. Because some allotypes have proven to be immunogenic, they may be relevant to consider when developing therapeutic antibodies. Treatment using therapeutic monoclonal antibodies can in principle also lead to an anti-allotype response; however, to date, little evidence has been found for significant anti-allotype responses (e.g., adalimumab[38] or infliximab[39]). There are no known allotypic variations that result in a functionally different antibody, except for IgG_3, for which a few isoallotypic variants result in an extended half-life (discussed below). Interestingly, plasma IgG concentrations of an individual appear to correlate with Gm allotype.[40–42]

BINDING TO EFFECTOR MOLECULES

Antibodies link the adaptive immune system with the effector mechanisms of the innate immune system. They literally form a bridge by combining in one molecule antigen-binding sites and binding sites for a range of innate receptors and adaptor molecules. The effector mechanisms that will be triggered vary between the different immunoglobulin classes and subclasses. Broadly speaking, IgG_1 and IgG_3 are potent triggers of effector mechanisms, whereas IgG_2 and IgG_4 are not and may induce responses only in certain cases; however, these antibodies remain capable of neutralizing virus particles and toxins. Below, binding to C1q and Fc receptors is discussed, with a focus on the structural aspects that differ among the subclasses (Table 9.1).

C1q

IgG, as well as IgM, can activate complement once bound to target surfaces. This occurs through binding and subsequent activation

of C1q, leading to deposition of C3b to further opsonize the target, as well as to the formation of the membrane attack complex (C5–C9), causing disruption of the targeted bilipid membranes.[43] IgG_1 and IgG_3 are efficient in triggering this classical route of complement,[44] but IgG_2 and IgG_4 are not. This is due in large part to the reduced binding of C1q to the latter subclasses,[44–46] although it has also been observed that, in addition to C1q binding, downstream events of the complement cascade (C4b deposition) are differentially affected by IgG subclass.[44] Residues in the CH2 region important for C1q binding include L235, D270, K322, P329, and P331.[46–49] In IgG_2, A235 (Leu in other subclasses) appears to account for reduced C1q binding,[48] whereas in IgG_4 P331 is (at least in part) responsible for the reduced or absent binding of C1q (Figure 9.3A).[46,49] Structural determinants in the middle, or core, hinge region (residues 226 to 230) can influence the binding of C1q;[50] rigidity in this region contributes favorably to C1q binding, whereas removal of cysteine bonds negatively affects binding. It has also been suggested that the relatively long hinge of IgG_3 makes the C1q binding site more accessible, resulting in more efficient complement activation.[51,52] However, IgG_3 engineered with a short IgG_4 hinge binds C1q efficiently, although complement activation was somewhat reduced.[53] Interestingly, engineered $IgG_{1/3}$ hybrids with an IgG_1 CH1 and hinge region were found to be more potent in complement activation compared to wild-type IgG_3, with the largest contribution arising from the CH1 domain swap.[54] The binding of C1q to IgG_4 may conversely be influenced by shielding of the potential binding site by Fab arms.[22,52,55,56] IgG_4 also results in less complement activation by forming small immune complexes and in this way can even reduce complement activation by IgG_1 antibodies.[57] Although the short hinge of IgG_2 may lead to similar shielding of the potential C1q binding site, a notion that fits with its general poor activation of the classical complement cascade, IgG_2 can activate this cascade at high densities of surface antigens, as is the case for polysaccharides, to which IgG_2 antibodies tend to form.[58–60]

Fcγ-Receptors

FcγR bind to a region partially overlapping the C1q binding site and are responsible for many of the effector functions of IgG as discussed elsewhere in this book. The binding of IgG to these receptors has been studied in detail. Each of the IgG subclasses has a unique binding profile to each FcγR (Table 9.1).[61] A major distinction can be made between IgG_1/IgG_3, which interact efficiently with most FcγR, and IgG_2/IgG_4, which show reduced affinity to a number of FcγR. Furthermore, monomeric IgG_3 binds more efficient than monomeric IgG_1 to FcγRIIa, FcγRIIIa, and FcγRIIIb; also, the binding efficiency of complexed IgG_3 to all Fc receptors exceeds that of IgG_1.[61] Structural determinants responsible for the differences between IgG_1 and IgG_3 are not well defined, although one might expect the length of the hinge to be of crucial importance. Below, structural differences that determine the subclass-specific variations are discussed. For all FcγR interactions, the stretch of amino acids comprising the N-terminus of the CH2 domains and strands adjacent in the three-dimensional immunoglobulin fold are important for binding. In general, this encompasses amino acids 234 to 239, 265 to 269, 297 to 299 and 327 to 330.[62,63] Subclass-specific variations in these regions are responsible for the weaker binding of IgG_2 in particular, but also of IgG_4 to all FcγR.

FcγRI

This receptor binds all human IgG subclasses except IgG_2 and, unlike the other FcγR, contains a unique third membrane-proximal immunoglobulin domain that probably is also responsible for its higher affinity to IgG. Mutations of IgG_1 in the lower hinge to the IgG_2 equivalents,

particularly E233P, L235A, and G236Delta, will abrogate binding.[64–68] Binding to FcγRI is reduced for IgG_4,[61] and both P331S and L234F are implicated to account for the reduced binding in comparison to IgG_3,[67] but P331 may not be important for binding of IgG_1.[66,68] An IgG_3 with a partially deleted hinge was found to have reduced binding to FcγRI and FcγRIIa.[69]

FcγRIIa

FcγRIIa is the only FcγR with significant binding to IgG_2.[61,70–72] Binding is more efficient for the 131H (low-responder, LR) variant than the 131R (high-responder, HR) variant (nomenclature based on differential binding to mouse IgG_1 which binds the HR much better).[70] Binding affinity varies among subclasses as follows: IgG_3>IgG_1>IgG_4=IgG_2. Recently, a crystal structure of the complex of IgG_1 Fc with FcγRIIa was published,[62] and contact residues relating to differences in subclass binding include L234, L235, and G236 in the lower hinge and the structurally adjacent A327. Significantly, the 131R site in FcγRIIa is also in close proximity to the lower hinge in this co-crystal structure. Thus, the lowered binding affinity of IgG_2 to FcγRIIa and the differential binding to the HR/LR form of FcγRIIa may also be attributed to differences in the hinge of IgG_2.

FcγRIIb/IIc

The extracellular domain of the inhibiting FcγRIIb is identical to the activating FcγRIIc that is expressed in some individuals.[73] Binding to the inhibitory receptor FcγRIIb or FcγRIIc is weak for all subclasses, generally preferring IgG_3=IgG_1=IgG_4>IgG_2. Interestingly, dissociation constants for binding of monomeric IgG_1 and IgG_3 are similar, but immune complexes of IgG_3 seem to bind more efficiently compared to IgG_1.[61] Binding to most activating Fc receptors is lower for IgG_4 compared to IgG_1, but this is not the case for the inhibitory receptor FcγRIIb. This altered balance between binding to activatory receptors in comparison to inhibitory receptors may be an important feature of IgG_4 that contributes to its low proinflammatory capacity.

FcγRIIIa

Two allotypic variants of FcγRIIIa exist: F158 and V158. The V158 variant has greater affinity for all subclasses, and for IgG_3 binding efficiency approaches that of FcγRI,[61] with general affinities following IgG_3>IgG_1 >> IgG_4>IgG_2. Besides changing amino acids 233 to 236 from IgG_1 to the IgG_2 equivalents, A327G (Ala present in IgG_1 and IgG_3; Gly in IgG_2 and IgG_4) also results in decreased binding.[66] The binding affinity of FcγRIIIa seems to be particularly sensitive to core fucosylation of the N-linked glycan at N297 of the Fc tail of IgG, as its binding affinity can be enhanced up to 50 times—with corresponding increase in effector function—if the Fc tail is not fucosylated.[74,75] Recent work by Ferrara et al.[76] has pinpointed this interaction to be due to carbohydrate–carbohydrate interactions between the glycan on N297 of the heavy chain and glycosylation of FcγRIIIa at position 162, a position unique to both FcγRIIIa and FcγRIIIb.

FcγRIIIb

There are also functional allotypic variations of the neutrophil FcγRIIIb, referred to as human neutrophil antigen 1 (NA1/HNA1a) and neutrophil antigen 2 (NA2/HNA1b).[77] The FcγRIIIb-NA1 form is capable of better ingestion of IgG_1- or IgG_3-opsonized particles than FcγRIIIb-NA2.[78] FcγRIIIb generally bind IgG_1 and IgG_3 but not IgG_2 and IgG_4, with IgG_3 binding being better than IgG_1.[61] A crystal structure of the complex of IgG_1 Fc with FcγRIIIb reveals amino acids 234 to 238 to be important contact residues, and the subclass-specific variation in this area again can explain the lack of binding of IgG_2 and IgG_4 to this receptor.[63,79]

FcRn

In the 1960s, the existence of a functionally distinct FcγR was first proposed by Brambell,[80,81] who suggested that the unusually long half-life of immunoglobulin G (three weeks; see Table 9.1) and efficient transport from mother to young were mediated by a single receptor. This was later confirmed by various groups.[82–85] Structurally, the neonatal IgG receptor, or FcRn, is strikingly similar to MHC-class I molecules.[86,87] Like MHC class-I and CD1, FcRn is coexpressed with the non-glycosylated 12-kD β_2-microglobulin encoded on chromosome 15. The α-chain of human FcRn, a 45-kD polypeptide chain, is encoded on chromosome 19 in the vicinity of various other immune receptors (e.g., KIR, LAIR-1, CD89, CEACAM). Unlike FcRn from mice and rat, human FcRn has only one potential glycosylation site (N102). It is located on the face opposite the IgG-binding site and is also shared with that of all known FcRn sequences (mouse, rat, human, macaque, pig, sheep, bovine, dromedary, and possum). FcRn does not bind its ligand at physiological pH (7.4). Only in the acidic environment of endocytic vacuoles ($pH \leq 6.5$), where solvent-exposed histidine residues in IgG are protonated, does binding to FcRn take place.[85,88,89] Histidine residues within the Fc tail of IgG (CH2–CH3 interface) are critical for high-affinity binding to residues within β_2M and FcRn α-chain.[66,86,87] H435 sits at the heart of this interface, and the lowered affinity of R435-containing allotypes of IgG_3 to FcRn likely explains their shortened half-life (see Table 9.1), as H435-containing IgG_3 allotypes have a normal half-life of three weeks.[90]

On mucosal cells, FcRn has been found to transport IgG and be involved in antigen sampling,[91–93] and its expression on phagocytic cells[91,94] has recently been found to enhance the phagocytosis capacity of IgG-opsonized particles.[95,96] On antigen-presenting cells, this ingestion of IgG complexes can lead to enhanced presentation.[97–99] Similar to phagocytosis responses, the enhanced presentation likely requires external sensing and cellular activation through FcγR and pattern-recognition receptors and handing the IgG–antigen cargo over to FcRn at low pH.[96,97,100] Thus, immunoglobulin activities including extended half-life, transport to young, and antigen sampling seem to be orchestrated through a single receptor, the MHC-class I-like FcRn. In contrast, other effector functions of IgG, such as phagocytosis and antigen-presentation seem to be mediated by both FcRn and classical FcγRs.

Protein A and Protein G

Protein A and protein G are bacterial proteins that bind human IgG, but also IgG from various other species. The proteins are widely used as affinity matrices for purification of IgG. For both proteins, the major binding site in IgG is located in the Fc part of the antibody at the CH2–CH3 interface and overlaps with the FcRn binding site.[101–103] Protein G binds all human subclasses (Kd ~2×10^{-10} M),[104,105] whereas protein A generally only binds IgG_1, IgG_2, and IgG_4 (Kd ~2×10^{-9} M), but not IgG_3. Because Arg435 in IgG_3 is responsible for the lack of binding to protein A, IgG_3 allotypes with a histidine rather than arginine at position 435 do bind protein A.[106] In addition to the high-affinity Fc binding site, protein G binds to a low-affinity site in the CH1 domain of IgG[107,108] that apparently is not present in IgG_2.[109] Protein A also binds variable domains from the VH3 family.[110]

IgG ANTIBODY RESPONSES

The nature of the antigen and the route by which it enters the body govern and steer the (secondary) immune reaction into preferential patterns of class switching. In addition to direct B-cell triggering by the antigen itself, a number of secondary signals will also influence differentiation of the B cell, including ligands for

Toll-like receptors and cytokines produced by other lymphocytes and antigen-presenting cells. For example, protein antigens usually trigger B cells receiving T-cell help through MHC-class II expressed by the B cell. For those antigens, class switching tends to be IgG_1 or IgG_3, but can also be IgG_4 or IgE. On the other hand, in the absence of T-cell help, polysaccharide antigens may induce class switching to IgG_2 in particular. B cells that have undergone class switching in a primary or secondary immune reaction can also go through subsequent class switching,[111] but those events are limited by the availability of remaining heavy-chain genes. The relatively terminal position of the Cγ4 cassette may be one of the reasons why IgG_4 responses tend to occur after repeated antigen exposure (Figure 9.1C).

Selective deficiency of one or more subclasses may occur in individuals. A complete isotype or subclass deficiency caused by deletions in chromosome 14 loci is rare.[112] More often, one or more of the IgG subclass levels (predominantly IgG_2 and/or IgG_4) are below the normal range in healthy individuals,[113] which sometimes leads to an impaired response to infections with encapsulated bacteria, as will be discussed below.

IgG_1

Antibody responses to soluble protein antigens such as tetanus toxoid and membrane proteins primarily induce IgG_1 but are accompanied by lower levels of the other subclasses.[114] IgG_1 deficiencies are seen in a variety of primary and secondary antibody deficiencies and, because of its high titer, result in a decreased level of total IgG levels (hypogammaglobulinemia). IgG_1 deficiencies alone, but also in combination with deficiencies of other subclasses, are associated with recurrent infections.[115] IgG_1 responses are often, but not always, formed together with IgG_3 and sometimes IgG_4. The latter case usually results from chronic or repeated antigen stimulation (see below).

IgG_2

Antibody responses to bacterial capsular polysaccharide antigens can be almost completely restricted to IgG_2,[114,116–118] and IgG_2 deficiency may result in the virtual absence of IgG anti-carbohydrate antibodies.[119] An increased susceptibility to certain bacterial infections is associated with IgG_2 deficiency, suggesting a role of IgG_2 in the defense to these pathogens.[120] Low concentrations of IgG_2 often occur in association with a deficiency in IgG_4 and IgA_1 and IgA_2. An extensive analysis of anti-carbohydrate reactivities in intravenous immunoglobulin revealed that, although IgG_2 indeed represents the bulk of the reactivity to many glycans, this is not always the case,[121] as IgG_1 antibodies can also prevail—for instance, to *Haemophilus influenza* b polysaccharide during natural infections.[114]

IgG_3

IgG_3 antibodies are particularly effective in the induction of effector functions. Being a potent proinflammatory antibody, its shorter half-life may function to limit the potential of excessive inflammatory responses. However, the finding that some individuals bearing the sG3m allotypic marker also have IgG_3 with a prolonged half-life may challenge that assumption.[90] Curiously, IgG_3 levels in these individuals do not seem to be increased, which may be explained by γ_3-promotor polymorphisms known to affect the frequency of class switching to IgG_3 in G3m(g) allotypes, explaining the low concentration in most G3m(g) homozygous individuals.[42,122] Viral infections in general lead to IgG antibodies of the IgG_1 and IgG_3 subclasses, with IgG_3 antibodies appearing first in the course of the infection.[114] IgG_3-dominated responses appear to be rare. A curious example is so-called anti-hinge antibodies,[123] which bind to the hinge region of Fab2 fragments but not intact IgG antibodies. Also, antibodies

to P and P^k blood group antigens are largely restricted to IgG_3.[124] Decreased IgG_3 levels are frequently associated with other IgG subclass deficiencies.[40]

IgG_4

Allergens are often good inducers of IgG_1 and IgG_4, in addition to IgE. IgG_4 antibodies are often formed following repeated or long-term exposure to antigen in a non-infectious setting and may become the dominant subclass. Examples are long-term beekeepers and allergic individuals who underwent immune therapy.[125–128] In immunotherapy, relief of symptoms appears to correlate with IgG_4 induction. Switching to IgG_4 may be modulated by IL-10, linking this subclass downregulation of immune responses or tolerance induction.[128,129] IgG_4 may also represent the dominant antibody subclass in immune responses to therapeutic proteins such as factor VIII and IX[130–132] and adalimumab.[133] Furthermore, helminth or filarial parasite infections may result in the formation of IgG_4 antibodies,[134,135] and high IgG_4 titers can be associated with an asymptomatic infection.[136]

Isolated IgG_4 deficiencies are rare and the consequences uncertain. On the other hand, a group of disorders today referred to as IgG_4-related diseases (IgG_4RD) is characterized by elevated serum IgG_4 concentration and tissue infiltration by IgG_4-positive plasma cells and may affect a number of organs.[137,138] The spectrum of IgG_4RD is wide and includes patients with autoimmune pancreatitis (AIP), Mikulicz's disease, hypophysitis, Riedel thyroiditis, interstitial pneumonitis, interstitial nephritis, prostatitis, lymphadenopathy, retroperitoneal fibrosis, inflammatory aortic aneurysm, and inflammatory pseudotumor. In AIP patients, serum IgG_4 elevations (>1.4 g/L) are seen in 70 to 80% of the cases and in 10% of pancreatic cancer patients. However, as 5% of the normal population also have elevated IgG_4 levels, this makes it an unsuitable single marker for diagnosis. When combined with other features of AIP, it can be of great diagnostic value, although its utility in the monitoring of therapy or as a marker or predictor of relapse is limited.

THERAPEUTIC CONSIDERATIONS

In order to develop therapeutic antibodies with improved effector function profiles, binding profiles to FcγR, FcRn, and C1q have been optimized by engineering the constant domains of the heavy chains. Antibodies with improved CDC activity[54] have been developed by combining structural elements from different subclasses to achieve enhanced effector functions (e.g., IgG_1 with elements from IgG_3). Likewise, attempts have been made to alleviate binding to the effector arm of these molecules (FcγR and C1q) by transferring amino acids found in IgG_2 and IgG_4 onto an IgG_1 backbone.[68,139–143] Similarly, antibodies engineered with increased affinity for FcRn, aimed to increase the half-life of IgG for increased bioavailability of therapeutic antibodies, may eventually lead to IgG molecules with increased half-life, but these may come at a cost because they have mutations that are not naturally occurring and may have immunogenicity issues.[144,145] Some of these modifications also increased binding at pH 7.5, actually resulting in blockade of FcRn; thus, they may have therapeutic benefits on their own by blocking recycling of endogenous autoantibodies.[146] In contrast, reduced affinity to FcRn may also be beneficial in imaging applications due to the increased rate of clearance of nonbinding excess antibody and label.[147] In conclusion, tailored effector functions of IgG may be achieved while avoiding immunogenicity by making use of isotypic and isoallotypic variations existing within all individuals. This seems to have been achieved for IgG_1, with IgG_2/IgG_4 containing alterations for diminished activities,[68,143] but also for isoallotypic variants of IgG_3 with long half-lives and improved effector

functions compared to IgG_1.[90] Importantly, glyco-engineering of IgG may also be used to fine-tune IgG effetor functions (discussed elsewhere in this book). Blocking antibodies based on an IgG_4 backbone may participate in Fab arm exchange *in vivo*, resulting in monovalent IgG_4. This may not be problematic and may even be beneficial, provided that the affinity is sufficient, as seems to be the case for the blocking anti-α_4-integrin IgG_4 antibody (natalizumab), which is used with success for the treatment of remitting-relapsing multiple sclerosis.[148] To avoid Fab arm exchange, an S228P mutation and/or the isoallotypic R409K can be introduced (Figure 9.3B),[149] which appears to be sufficient for abrogating Fab arm exchange *in vivo*.[22,25,150,151]

Acknowledgment

The authors would like to thank Ed Nieuwenhuys for his critical reading of the manuscript.

References

1. Schur PH. IgG subclasses. A historical perspective. *Monogr Allergy* 1988;**23**:1–11.
2. Schatz DG, Swanson PC. V(D)J recombination: mechanisms of initiation. *Annu Rev Genet* 2011;**45**:167–202.
3. Bachl J, Turck CW, Wabl M. Translatable immunoglobulin germ-line transcript. *Eur J Immunol* 1996;**26**(4):870–4.
4. Kracker S, Durandy A. Insights into the B cell specific process of immunoglobulin class switch recombination. *Immunol Lett* 2011;**138**(2):97–103.
5. Nezlin R. *The Immunoglobulins*. New York: Academic Press; 1998.
6. Schroeder Jr HW, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010;**125** (2 Suppl. 2):S41–52.
7. Hamilton RG. Human IgG subclass measurements in the clinical laboratory. *Clin Chem* 1987;**33**(10):1707–25.
8. Wuhrer M, Porcelijn L, Kapur R, Koeleman CA, Deelder A, de Haas M, et al. Regulated glycosylation patterns of IgG during alloimmune responses against human platelet antigens. *J Proteome Res* 2009;**8**(2):450–6.
9. Guhr T, Bloem J, Derksen NI, Wuhrer M, Koenderman AH, Aalberse RC, et al. Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. *PLoS One* 2011;**6**(6):e21246.
10. Stadlmann J, Weber A, Pabst M, Anderle H, Kunert R, Ehrlich HJ, et al. A close look at human IgG sialylation and subclass distribution after lectin fractionation. *Proteomics* 2009;**9**(17):4143–53.
11. Michaelsen TE, Naess LM, Aase A. Human IgG3 is decreased and IgG1, IgG2 and IgG4 are unchanged in molecular size by mild reduction and reoxidation without any major change in effector functions. *Mol Immunol* 1993;**30**(1):35–45.
12. Saluk PH, Clem LW. The unique molecular weight of the heavy chain from human IgG3. *J Immunol* 1971;**107**(1):298–301.
13. Roux KH, Strelets L, Michaelsen TE. Flexibility of human IgG subclasses. *J Immunol* 1997;**159**(7):3372–82.
14. Carrasco B, Garcia de la Torre J, Davis KG, Jones S, Athwal D, Walters C, et al. Crystallohydrodynamics for solving the hydration problem for multi-domain proteins: open physiological conformations for human IgG. *Biophys Chem* 2001;**93**(2–3):181–96.
15. Wypych J, Li M, Guo A, Zhang Z, Martinez T, Allen MJ, et al. Human IgG2 antibodies display disulfide-mediated structural isoforms. *J Biol Chem* 2008;**283**(23):16194–205.
16. Zhang B, Harder AG, Connelly HM, Maheu LL, Cockrill SL. Determination of Fab-hinge disulfide connectivity in structural isoforms of a recombinant human immunoglobulin G2 antibody. *Anal Chem* 2010;**82**(3):1090–9.
17. Lightle S, Aykent S, Lacher N, Mitaksov V, Wells K, Zobel J, et al. Mutations within a human IgG2 antibody form distinct and homogeneous disulfide isomers but do not affect Fc gamma receptor or C1q binding. *Protein Sci* 2010;**19**(4):753–62.
18. Yoo EM, Wims LA, Chan LA, Morrison SL. Human IgG2 can form covalent dimers. *J Immunol* 2003;**170**(6):3134–8.
19. Chivers PT, Prehoda KE, Raines RT. The CXXC motif: a rheostat in the active site 2. *Biochemistry* 1997;**36**(14):4061–6.
20. Bloom JW, Madanat MS, Marriott D, Wong T, Chan SY. Intrachain disulfide bond in the core hinge region of human IgG4. *Protein Sci* 1997;**6**(2):407–15.
21. Angal S, King DJ, Bodmer MW, Turner A, Lawson AD, Roberts G, et al. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody. *Mol Immunol* 1993;**30**(1):105–8.
22. Rispens T, Ooijevaar-de HP, Bende O, Aalberse RC. Mechanism of immunoglobulin G4 Fab-arm exchange. *J Am Chem Soc* 2011;**133**(26):10302–11.
23. Van der Zee JS, Van Swieten P, Aalberse RC. Serologic aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. *J Immunol* 1986;**137**(11):3566–71.

24. van der Neut KM, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 2007;**317**(5844):1554–7.
25. Labrijn AF, Rispens T, Meesters J, Rose RJ, den Bleker TH, Loverix S, et al. Species-specific determinants in the IgG CH3 domain enable Fab-arm exchange by affecting the noncovalent CH3–CH3 interaction strength. *J Immunol* 2011;**187**(6):3238–46.
26. Gergely J, Fudenberg HH, Van LE. The papain susceptibility of IgG myeloma proteins of different heavy chain subclasses. *Immunochemistry* 1970;**7**(1):1–6.
27. Gergely J, Stanworth DR, Jefferis R, Normansell DE, Henney CS, Pardo GI. Structural studies of immunoglobulins. I. The role of cysteine in papain hydrolysis. *Immunochemistry* 1967;**4**:101–11.
28. Skvaril F, Theilkas L, Probst M, Morell A, Barundun S. IgG subclass composition and immunochemical characteristics of plasmin-treated human gamma-globulin. *Vox Sang* 1976;**30**:334–8.
29. Gergely J, Medgyesi GA, Wang AC, Fudenberg HH. IgG myeloma subclass-typing by tryptic digestion of whole sera. *Immunochemistry* 1972;**9**(5):589–92.
30. Medgyesi GA, Gergely J. Structural studies of immunoglobulins. 3. Susceptibility of human gamma G globulins to peptic hydrolysis and papain sensitivity of F(ab′)2 fragments. *Immunochemistry* 1969;**6**(3):473–9.
31. Ryan MH, Petrone D, Nemeth JF, Barnathan E, Bjorck L, Jordan RE. Proteolysis of purified IgGs by human and bacterial enzymes *in vitro* and the detection of specific proteolytic fragments of endogenous IgG in rheumatoid synovial fluid 3. *Mol Immunol* 2008;**45**(7):1837–46.
32. de Lange GG. Polymorphisms of human immunoglobulins: Gm, Am, Em and Km allotypes. *Exp Clin Immunogenet* 1989;**6**(1):7–17.
33. Jefferis R, Lefranc MP. Human immunoglobulin allotypes: possible implications for immunogenicity. *MAbs* 2009;**1**(4):332–8.
34. Dard P, Lefranc MP, Osipova L, Sanchez-Mazas A. DNA sequence variability of IGHG3 alleles associated to the main G3m haplotypes in human populations. *Eur J Hum Genet* 2001;**9**(10):765–72.
35. Brusco A, Saviozzi S, Cinque F, DeMarchi M, Boccazzi C, De LG, et al. Molecular characterization of immunoglobulin G4 gene isoallotypes. *Eur J Immunogenet* 1998;**25**(5):349–55.
36. Brusco A, de Lange GG, Boccazzi C, Carbonara AO. Molecular characterization of Gm(n+) and G2m(n–) allotypes. *Immunogenetics* 1995;**42**(5):414–7.
37. de Lange G. *Monoclonal antibodies against human immunoglobulin allotypes*. PhD thesis, University of London, 1988.
38. Bartelds GM, de GE, Nurmohamed MT, Hart MH, van Eede PH, Wijbrandts CA, et al. Surprising negative association between IgG1 allotype disparity and anti-adalimumab formation: a cohort study 1. *Arthritis Res Ther* 2010;**12**(6):R221.
39. Magdelaine-Beuzelin C, Vermeire S, Goodall M, Baert F, Noman M, Assche GV, et al. IgG1 heavy chain-coding gene polymorphism (G1m allotypes) and development of antibodies-to-infliximab 8. *Pharmacogenet Genomics* 2009;**19**(5):383–7.
40. Meyts I, Bossuyt X, Proesmans M, Boeck KD. Isolated IgG3 deficiency in children: to treat or not to treat? Case presentation and review of the literature. *Pediatr Allergy Immunol* 2012;**2006**(17):544–50.
41. Morell A, Skvaril F, Steinberg AG, Van LE, Terry WD. Correlations between the concentrations of the four sub-classes of IgG and Gm allotypes in normal human sera. *J Immunol* 1972;**108**(1):195–206.
42. Seppala IJ, Sarvas H, Makela O. Low concentrations of Gm allotypic subsets G3 mg and G1 mf in homozygotes and heterozygotes. *J Immunol* 1993;**151**(5):2529–37.
43. Sarma JV, Ward PA. The complement system. *Cell Tissue Res* 2011;**343**(1):227–35.
44. Bindon CI, Hale G, Bruggemann M, Waldmann H. Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q. *J Exp Med* 1988;**168**(1):127–42.
45. Schumaker VN, Calcott MA, Spiegelberg HL, Muller-Eberhard HJ. Ultracentifuge studies of the binding of IgG of different subclasses to the Clq subunit of the first component of complement. *Biochemistry* 1976;**15**(23):5175–81.
46. Tao MH, Smith RI, Morrison SL. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J Exp Med* 1993;**178**(2):661–7.
47. Idusogie EE, Presta LG, Gazzano-Santoro H, Totpal K, Wong PY, Ultsch M, et al. Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. *J Immunol* 2000;**164**(8):4178–84.
48. Morgan A, Jones ND, Nesbitt AM, Chaplin L, Bodmer MW, Emtage JS. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc gamma RI and Fc gamma RIII binding. *Immunology* 1995;**86**(2):319–24.
49. Brekke OH, Michaelsen TE, Aase A, Sandin RH, Sandlie I. Human IgG isotype-specific amino acid residues affecting complement-mediated cell lysis and phagocytosis. *Eur J Immunol* 1994;**24**(10):2542–7.
50. Dall'Acqua WF, Cook KE, Damschroder MM, Woods RM, Wu H. Modulation of the effector functions of a human IgG1 through engineering of its hinge region. *J Immunol* 2006;**177**(2):1129–38.
51. Dangl JL, Wensel TG, Morrison SL, Stryer L, Herzenberg LA, Oi VT. Segmental flexibility and

complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies. *EMBO J* 1988;**7**(7):1989–94.
52. Lu Y, Harding SE, Michaelsen TE, Longman E, Davis KG, Ortega A, et al. Solution conformation of wild-type and mutant IgG3 and IgG4 immunoglobulins using crystallohydrodynamics: possible implications for complement activation. *Biophys J* 2007;**93**(11):3733–44.
53. Tan LK, Shopes RJ, Oi VT, Morrison SL. Influence of the hinge region on complement activation, C1q binding, and segmental flexibility in chimeric human immunoglobulins. *Proc Natl Acad Sci USA* 1990;**87**(1):162–6.
54. Natsume A, In M, Takamura H, Nakagawa T, Shimizu Y, Kitajima Engineered antibodies of IgG1/IgG3 mixed isotype with enhanced cytotoxic activities. *Cancer Res* 2008;**68**(10):3863–72.
55. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002;**105**(1):9–19.
56. Abe Y, Gor J, Bracewell DG, Perkins SJ, Dalby PA. Masking of the Fc region in human IgG4 by constrained x-ray scattering modelling: implications for antibody function and therapy 1. *Biochem J* 2010;**432**(1):101–11.
57. Van der Zee JS, Van Swieten P, Aalberse RC. Inhibition of complement activation by IgG4 antibodies. *Clin Exp Immunol* 1986;**64**(2):415–22.
58. Saeland E, Vidarsson G, Leusen JH, Van Garderen E, Nahm MH, Vile-Weekhout H, et al. Central role of complement in passive protection by human IgG1 and IgG2 anti-pneumococcal antibodies in mice. *J Immunol* 2003;**170**(12):6158–64.
59. Vidarsson G, Sigurdardottir ST, Gudnason T, Kjartansson S, Kristinsson KG, Ingolfsdottir G, et al. Isotypes and opsonophagocytosis of pneumococcus type 6B antibodies elicited in infants and adults by an experimental pneumococcus type 6B-tetanus toxoid vaccine. *Infect Immun* 1998;**66**(6):2866–70.
60. Vidarsson G, Jonsdottir I, Jonsson S, Valdimarsson H. Opsonization and antibodies to capsular and cell wall polysaccharides of *Streptococcus pneumoniae*. *J Infect Dis* 1994;**170**:592–9.
61. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* 2009;**113**(16):3716–25.
62. Ramsland PA, Farrugia W, Bradford TM, Sardjono CT, Esparon S, Trist HM, et al. Structural basis for Fc gammaRIIa recognition of human IgG and formation of inflammatory signaling complexes. *J Immunol* 2011;**187**(6):3208–17.
63. Sondermann P, Huber R, Oosthuizen V, Jacob U. The 3.2-A crystal structure of the human IgG1 Fc fragment–Fc gammaRIII complex. *Nature* 2000;**406**(6793):267–73.
64. Canfield SM, Morrison SL. The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. *J Exp Med* 1991;**173**(6):1483–91.
65. Chappel MS, Isenman DE, Everett M, Xu YY, Dorrington KJ, Klein MH. Identification of the Fc gamma receptor class I binding site in human IgG through the use of recombinant IgG1/IgG2 hybrid and point-mutated antibodies. *Proc Natl Acad Sci USA* 1991;**88**(20):9036–40.
66. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *J Biol Chem* 2001;**276**(9):6591–604.
67. Lund J, Winter G, Jones PT, Pound JD, Tanaka T, Walker MR, et al. Human Fc gamma RI and Fc gamma RII interact with distinct but overlapping sites on human IgG. *J Immunol* 1991;**147**(8):2657–62.
68. Armour KL, Clark MR, Hadley AG, Williamson LM. Recombinant human IgG molecules lacking Fcgamma receptor I binding and monocyte triggering activities. *Eur J Immunol* 1999;**29**(8):2613–24.
69. Redpath S, Michaelsen TE, Sandlie I, Clark MR. The influence of the hinge region length in binding of human IgG to human Fcgamma receptors. *Hum Immunol* 1998;**59**(11):720–7.
70. Parren PW, Warmerdam PA, Boeije LC, Arts J, Westerdaal NA, Vlug A, et al. On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. *J Clin Invest* 1992;**90**(4):1537–46.
71. Armour KL, van de Winkel JG, Williamson LM, Clark MR. Differential binding to human FcgammaRIIa and FcgammaRIIb receptors by human IgG wildtype and mutant antibodies. *Mol Immunol* 2003;**40**(9):585–93.
72. Wines BD, Powell MS, Parren PW, Barnes N, Hogarth PM. The IgG Fc contains distinct Fc receptor (FcR) binding sites: the leukocyte receptors Fc gamma RI and Fc gamma RIIa bind to a region in the Fc distinct from that recognized by neonatal FcR and protein A. *J Immunol* 2000;**164**(10):5313–8.
73. Breunis WB, van ME, Bruin M, Geissler J, de BM, Peters M, et al. Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. *Blood* 2008;**111**(3):1029–38.
74. Li H, Sethuraman N, Stadheim TA, Zha D, Prinz B, Ballew N, et al. Optimization of humanized IgGs in glycoengineered *Pichia pastoris*. *Nat Biotechnol* 2006;**24**(2):210–5.
75. Siberil S, de Romeuf C, Bihoreau N, Fernandez N, Meterreau JL, Regenman A, et al. Selection of a

human anti-RhD monoclonal antibody for therapeutic use: impact of IgG glycosylation on activating and inhibitory Fc gamma R functions. *Clin Immunol* 2006;**118**(2–3):170–9.
76. Ferrara C, Grau S, Jager C, Sondermann P, Brunker P, Waldhauer I, et al. Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. *Proc Natl Acad Sci USA* 2011;**108**(31):12669–74.
77. Huizinga TW, Kleijer M, Tetteroo PA, Roos D, von dem Borne AE. Biallelic neutrophil Na-antigen system is associated with a polymorphism on the phosphoinositol-linked Fc gamma receptor III (CD16). *Blood* 1990;**75**(1):213–7.
78. Huizinga TW, Kerst M, Nuyens JH, Vlug A, von dem Borne AE, Roos D, et al. Binding characteristics of dimeric IgG subclass complexes to human neutrophils. *J Immunol* 1989;**142**(7):2359–64.
79. Radaev S, Motyka S, Fridman WH, Sautes-Fridman C, Sun PD. The structure of a human type III Fcgamma receptor in complex with Fc. *J Biol Chem* 2001;**276**(19):16469–77.
80. Brambell FW, Hemmings WA, Morris IG. A theoretical model of gamma-globulin catabolism. *Nature* 1964;**203**:1352–4.
81. Brambell FW. The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet* 1966;**2**(7473):1087–93.
82. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee Y, Ward ES. Abnormally short serum half-lives of IgG in beta 2-microglobulin-deficient mice. *Eur J Immunol* 1996;**26**(3):690–6.
83. Israel EJ, Wilsker DF, Hayes KC, Schoenfeld D, Simister NE. Increased clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn. *Immunology* 1996;**89**(4):573–8.
84. Junghans RP, Anderson CL. The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. *Proc Natl Acad Sci USA* 1996;**93**(11):5512–6.
85. Roopenian DC, Christianson GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, et al. The MHC class i-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. *J Immunol* 2003;**170**(7):3528–33.
86. Burmeister WP, Gastinel LN, Simister NE, Blum ML, Bjorkman PJ. Crystal structure at 2.2 A resolution of the MHC-related neonatal Fc receptor. *Nature* 1994;**372**(6504):336–43.
87. West Jr AP, Bjorkman PJ. Crystal structure and immunoglobulin G binding properties of the human major histocompatibility complex-related Fc receptor. *Biochemistry* 2000;**39**(32):9698–708.
88. Chaudhury C, Mehnaz S, Robinson JM, Hayton WL, Pearl DK, Roopenian DC, et al. The major histocompatibility complex-related Fc receptor for IgG (FcRn) binds albumin and prolongs its lifespan. *J Exp Med* 2003;**197**(3):315–22.
89. Gastinel LN, Simister NE, Bjorkman PJ. Expression and crystallization of a soluble and functional form of an Fc receptor related to class I histocompatibility molecules. *Proc Natl Acad Sci USA* 1992;**89**(2):638–42.
90. Stapleton NM, Andersen JT, Stemerding AM, Bjarnarson SP, Verheul RC, Gerritsen J, et al. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. *Nat Commun* 2011;**5**:599.
91. Spiekermann GM, Finn PW, Ward ES, Dumont J, Dickinson BL, Blumberg RS, et al. Receptor-mediated immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. *J Exp Med* 2002;**196**(3):303–10.
92. Yoshida M, Claypool SM, Wagner JS, Mizoguchi E, Mizoguchi A, Roopenian DC, et al. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. *Immunity* 2004;**20**(6):769–83.
93. Yoshida M, Kobayashi K, Kuo TT, Bry L, Glickman JN, Claypool SM, et al. Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. *J Clin Invest* 2006;**116**(8):2142–51.
94. Zhu X, Meng G, Dickinson BL, Li X, Mizoguchi E, Miao L, et al. MHC class I-related neonatal Fc receptor for IgG is functionally expressed in monocytes, intestinal macrophages, and dendritic cells. *J Immunol* 2001;**166**(5):3266–76.
95. Cervenak J, Bender B, Schneider Z, Magna M, Carstea BV, Liliom K, et al. Neonatal FcR overexpression boosts humoral immune response in transgenic mice. *J Immunol* 2011;**186**(2):959–68.
96. Vidarsson G, Stemerding AM, Stapleton NM, Spliethoff SE, Janssen H, Rebers FE, et al. FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. *Blood* 2006;**108**(10):3573–9.
97. Baker K, Qiao SW, Kuo TT, Aveson VG, Platzer B, Andersen JT, et al. Neonatal Fc receptor for IgG (FcRn) regulates cross-presentation of IgG immune complexes by CD8–CD11b+ dendritic cells. *Proc Natl Acad Sci USA* 2011;**108**(24):9927–32.
98. Mi W, Wanjie S, Lo ST, Gan Z, Pickl-Herk B, Ober RJ, et al. Targeting the neonatal fc receptor for antigen delivery using engineered Fc fragments. *J Immunol* 2008;**181**(11):7550–61.
99. Qiao SW, Kobayashi K, Johansen FE, Sollid LM, Andersen JT, Milford E, et al. Dependence of antibody-mediated presentation of antigen on FcRn. *Proc Natl Acad Sci USA* 2008;**105**(27):9337–42.

100. Heijnen IA, Van Vugt MJ, Fanger NA, Graziano RF, de Wit TP, Hofhuis FM, et al. Antigen targeting to myeloid-specific human Fc gamma RI/CD64 triggers enhanced antibody responses in transgenic mice. *J Clin Invest* 1996;**97**(2):331–8.
101. Sauer-Eriksson AE, Kleywegt GJ, Uhlen M, Jones TA. Crystal structure of the C2 fragment of streptococcal protein G in complex with the Fc domain of human IgG. *Structure* 1995;**3**(3):265–78.
102. Deisenhofer J. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8-A resolution. *Biochemistry* 1981;**20**(9):2361–70.
103. Jefferis R, Lund J, Pound JD. IgG-Fc-mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. *Immunol Rev* 1998;**163**:59–76.
104. Bjorck L, Kronvall G. Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. *J Immunol* 1984;**133**(2):969–74.
105. Stone GC, Sjobring U, Bjorck L, Sjoquist J, Barber CV, Nardella FA. The Fc binding site for streptococcal protein G is in the C gamma 2–C gamma 3 interface region of IgG and is related to the sites that bind staphylococcal protein A and human rheumatoid factors. *J Immunol* 1989;**143**(2):565–70.
106. Van LE, Frangione B, Recht B, Franklin EC. Staphylococcal protein A and human IgG subclasses and allotypes. *Scand J Immunol* 1982;**15**(3):275–8.
107. Lian LY, Barsukov IL, Derrick JP, Roberts GC. Mapping the interactions between streptococcal protein G and the Fab fragment of IgG in solution. *Nat Struct Biol* 1994;**1**(6):355–7.
108. Derrick JP, Wigley DB. The third IgG-binding domain from streptococcal protein G. An analysis by x-ray crystallography of the structure alone and in a complex with Fab. *J Mol Biol* 1994;**243**(5):906–18.
109. Perosa F, Luccarelli G, Dammacco F. Absence of streptococcal protein G (PG)-specific determinant in the Fab region of human IgG2. *Clin Exp Immunol* 1997;**109**(2):272–8.
110. Silverman GJ. B-cell superantigens. *Immunol Today* 1997;**18**(8):379–86.
111. Berkowska MA, Driessen GJ, Bikos V, Grosserichter-Wagener C, Stamatopoulos K, Cerutti A, et al. Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways. *Blood* 2011;**118**(8):2150–8.
112. Pan Q, Hammarstrom L. Molecular basis of IgG subclass deficiency. *Immunol Rev* 2000;**178**:99–110.
113. Vlug A, Nieuwenhuys EJ, van Eijk RV, Geertzen HG, van Houte AJ. Nephelometric measurements of human IgG subclasses and their reference ranges. *Ann Biol Clin (Paris)* 1994;**52**(7–8):561–7.
114. Ferrante A, Beard LJ, Feldman RG. IgG subclass distribution of antibodies to bacterial and viral antigens. *Pediatr Infect Dis J* 1990;**9**(8 Suppl.):S16–24.
115. Jefferis R, Kumararatne DS. Selective IgG subclass deficiency: quantification and clinical relevance. *Clin Exp Immunol* 1990;**81**(3):357–67.
116. Siber GR, Schur PH, Aisenberg AC, Weitzman SA, Schiffman G. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. *N Engl J Med* 1980;**303**(4):178–82.
117. Barrett DJ, Ayoub EM. IgG2 subclass restriction of antibody to pneumococcal polysaccharides. *Clin Exp Immunol* 1986;**63**(1):127–34.
118. Schauer U, Stemberg F, Rieger CH, Buttner W, Borte M, Schubert S, et al. Levels of antibodies specific to tetanus toxoid, *Haemophilus influenzae* type b, and pneumococcal capsular polysaccharide in healthy children and adults. *Clin Diagn Lab Immunol* 2003;**10**(2):202–7.
119. Hammarstrom L, Smith CI. IgG2 deficiency in a healthy blood donor. Concomitant lack of IgG2, IgA and IgE immunoglobulins and specific anti-carbohydrate antibodies. *Clin Exp Immunol* 1983;**51**(3):600–4.
120. Kuijpers TW, Weening RS, Out TA. IgG subclass deficiencies and recurrent pyogenic infections, unresponsiveness against bacterial polysaccharide antigens. *Allergol Immunopathol (Madr)* 1992;**20**(1):28–34.
121. von GS, Smith DF, Cummings RD, Riedel S, Miescher S, Schaub A, et al. Intravenous immunoglobulin contains a broad repertoire of anticarbohydrate antibodies that is not restricted to the IgG2 subclass. *J Allergy Clin Immunol* 2009;**123**(6):1268–76.
122. Hassan MS, Islam KB, Hammarstrom L, Smith CI. Regulation of C gamma 3 expression. Role of switch in the allotype-associated variation of human serum IgG3 levels. *J Immunol* 1992;**148**(8):2555–62.
123. Brezski RJ, Jordan RE. Cleavage of IgGs by proteases associated with invasive diseases: an evasion tactic against host immunity? *MAbs* 2010;**2**(3):212–20.
124. Soderstrom T, Enskog A, Samuelsson BE, Cedergren B. Immunoglobulin subclass (IgG3) restriction of anti-P and anti-Pk antibodies in patients of the rare p blood group. *J Immunol* 1985;**134**(1):1–3.
125. Aalberse RC, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983;**130**:722–6.
126. Aalberse RC, Dieges PH, Knul-Bretlova V, Vooren P, Aalbers M, van LJ. IgG4 as a blocking antibody. *Clin Rev Allergy* 1983;**1**(2):289–302.

127. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;**172**(5):3252–9.
128. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* 2009;**39**(4):469–77.
129. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. *Allergy* 2011;**66**(6):725–32.
130. van Helden PM, van den Berg HM, Gouw SC, Kaijen PH, Zuurveld MG, Mauser-Bunschoten EP, et al. IgG subclasses of anti-FVIII antibodies during immune tolerance induction in patients with hemophilia A. *Br J Haematol* 2008;**142**(4):644–52.
131. Andersen BR, Terry WD. Gamma G4 globulin Ab causing inhibition of clotting factor VIII. *Nature* 1968;**217**:174.
132. Iizuka A, Nagao T. Analysis of IgG heavy chain subclasses of alloantibodies to factor IX by crossed immunoelectrophoresis of factor IX using the intermediate gel technique. *Br J Haematol* 1983;**53**(4):687–8.
133. Hart MH, de VH, Wouters D, Wolbink GJ, Killestein J, de Groot ER, et al. Differential effect of drug interference in immunogenicity assays. *J Immunol Methods* 2011;**372**(1–2):196–203.
134. Ottesen EA, Skvaril F, Tripathy SP, Poindexter RW, Hussain R. Prominence of IgG4 in the IgG antibody response to human filariasis. *J Immunol* 1985; **134**(4):2707–12.
135. Adjobimey T, Hoerauf A. Induction of immunoglobulin G4 in human filariasis: an indicator of immunoregulation. *Ann Trop Med Parasitol* 2010;**104**(6):455–64.
136. Kurniawan A, Yazdanbakhsh M, van RR, Aalberse R, Selkirk ME, Partono F, et al. Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. *J Immunol* 1993;**150**(9):3941–50.
137. Sah RP, Chari ST. Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis. *Curr Opin Rheumatol* 2011;**23**(1):108–13.
138. Yamamoto M, Tabeya T, Naishiro Y, Yajima H, Ishigami K, Shimizu Y, et al. Value of serum IgG4 in the diagnosis of IgG4-related disease and in differentiation from rheumatic diseases and other diseases. *Mod Rheumatol* 2012;**22**(3):419–25.
139. Oganesyan V, Gao C, Shirinian L, Wu H, Dall'Acqua WF. Structural characterization of a human Fc fragment engineered for lack of effector functions. *Acta Crystallogr D Biol Crystallogr* 2008;**64**(Pt. 6):700–4.
140. An Z, Forrest G, Moore R, Cukan M, Haytko P, Huang L, et al. IgG2m4, an engineered antibody isotype with reduced Fc function. *MAbs* 2009;**1**(6):572–9.
141. Salfeld JG. Isotype selection in antibody engineering. *Nat Biotechnol* 2007;**25**(12):1369–72.
142. Mueller JP, Giannoni MA, Hartman SL, Elliott EA, Squinto SP, Matis LA, et al. Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells. *Mol Immunol* 1997;**34**(6):441–52.
143. Ghevaert C, Wilcox DA, Fang J, Armour KL, Clark MR, Ouwehand WH, et al. Developing recombinant HPA-1a-specific antibodies with abrogated Fcgamma receptor binding for the treatment of feto-maternal alloimmune thrombocytopenia. *J Clin Invest* 2008;**118**(8):2929–38.
144. Dall'Acqua WF, Woods RM, Ward ES, Palaszynski SR, Patel NK, Brewah YA, et al. Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* 2002;**169**(9):5171–80.
145. Ward ES, Zhou J, Ghetie V, Ober RJ. Evidence to support the cellular mechanism involved in serum IgG homeostasis in humans. *Int Immunol* 2003;**15**(2):187–95.
146. Patel DA, Puig-Canto A, Challa DK, Montoyo HP, Ober RJ, Ward ES. Neonatal Fc receptor blockade by Fc engineering ameliorates arthritis in a murine model. *J Immunol* 2011;**187**(2):1015–22.
147. Jaggi JS, Carrasquillo JA, Seshan SV, Zanzonico P, Henke E, Nagel A, et al. Improved tumor imaging and therapy via i.v. IgG-mediated time-sequential modulation of neonatal Fc receptor. *J Clin Invest* 2007;**117**(9):2422–30.
148. Materials and methods are available as supporting material on ScienceOnline.com.
149. Broug E, Bland-Ward PA, Powell J, Johnson KS. Fab–arm exchange. *Nat Biotechnol* 2010;**28**(2):123–5.
150. Labrijn AF, Buijsse AO, van den Bremer ET, Verwilligen AY, Bleeker WK, Thorpe SJ, et al. Therapeutic IgG4 antibodies engage in Fab–arm exchange with endogenous human IgG4 *in vivo*. *Nat Biotechnol* 2009;**27**(8):767–71.
151. Stubenrauch K, Wessels U, Regula JT, Kettenberger H, Schleypen J, Kohnert U. Impact of molecular processing in the hinge region of therapeutic IgG4 antibodies on disposition profiles in cynomolgus monkeys. *Drug Metab Dispos* 2010;**38**(1):84–91.