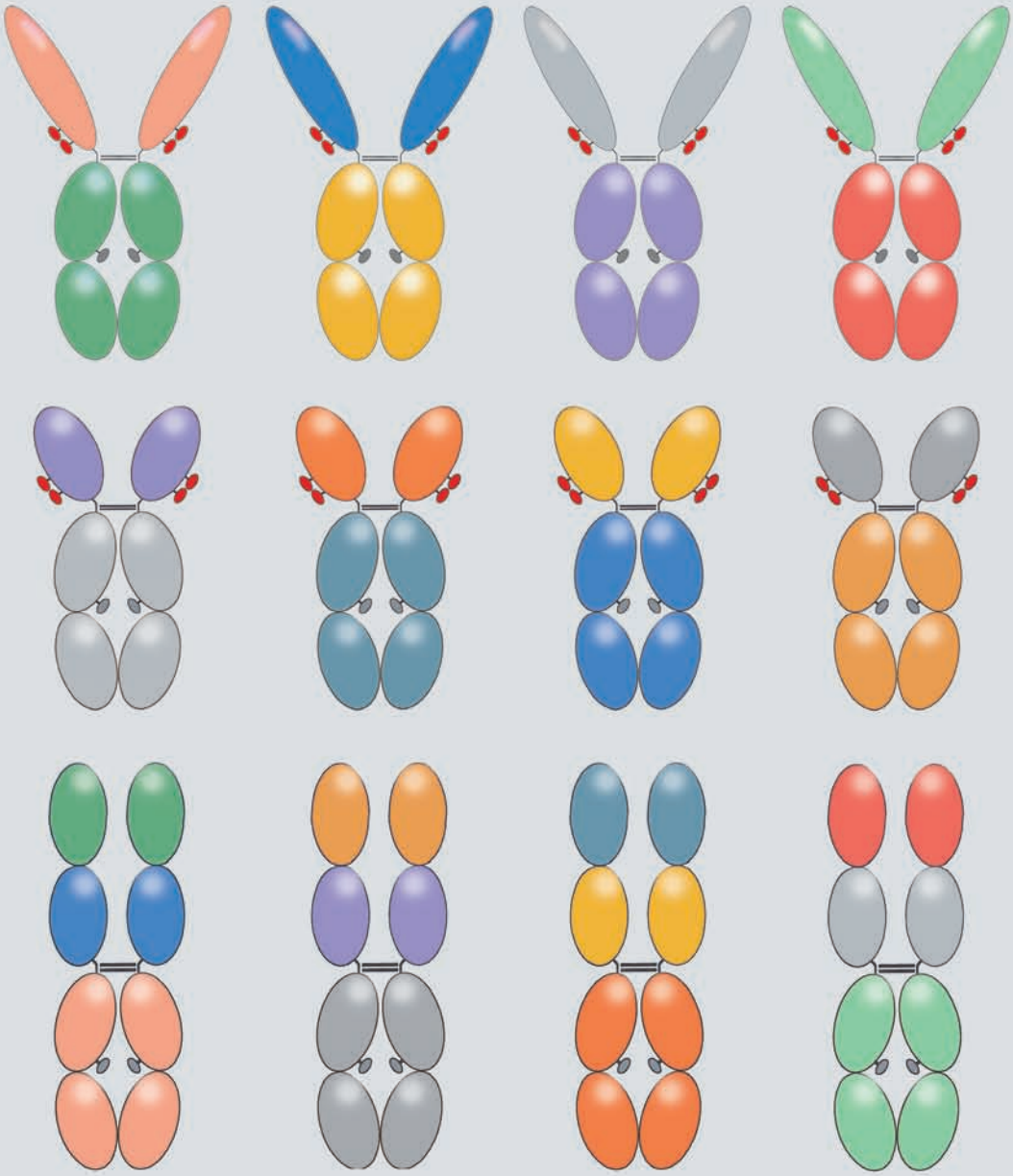
Therapeutic Fc-Fusion Proteins

Edited by Steven M. Chamow,
Thomas Ryll, Henry B. Lowman, and Deborah Farson



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Top row: Tumor necrosis factor receptor-immunoglobulin G1 (TNFR-Fc) fusion protein; middle row: Interleukin receptor 1-immunoglobulin G1 (L1R-Fc) fusion protein; bottom row: Vascular endothelial growth factor receptor-immunoglobulin G1 (VEGFTrap) Fc fusion protein.

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Contents

Preface

Fc-fusion proteins – engineered polypeptides that combine biologically active peptides or protein domains with the crystallizable fragment (Fc) domain of an antibody – have become widely used agents both in research and in clinical practice. The fact that these molecules resemble antibodies in so many aspects of structure, function, expression, purification, and pharmacology has enabled them to be rapidly integrated into a variety of assays, preclinical studies, and clinical applications through leveraging the prior experience with monoclonal antibodies. In the years following the 1989 report from Genentech by Dan Capon and colleagues on an Fc-fusion protein or "immunoadhesin" composed of CD4 linked to an antibody Fc, a variety of different receptor extracellular domains were produced in this format. An earlier volume, *Antibody Fusion Proteins*, by Chamow and Ashkenazi (Wiley, 1999) highlighted progress up to the stage of the first therapeutic Fc fusions progressing through clinical trials. Etanercept became the first FDA-approved therapeutic fusion protein in 1998 and has since become one of the most clinically and commercially successful therapeutics. However, the story of therapeutic Fc fusions does not end here. On the contrary, a growing number of these molecules are being developed as biotherapeutics, including Fc-fusion proteins composed of heterodimeric polypeptide chains and others containing novel peptide mimotopes attached to Fc fragments. We therefore thought it important to review the literature and experience in developing this novel class of biologics – hence the current volume, *Therapeutic Fc-Fusion Proteins*, which brings up-to-date information on the processes of designing and producing these molecules and highlights some of the most prominent case studies from clinical experience.

Owing to the crucial components of antibody structure and function in the design, production, and use of therapeutic Fc fusions, we begin the book with an extensive introduction to the structure and function of IgG molecules (Chapter 1). This is followed by Part One, a series of chapters summarizing state-of-the-art approaches for producing therapeutic Fc proteins: Chapter 2 presents the principles of design and expression systems; Chapter 3, cell culture production; Chapter 4, downstream processing; Chapter 5, formulation and delivery; Chapter 6, quality by design; and Chapter 7, analytical characterization. These chapters provide a roadmap for the development and life cycle of manufacturing processes for therapeutic Fc fusions. Part Two begins with a synopsis (Chapter 8) of clinically significant Fc-fusion

proteins that have been approved or are in late-stage clinical trials. Subsequent chapters present case studies of a subset of these, selected for their unique features in terms of molecular design and/or mechanism of action: alefacept, a lymphocyte function-associated antigen 3 (LFA-3) fusion (Chapter 9); etanercept, a tumor necrosis factor (TNF) receptor fusion (Chapter 10); abatacept and belatacept, cytotoxic T-lymphocyte antigen 4 (CTLA-4) fusions (Chapter 11); aflibercept, a vascular endothelial growth factor (VEGF) receptor fusion (Chapter 12); and factor VIII/IX fusions (Chapter 13). In several cases, we have included authors who were involved directly in development of the Fc-fusion protein products about which they have written. We believe that these accounts of the biologics development process in the context of a range of biological mechanisms and disease indications provide important lessons for the development of future therapeutic Fc-fusion proteins.

We thank all of the contributors to this book for taking the time to write what we hope you will find are useful discussions of these topics. We also thank Laura Shih, Wendy Lin, and Anne Chassin du Guerny and the editorial staff of Wiley-Blackwell for their editing support.

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1
Introduction: Antibody Structure and Function

Arvind Rajpal, Pavel Strop, Yik Andy Yeung, Javier Chaparro-Riggers, and Jaume Pons

1.1
Introduction to Antibodies

Antibodies, a central part of humoral immunity, have increasingly become a dominant class of biotherapeutics in clinical development and are approved for use in patients. As with any successful endeavor, the history of monoclonal antibody therapeutics benefited from the pioneering work of many, such as Paul Ehrlich who in the late nineteenth century demonstrated that serum components had the ability to protect the host by "passive vaccination" [1], the seminal invention of monoclonal antibody generation using hybridoma technology by Kohler and Milstein [2], and the advent of recombinant technologies that sought to reduce the murine content in therapeutic antibodies [3].

During the process of generation of humoral immunity, the B-cell receptor (BCR) is formed by recombination between variable (V), diversity (D), and joining (J) exons, which define the antigen recognition element. This is combined with an immunoglobulin (Ig) constant domain element (μ for IgM, δ for IgD, γ for IgG (gamma immunoglobulin), α for IgA, and ε for IgE) that defines the isotype of the molecule. Sequences for these V, D, J, and constant domain genes for disparate organisms can be found through the International ImMunoGeneTics Information System® [4]. The different Ig subtypes are presented at different points during B-cell maturation. For instance, all naïve B cells express IgM and IgD, with IgM being the first secreted molecule. As the B cells mature and undergo class switching, a majority of them secrete either IgG or IgA, which are the most abundant class of Ig in plasma.

Characteristics like high neutralizing and recruitment of effector mechanisms, high affinity, and long resident half-life in plasma make the IgG isotype an ideal candidate for generation of therapeutic antibodies. Within the IgG isotype, there are four subtypes (IgG1–IgG4) with differing properties (Table 1.1). Most of the currently marketed IgGs are of the subtype IgG1 (Table 1.2).

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Table 1.1 Subtype properties.

Property	IgG1	IgG2	IgG3	IgG4
Heavy chain constant gene	γ1	γ2	γ3	γ4
Approximate molecular weight (kDa)	150	150	170	150
Mean serum level (mg/ml)	9	3	1	0.5
Half-life in serum (days)	21	21	7	21
ADCC	+	−	+	+/−
CDC	++	+	+++	−
Number of disulfides in hinge	2	4	11	2
Number of amino acids in hinge	15	12	62	12
Gm allotypes	4	1	13	−
Protein A binding	+++	+++	+	+++
Protein G binding	+++	+++	+++	+++

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity.

Table 1.2 Marketed antibodies and antibody derivatives by target.

Trade name	International non-proprietary name	Target	Type	Indication
Benlysta®	Belimumab	BLyS	Human IgG1λ	SLE
Soliris®	Eculizumab	C5	Humanized IgG2/4	PNH
Raptiva®	Efalizumab	CD11a	Humanized IgG1κ	Psoriasis
Amevive®	Alefacept	CD2	CD2-binding domain of LFA3–IgG1 Fc fusion	Psoriasis
Rituxan®	Rituximab	CD20	Chimeric IgG1κ	NHL, CLL, RA, GPA/MPA
Zevalin®	Ibritumomab tiuxetan	CD20	Murine IgG1κ–Y90/In111 conjugate	NHL
Bexxar®	Tositumomab-I131	CD20	Murine IgG2aλ–I131 conjugate	NHL
Arzerra®	Ofatumumab	CD20	Human IgG1κ	CLL
Orthoclone-OKT3®	Muromonab-CD3	CD3	Murine IgG2a	Transplant rejection
Adcetris®	Brentuximab vedotin	CD30	Chimeric IgG1κ-conjugated MMAE	Hodgkin's lymphoma
Mylotarg®	Gemtuzumab ozogamicin	CD33	Humanized IgG4κ–calicheamicin conjugate	Leukemia
Campath-1H®	Alemtuzumab	CD52	Humanized IgG1κ	Leukemia
Orencia®	Abatacept	CD80/CD86	CTLA4–IgG1 Fc fusion	RA
Nulojix®	Belatacept	CD80/CD86	CTLA4–IgG1 Fc fusion	Transplant rejection
Yervoy®	Ipilimumab	CTLA4	Human IgG1κ	Metastatic melanoma

Erbitux®	Cetuximab	EGFR	Chimeric IgG1κ	Colorectal cancer
Vectibix®	Panitumumab	EGFR	Human IgG2κ	Colorectal cancer
Removab®	Catumaxomab	EpCAM/CD3	Rat IgG2b/mouse IgG2a	Malignant ascites
ReoPro®	Abciximab	gPIIb/IIIa	Chimeric Fab	PCI complications
Herceptin®	Trastuzumab	Her2	Humanized IgG1κ	Breast cancer
Kadcyla®	Trastuzumab emtansine	Her2	Humanized IgG1κ–DM1 conjugate	Breast cancer
Perjeta®	Pertuzumab	Her2	Humanized IgG1κ	Breast cancer
Xolair®	Omalizumab	IgE	Humanized IgG1κ	Asthma
Ilaris®	Canakinumab	IL-1b	Human IgG1κ	CAPS, FCAS, MWS
Arcalyst®	Rilonacept	IL1	IL1R1–IL1RAcP–IgG1 Fc fusion	CAPS
Stelara®	Ustekinumab	IL12/IL23	Human IgG1κ	Psoriasis
Zenapax®	Daclizumab	IL2ra	Humanized IgG1	Transplant rejection
Simulect®	Basiliximab	IL2ra	Chimeric IgG1κ	Transplant rejection
Actemra®	Tocilizumab	IL6r	Humanized IgG1κ	RA
Tysabri®	Natalizumab	LFA4	Humanized IgG4κ	MS
Prolia®	Denosumab	RANKL	Human IgG2κ	Bone metastases
Synagis®	Pavilizumab	RSV F protein	Chimeric IgG1κ	RSV
Remicade®	Infliximab	TNFα	Chimeric IgGκ	RA
Enbrel®	Etanercept	TNFα	TNFrII–p75 ECD–IgG1 Fc fusion	RA
Humira®	Adalimumab	TNFα	Human IgG1κ	RA, Crohn's disease
Cimzia®	Certolizumab pegol	TNFα	Humanized IgG1κ Fab–PEG conjugate	RA
Simponi®	Golimumab	TNFα	Human IgG1κ	RA, PA, AS
Nplate®	Romiplostim	TPOr	Peptide–IgG1 Fc fusion	TCP, UC
Avastin®	Bevacizumab	VEGF	Humanized IgG1κ	Colorectal cancer
Lucentis®	Ranibizumab	VEGF	Humanized IgG1κ Fab	wAMD
Eylea®	Aflibercept	VEGF-A	VEGFr1 and VEGFr2–IgG1 Fc fusion	wAMD

Abbreviations: AS, ankylosing spondylitis; CAPS, cryopyrin-associated periodic syndrome; CLL, chronic lymphocytic leukemia; FCAS, familial cold autoinflammatory syndrome; GPA/MPA, granulomatosis with polyangiitis (Wegener's granulomatosis)/microscopic polyangiitis; MS, multiple sclerosis; MWS, Muckle–Wells syndrome; NHL, non-Hodgkin's lymphoma; PA, psoriatic arthritis; PCI, percutaneous coronary intervention; PNH, paroxysmal nocturnal hemoglobinuria; RA, rheumatoid arthritis; RSV, respiratory syncytial virus; SLE, systemic lupus erythematosus; TCP, thrombocytopenia; UC, ulcerative colitis; wAMD, neovascular (wet) age-related macular degeneration.

The ability of antibodies to recognize their antigens with exquisite specificity and high affinity makes them an attractive class of molecules to bind extracellular targets and generate a desired pharmacological effect. Antibodies also benefit from their ability to harness an active salvage pathway, mediated by the neonatal Fc receptor (FcRn), thereby enhancing their pharmacokinetic (PK) life span and mitigating the need for frequent dosing. The antibodies and antibody derivatives approved in the United States and the European Union (Table 1.2) span a wide range of therapeutic areas, including oncology, autoimmunity, ophthalmology, and transplant rejection. They also harness disparate modes of action like blockade of ligand binding and subsequent signaling, and receptor and signal activation, which target effector functions (antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)), and delivery of cytotoxic payload.

Antibodies are generated by the assembly of two heavy chains and two light chains to produce two antigen-binding sites and a single constant domain region (Figure 1.1, panel a). The constant domain sequence in the heavy chain designates the subtype (Table 1.1). The light chains can belong to two families (λ and κ), with most of the currently marketed antibodies belonging to the κ family.

The antigen-binding regions can be derived by proteolytic cleavage of the antibody to generate antigen-binding fragments (Fab) and the constant fragment (Fc, also known as the fragment of crystallization). The Fab comprises the variable regions (variable heavy (VH) [11] and variable light (VL)) and constant regions (C_H1 and Cκ/Cλ). Within these variable regions reside loops called complementarity determining regions (CDRs) responsible for direct interaction with the antigen (Figure 1.1, panel b). Because of the significant variability in the number of amino acids in these CDRs, there are multiple numbering schemes for the variable domains [12,13] but only one widely used numbering scheme for the constant domain (including portions of the C_H1, hinge, and the Fc) called the EU numbering system [14].

There are two general methods to generate antibodies in the laboratory. The first utilizes the traditional methodology employing immunization followed by recovery of functional clones either by hybridoma technology or, more recently, by recombinant cloning of variable domains from previously isolated B cells displaying and expressing the desired antigen-binding characteristics. There are several variations of these approaches. The first approach includes the immunization of transgenic animals expressing subsets of the human Ig repertoire (see review by Lonberg [15]) and isolation of rare B-cell clones from humans exposed to specific antigens of interest [16]. The second approach requires selecting from a large *in vitro* displayed repertoire either amplified from natural sources (i.e., human peripheral blood lymphocytes in Ref. [17]) or designed synthetically to reflect natural and/or desired properties in the binding sites of antibodies [18,19]. This approach requires the use of a genotype–phenotype linkage strategy, such as phage or yeast display, which allows for the recovery of genes for antibodies displaying appropriate binding characteristics for the antigen.

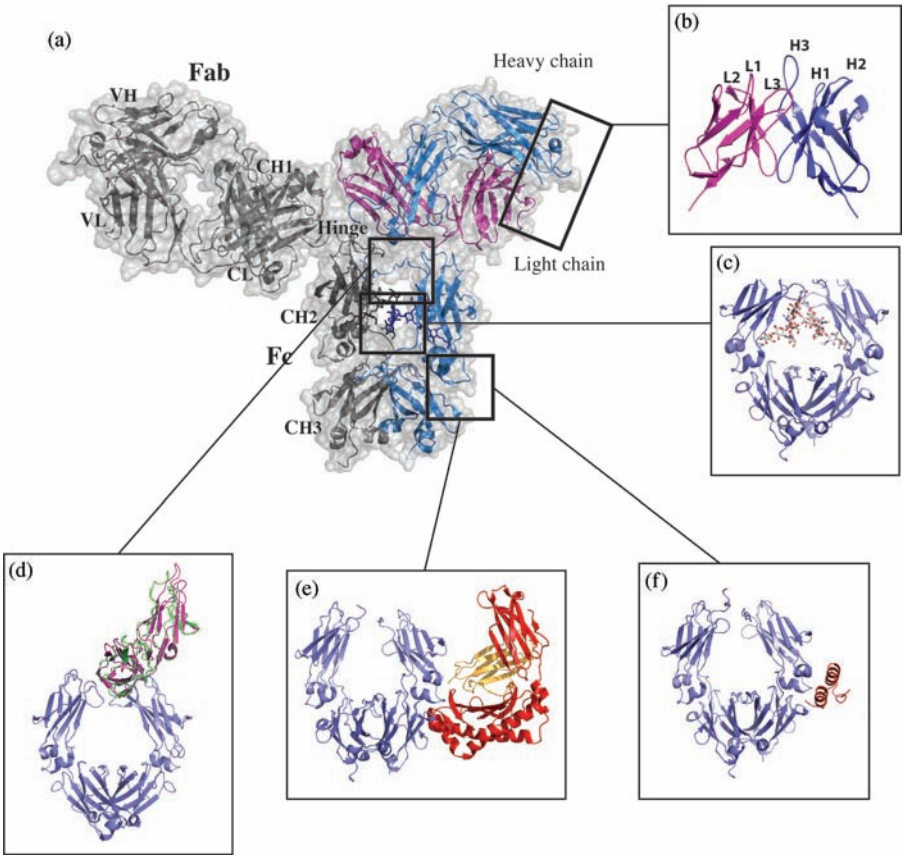

Figure 1.1 Structure and features of the IgG and its interactions. (a) The structure of a full-length IgG is shown in ribbon representation with transparent molecular surface. One heavy chain is shown in blue and one light chain in magenta. The other heavy chain and light chain are shown in gray for clarity. In this orientation, two Fab domains sit on top of the Fc domain and are connected in the middle by the hinge region. The Fab domain is composed of the heavy chain V_H and C_H1 domains and the light chain V_L and C_L domains—Protein Data Bank (PDB) [5] code 1HZH [6]. (b) Each variable domain contains three variable loops (L1–L3 on light chain and H1–H3 on heavy chain) that make up the antigen-binding site—PDB code 1HZH [6]. (c) The Fc region is composed of the dimer of C_H2 and C_H3 domains. The C_H3 domains form a tight interaction while the C_H2 domains interact through protein–protein, protein–carbohydrate, and carbohydrate–carbohydrate contacts—PDB code 1HZH [6]. (d) The hinge region is composed of a flexible region covalently tied together through disulfide bridges. Structures of the FcγRIIIa and FcγRIIa bound to the Fc are shown. The structures reveal that both receptors bind to the C_H2 domain near the hinge and carbohydrates and upon their binding create an asymmetry such that the second FcγR is unable to bind. In this panel, FcγRIII is shown in green, and the FcγRII is shown in purple—PDB codes 3RY6 [7] and 1T83 [8]. (e) The crystal structure of the complex between the Fc and FcRn reveals that FcRn binds between the C_H2 and C_H3 domains in the Fc. FcRn chains are shown in red and orange—PDB code 1FRT [9]. (f) Interestingly, the same region also binds to bacterial Protein A commonly used for purification—PDB code 1FC2 [10].

1.2 General Domain and Structure of IgG

Topologically, the IgG is composed of two heavy chains (50 kDa each) and two light chains (25 kDa each) with total molecular weight of approximately 150 kDa. Each heavy chain is composed of four domains: the variable domain (VH), C_H1, C_H2, and C_H3. The light chain is composed of variable domain (VL) and constant domain (CL). All domains in the IgG are members of the Ig-like domain family and share a common Greek-key beta-sandwich structure with conserved intradomain disulfide bonds. The CLs contain seven strands with three in one sheet, and four in the other, while the VLs contain two more strands, resulting in two sheets of four and five strands.

The light chain pairs up with the heavy chain VH and C_H1 domains to form the Fab fragment, while the heavy chain C_H2 and C_H3 domains dimerize with additional heavy chain C_H2 and C_H3 domains to form the Fc region (Figure 1.1, panel c). The Fc domain is connected to the Fab domain via a flexible hinge region that contains several disulfide bridges that covalently link the two heavy chains together. The light chain and heavy chains are also connected by one disulfide bridge, but the connectivity differs among the IgG subclasses (Figure 1.2). The overall structure of IgG resembles a Y-shape, with the Fc region forming the base while the two Fab domains are available for binding to the antigen [6]. Studies have shown that in solution the Fab domains can adopt a variety of conformations with regard to the Fc region.

1.2.1 Structural Aspects Important for Fc Fusion(s)

1.2.1.1 Fc Protein–Protein Interactions

While the Fab region of an antibody is responsible for binding and specificity to a given target, the Fc region has many important functions outside its role as a structural scaffold. The Fc region is responsible for the long half-life of antibodies as well as for their effector functions including ADCC, CDC, and phagocytosis [20].

The long half-life of human IgGs relative to other serum proteins is a consequence of the pH-dependent interaction with the FcRn [21–23]. In the endosome, FcRn binds to the Fc region and recycles the antibody back to the plasma membrane, where the increase in pH releases the antibody back to the serum, thus rescuing it from degradation. The details of FcRn binding and its effects on antibody pharmacokinetics, including results from modulating FcRn interaction by protein engineering, are discussed in Section 1.3.3. One FcRn binds between the C_H2 and C_H3 domains of an Fc dimer half (Figure 1.1, panel e) [21]; therefore, up to two FcRns can bind to a single Fc.

Fc region is also responsible for binding to bacterial Protein A [10] and Protein G [24], which are commonly used for purification of Fc-containing proteins. Although Protein A binds to Fc mainly through hydrophobic interactions and

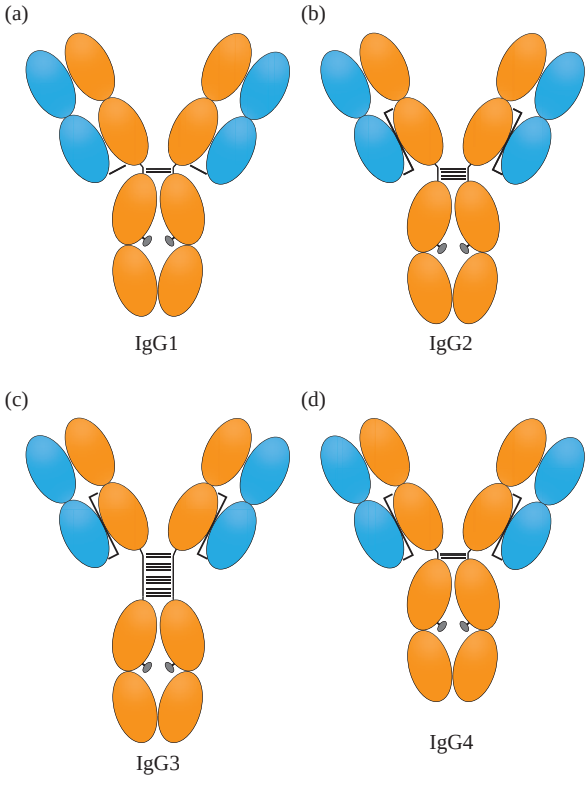

Figure 1.2 Interchain disulfide topology in human IgG subclasses. Only H–H hinge and H–L chain disulfides are shown. (a) IgG1, (b), IgG2, (c) IgG3, and (d) IgG4.

Protein G through charged and polar interactions, Proteins A and G bind to a similar site on Fc domain and compete with each other (Figure 1.1, panel f). Interestingly, the binding occurs between the C_H2 and C_H3 domains of the Fc and largely overlaps with the FcRn binding site.

ADCC function is mediated by the interaction of the Fc region with Fcγ receptors (FcγRs). Biochemical data and structures of Fc in complex with FcγRIII and FcγRII reveal that the FcγRs bind to the combination of the Fc C_H2 domain and the lower hinge region (Figure 1.1, panel d) [7,8,25]. Members of the Fcγ family have been found to bind to the same region of Fc [20,26,27] and form a 1 : 1 asymmetric complex where one FcγR interacts with the dimer of Fc. The binding of one FcγRIII to Fc induces asymmetry in the Fc region and prevents a second interaction. While the detailed structural understanding is not available for the Fc–C1q interaction, biochemical data suggest that C1q binds mainly to the C_H2 domain with an overlapping, but nonidentical, binding site of FcγRIII [28]. The

details of the interaction between the Fc and Fcγ receptors, as well as the engineering of effector function, are further discussed in Section 1.4.2.1.

1.2.1.2 Fc Glycosylation

The Fc region of IgG has a conserved glycosylation site in the C_H2 domain at position N297 (Figure 1.1, panel c). Glycosylation of the C_H2 domain is important in achieving optimal effector function [29] and complement activation; it also contributes to overall IgG stability [30]. Antibodies purified from human serum have been found to contain heterogeneous oligosaccharides where each C_H2 domain can contain one of many potential glycans [31]. Therapeutic Fc-containing proteins that are expressed in Chinese hamster ovary (CHO) or human embryo kidney 293 (HEK293) cells typically contain a mixture of glycoforms, with G0F being the most abundant, followed by G1F and G2F [32,33]. The attachment of the glycans at position Asn297 in the C_H2 domain positions the carbohydrates to interact with each other and to form a part of the Fc dimer interface. Because of carbohydrate sequestration into the space between the two C_H2 domains and significant carbohydrate–carbohydrate and carbohydrate–protein contacts, the carbohydrates in the Fc crystal structures are relatively well ordered.

The glycosylation of the Fc has been found to influence biological activity as well as stability of IgGs [34,35]. The removal of the core fucose enhances ADCC activation of FcγRIIIa on natural killer (NK) cells but does not change the binding of FcγRI or C1q [36]. Increased ADCC has also been observed with the presence of bisecting *N*-acetylglucosamine in the context of fucosylated IgG, although the effect appears to be smaller than removal of the core fucose [37]. Sialylated IgGs have been suggested to enhance anti-inflammatory properties [38]; however, more work is needed to understand this effect and potential mechanism.

1.2.1.3 Hinge and Interchain Disulfide Bonds

The hinge region of human IgGs (IgG1, IgG2, and IgG4) differs between the subtypes both in the hinge length (12–15 residues) and in number of disulfides linking the two heavy chains together (2–4 residues) (Figure 1.2). In addition, the position of the light chain–heavy chain linkage differs among the human IgG subtypes (Figure 1.2). In human IgG1, two disulfides link the heavy chains together while human IgG2 contains four disulfides and a shorter hinge. The presence of an increased number of disulfides as well as a shorter hinge likely decreases the flexibility of hIgG2 Fab regions relative to hIgG1. The hinge can have a profound impact on antibody properties. For example, the sequence in the hinge near the disulfides has been found to be important in the ability of IgG4s to exchange half molecules *in vivo* and under certain conditions *in vitro* [39,40]. The absence of one of the proline residues in the hinge of IgG4 coupled with substitution in the C_H3 domain allows IgG4 to form half-antibodies and form bispecific antibodies by exchanging with other IgG4s (Figure 1.2).

1.3 The Neonatal Fc Receptor

1.3.1 FcRn Function and Expression

One major characteristic of IgG, which differs from other Ig isotypes and most of the other serum proteins, is its long serum half-life. Typically, serum proteins and other Ig isotypes have half-lives of <1 week, for example, fibrinogen (1–3 days), IgD (2–5 days), IgM (4–6 days), IgA (3–7 days), and haptoglobin (~5 days) [41–44]; however, serum IgGs have half-lives of ~3 weeks (Table 1.3). The prolonged half-lives of IgGs are mainly due to the protective and recycling action of the FcRn [22,45,46].

Table 1.3 Summary of the pharmacokinetics of antibody variants engineered for increased FcRn binding in nonhuman primates.

Mutation(s) (EU numbering)	IgG isotype	Target antigen	FcRn affinity increase at pH 6.0 (fold)	Serum half-life (fold of WT)	Clearance (Fold of WT)	Source
M428L	IgG2	α-HBV OST577	~7× (human)[a)] ~8× (rhesus)[a)]	1.8× (rhesus)	0.56× (rhesus)	[47]
T250Q/ M428L	IgG2	α-HBV OST577	~28× (human)[a)] ~27× (rhesus)[a)]	1.8× (rhesus)	0.36× (rhesus)	[47]
M252Y/ S254T/ T256E	IgG1	α-RSV	~11× (human)[b)] ~9× (rhesus)[b)]	3.5× (cyno)	N.A.	[48]
T250Q/ M428L	IgG1	α-HBV OST577	~29× (human)[a)] ~37× (rhesus)[a)]	2.5× (rhesus)	0.42× (rhesus)	[49]
P257I/ N434H	IgG1	TNFα	~16× (human)[c)] ~52× (cyno)[c)]	0.8×[d)] (cyno)	1.1×[d)] (cyno)	[50]
D376V/ N434H	IgG1	TNFα	~15× (human)[c)] ~52× (cyno)[c)]	0.7× (cyno)	1.3×[d)] (cyno)	[50]
T250Q/ M428L	IgG1	TNFα	~40× (cyno)[c)]	0.9× (cyno)	1.1×[d)] (cyno)	[51]
P257I/ Q311I	IgG1	TNFα	~19× (human)[c)] ~80× (cyno)[c)]	0.8× (cyno)	0.8×[d)] (cyno)	[50,51]
N434A	IgG1	Unknown	~3× (human)[b)]	1.6× (cyno)	0.54× (cyno)	[52]
N434W	IgG1	Unknown	~38× (human)[b)]	1.0×[d)] (cyno)	1.2×[d)] (cyno)	[52]

(*continued*)

Table 1.3 (*Continued*)

Mutation(s) (EU numbering)	IgG isotype	Target antigen	FcRn affinity increase at pH 6.0 (fold)	Serum half-life (fold of WT)	Clearance (Fold of WT)	Source
M428L/ N434S	IgG1	α-VEGF	∼11× (human)[b)]	3.2× (cyno)	0.32× (cyno)	[53]
V259I/ V308F	IgG1	α-VEGF	∼6× (human)[b)]	1.7× (cyno)	0.63× (cyno)	[53]
M252Y/ S254T/ T256E	IgG1	α-VEGF	∼7× (human)[b)]	2.5× (cyno)	0.42× (cyno)	[53]
V259I/ V308F/ M428L	IgG1	α-VEGF	∼20× (human)[b)]	2.6× (cyno)	0.39× (cyno)	[53]
M428L/ N434S	IgG1	α-EGFR	∼11× (human)[b)]	3.1× (cyno)	0.31× (cyno)	[53]
N434H	IgG1	α-VEGF	∼4× (human)[b)] ∼5× (cyno)[b)]	1.6× (cyno)	0.62× (cyno)	[54]
T307Q/ N434A	IgG1	α-VEGF	∼18× (human)[b)] ∼10× (cyno)[b)]	2.2× (cyno)	0.52× (cyno)	[54]
T307Q/ N434S	IgG1	α-VEGF	∼10× (human)[b)] ∼12× (cyno)[b)]	2.0× (cyno)	0.49× (cyno)	[54]
T307Q/ E380A/ N434A	IgG1	α-VEGF	∼13× (human)[b)] ∼15× (cyno)[b)]	1.9× (cyno)	0.57× (cyno)	[54]
V308P/ N434A	IgG1	α-VEGF	∼26× (human)[b)] ∼34× (cyno)[b)]	1.8× (cyno)	0.57× (cyno)	[54]
N434H	IgG1 (N297A)	CD4	∼3× (human)[b)] ∼3× (baboon)[b)]	N/A	0.50× (baboon)	[55]
V308P	IgG4	5 unknown targets	∼40–390× (cyno)[c)]	2.0–3.3× (cyno)	0.22–0.74× (cyno)	[56]
T250Q/ M428L	IgG4	5 unknown targets	∼11–110× (cyno)[c)]	0.9–2.6× (cyno)	0.31–0.89× (cyno)	[56]

Abbreviations: EGFR, endothelial cell growth factor receptor; FcRn, neonatal Fc receptor; HBV, hepatitis B virus; N/A: not available; RSV, respiratory syncytial virus; TNF, tumor necrosis factor; VEGF, vascular endothelial cell growth factor.

a) IC_{50} binding ratio performed on FcRn-transfected cells.

b) Monovalent interaction: injecting FcRn over surface-conjugated antibodies.

c) Bivalent interaction: injecting antibodies over surface-conjugated FcRn.

d) No statistically significant difference.