Trends in Immunology

CellPress
REVIEWS

Review

Role of IgG3 in Infectious Diseases

Timon Damelang,[1] Stephen J. Rogerson,[2] Stephen J. Kent,[1] and Amy W. Chung[1,*]

IgG3 comprises only a minor fraction of IgG and has remained relatively understudied until recent years. Key physiochemical characteristics of IgG3 include an elongated hinge region, greater molecular flexibility, extensive polymorphisms, and additional glycosylation sites not present on other IgG subclasses. These characteristics make IgG3 a uniquely potent immunoglobulin, with the potential for triggering effector functions including complement activation, antibody (Ab)-mediated phagocytosis, or Ab-mediated cellular cytotoxicity (ADCC). Recent studies underscore the importance of IgG3 effector functions against a range of pathogens and have provided approaches to overcome IgG3-associated limitations, such as allotype-dependent short Ab half-life, and excessive proinflammatory activation. Understanding the molecular and functional properties of IgG3 may facilitate the development of improved Ab-based immunotherapies and vaccines against infectious diseases.

Highlights

IgG3 has been associated with enhanced control or protection against a range of intracellular bacteria, parasites, and viruses.

IgG3 Abs are potent mediators of effector functions, including enhanced ADCC, opsonophagocytosis, complement activation, and neutralization, compared with other IgG subclasses.

Future Ab-based therapeutics and vaccines should consider utilizing IgG3, based on features of enhanced functional capacity.

Investigating the impact of glycosylation patterns and allotypes on IgG3 function may expand our understanding of IgG3 responses and their therapeutic potential.

Human IgG3 an Understudied but Highly Potent Immunoglobulin

Antibodies (Abs) play a major role in protection against infections by binding to and inactivating invading pathogens. Although IgG3 constitutes a minor proportion of total human immunoglobulins, a growing number of recent studies have highlighted IgG3 as critical for the control and/or protection of a range of pathogens, including viruses (e.g., HIV [1–5]), bacteria (e.g., *Neisseria* spp. [6–8]), and parasites (e.g., *Plasmodium* spp. [9–13]). This review describes the unique characteristics of human IgG3 that contribute to its potent anti-pathogenic functions, including enhanced neutralization, **Fcγ receptor (FcγR)** (see Glossary) affinity, and Fc-mediated activity. Of relevance, the structure and enhanced functionality of human IgG3 is unique compared with most mammals, including macaque species, which do not have an equivalent analog among their IgG subclasses [14–16]. This is particularly significant for non-human primate models, where macaque species are commonly used for pre-human clinical trials and may historically have contributed to why human IgG3 has been understudied in the control of infectious diseases, until recently. Furthermore, this review discusses how the unique properties of human IgG3 can be harnessed for future preventative and therapeutic manipulations, highlighting the major obstacles and outstanding questions that are pertinent.

Human IgG3: a Unique Antibody Subclass

The glycoprotein IgG is the most abundant isotype in healthy human plasma, and can be separated into four subclasses: IgG1 (60–70% in plasma), IgG2 (20–30%), IgG3 (5–8%), and IgG4 (1–3%) [17]. The amino acid sequences of the human IgG subclasses are >95% homologous in the constant domains of their heavy chains (Figure 1). IgG3 has a distinct amino acid composition and structure in the **hinge region** between C_H1 and C_H2 [18]. The flexibility of the hinge region decreases in the order IgG3 > IgG1 > IgG4 > IgG2 [19]. Furthermore, IgG3 has an extended hinge region (Figure 1 and Table 1), consisting of up to 62 amino acids, forming a polyproline double helix consisting of 11 disulfide bridges (compared with two in the hinge regions of IgG1 and IgG4, and four in the hinge region of IgG2) [20]. This is the result of duplications of a hinge exon, encoded by one single exon in IgG1, IgG2, and IgG4, but up to

[1]Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia
[2]Department of Medicine, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia

*Correspondence:
awchung@unimelb.edu.au
(A.W. Chung).

four exons in IgG3. The extended hinge region of IgG3 expands the **Fab** arms further away from the **Fc**. This distance enables high rotational freedom, which provides the molecule with greater flexibility and reach [21]. The longer hinge region and the greater reach of the Fab arms together might explain why this subclass can probe into less exposed antigens and could contribute to the high potential of IgG3 to activate effector functions [20,21].

IgG3 Allotypes

Allotypes are **polymorphisms** in the constant regions of immunoglobulin heavy and light chains (Figure 1B). IgG3 is the most polymorphic subclass with 13 Gm allotypes termed G3m; thought to be due to its higher rapid evolutionary diversification compared with other subclasses [20]. G3 m allotypes are inherited in different combinations or G3 m alleles (encoded by one or several *IGHG3* alleles) shared among individuals within populations (Table 2). Several G3 m allotypes consist of a combination of multiple amino acids, which lead to changes of their tertiary protein structures, with all known G3 m allotypes located within the C_H2 and C_H3 domains [22]. IgG3 allotypic variations can have structural and functional consequences, such as shorter hinge regions and extended **half-life** compared with other allotypes [23]. In addition, polymorphisms in the C_H3 domain affect the C_H3–C_H3 interdomain interactions [24], with potential consequences for **C1q** binding in **complement activation** [24,25]. Specific allotypes also contribute to underappreciated difficulties in purifying human IgG3 from serum samples, where only allotypes containing a histidine residue at position 435 can be purified by protein A [26].

Glycosylation of IgG3

Glycosylation is a post-translational modification of Abs, which can be regulated by a range of B cell stimuli, including environmental factors, such as stress or disease, cytokine activity, and innate immune signaling receptors, such as **Toll-like receptors**. Hence, exposure to specific pathogens, antigens, or vaccination has the potential to skew Ab glycan profiles [27,28]. Glycosylation is an inherent mechanism of Ab diversification, on top of **V(D)J recombination**, **somatic hypermutation (SHM)**, and **class switch recombination (CSR)**, thereby contributing to the extent of the Ab repertoire of B cells [29].

IgG3 Abs can include up to three potential glycosylation sites. The most well-described glycosylation site is found in all human IgG subclasses, where carbohydrate groups are attached to asparagine 297 in the C_H2 domain (Figure 1C). The glycans at this N-glycosylation site can influence Ab stability [30], binding to FcγRs and complement [31], consequently modulating effector functions, such as **complement-dependent cytotoxicity (CDC)** and **Ab-dependent cell cytotoxicity (ADCC)** [32–35]. For instance, IgG3 **monoclonal Abs (mAbs)** expressed from α1,6-fucosyltransferase gene knockout Chinese hamster ovary (CHO) cell lines (lacking the ability to attach fucose to asparagine 297 in Abs), have demonstrated increased binding to FcγRIIIa relative to wild-type controls [25,34]. Furthermore, a high degree of agalactosylation has been associated with disease progression for various infections, including chronic HIV, hepatitis B, leishmaniasis, and active tuberculosis (TB) [28,36,50], while the modulation of sialic acid has been linked to anti-inflammatory Ab-based treatments in autoimmune diseases such as rheumatoid arthritis [37]. A second N-glycosylation site in the heavy and light chain variable regions V_H and V_L has been observed in 15–25% of all serum IgG, which can contribute to Ab stability [38], and can also modulate antigen binding. This was demonstrated in a recent study where the presence of Fab glycans on human monoclonal Abs could increase Fab binding affinity up to twofold relative to controls [29].

Unique to IgG3, is the presence of O-glycosylation sites in the hinge region [39]. Approximately 10% of IgG3 derived from polyclonal serum samples and ~13% of monoclonal IgG3 Abs

Glossary

Antibody-dependent cell cytotoxicity (ADCC): killing of an Ab-coated target cell by a cytotoxic effector cell through a nonphagocytic process.
Antibody-dependent cellular inhibition (ADCI): a process by which Abs can inhibit *Plasmodium* growth in the presence of monocytes.
Antibody-dependent cellular viral inhibition (ADCVI): Fcγ-receptor-mediated antiviral activity, occurring when Ab bound to virus-infected target cells engages FcγR-bearing effector cells, (e.g., NK cells, monocytes, or macrophages).
Antibody-dependent phagocytosis (ADP): process by which innate immune phagocytic effector cells (e.g., neutrophils, macrophages, or monocytes) ingest or engulf other cells, organisms, or particles.
Allotype: amino acid differences in the constant region of either the heavy or light chains of an Ab within a subclass of Abs.
Avidity: the overall sum of binding strength of multiple affinities, often used to describe the accumulated strength of two Ab Fab arms with its antigens.
Bacteriolysis: destruction of bacterial cells, often be mediated by Abs.
Bispecific antibody: recombinant protein that can simultaneously bind two different types of antigens.
Broadly neutralizing antibody (bNAb): special type of Abs that can recognize and block many strains of a particular pathogen (e.g., virus) from entering healthy cells.
C-reactive protein: protein produced by the liver in response to inflammation or infection.
Class switch recombination: process by which proliferating B cells rearrange constant region genes in the immunoglobulin heavy chain locus, switching from one class of immunoglobulin to another.
C1q: protein complex involved in the complement system (innate immune system).
Complement activation: protein cascade within the innate immune system; it is responsible for a rapid and nonspecific clearance of

contain O-glycans [39]. Each IgG3 can contain up to three O-glycans at threonine residues at triple repeat regions within the hinge [39]. Indeed, the IgG3 hinge region has a high degree of surface accessibility associated with O-glycosylation [40]. Surface accessibility may be responsible for the lower degree of O-linked glycosylation observed in recombinant G3m15 IgG3, with a shorter hinge region than other IgG3 allotypes [39]. Although the function of O-glycosylation is still not fully understood, the hinge structure is hypothesized to be able to protect the immunoglobulin from proteolytic cleavage, and might also help maintain the extended conformation and flexibility of IgG3 hinge regions, though this has yet to be demonstrated [39]. Clearly, the role of IgG3-specific O-glycosylation is underexplored, but this is a growing area of interest that may provide new ways to enhance the potency of IgG3.

IgG3 Has Potent Effector Functions

IgG has a number of antigen-specific effector functions, such as immune cell-activation via FcγRs, neutralization, and activation of complement pathways [20,31]. IgG3 is the most functional subclass, closely followed by IgG1, due to its superior affinity to FcγR [41]. However, IgG2 also plays an important role in targeting polysaccharides and is commonly induced during bacterial infections [42], while IgG4 is often induced in response to allergens [43] (Table 1 and Figure 2, Key Figure).

Complement Activation

IgG can form complexes with C1q to activate the classical complement pathway [31], while agalactosylated IgG can activate the lectin complement pathway, inducing a range of functions including increased **opsonophagocytosis** and CDC [44,45]. IgG3 binds with a higher affinity to C1q compared with other IgG subclasses (Figure 2) [46]. While this activity is thought to be linked to the increased flexibility of IgG3, the interaction between C1q and IgG3 is not dependent on the length of its hinge region, as shown from studies where engineered IgG3 with shorter IgG1 or IgG4 hinge regions exhibited even greater binding affinity for C1q than wild-type IgG3 did [25,39]. Instead, Ab-dependent complement-mediated lysis reports have shown that one amino acid (Lys 322) in the human IgG3 C_H2 domain is critical for C1q interactions and enhanced CDC [47]. Nevertheless, the importance of IgG3 in the lectin pathway and the relevance of IgG3 glycosylation on CDC is still unknown.

FcγR Binding

Abs forming complexes with antigens, termed opsonization, can initiate important cell-based functions such as: ADCC, **Ab-dependent phagocytosis (ADP)**, **Ab-dependent cellular inhibition (ADCI)**, and **Ab-dependent cellular viral inhibition (ADCVI)**, inducing the respiratory burst, triggering the release of pro- and anti-inflammatory mediators and enzymes, as well as modulating antigen presentation and the clearance of pathogenic complexes (Figure 2) [41,48]. The efficiency of monomeric IgG3 binding to FcγRIIa, FcγRIIIa, and FcγRIIIb on effector cells such as neutrophils, monocytes, macrophages, or natural killer (NK) cells is even higher than that of monomeric IgG1 (Table 1) [49]. Thus, the interaction of IgG3 with FcγRs on these effector cells has been increasingly recognized as a critical immune response against a range of infections from viruses, bacteria, and parasitic pathogens [1,33,50–52].

Neonatal FcR Binding

The interaction between IgG and the **neonatal Fc receptor (FcRn)** is important for IgG transplacental passage of IgG Abs and Ab half-life in humans [53]. FcRn binds to IgG at an acidic pH in the endosomes of vascular endothelial cells and recycles it back to circulation by dissociating at physiological pH [54]. FcRn-mediated transport of IgG3 is inhibited with the presence of IgG1 due to intracellular competition [23]. Therefore, the unbound IgG3 is

pathogens by attracting phagocytic cells.

Complement-dependent cytotoxicity (CDC): C1q binds an Ab, triggering the complement cascade via the classical pathway of complement activation.

Envelope (Env) protein: expressed on the surface of enveloped viruses.

Fab region (fragment antigen binding): Ab domain that binds to antigens; involved in neutralization.

Fc region (fragment crystallizable): tail region of an Ab that determines its innate immune effector functions.

FcγRs: surface protein receptors for IgGs (broadly expressed by cells of hematopoietic origin); they are defined as either activating (FcγRI, FcγRIIa/c, FcγRIII) or inhibitory (FcγRIIb); they elicit or inhibit immune functions, respectively.

Glycosylation: post-translational modification whereby carbohydrates are attached to proteins (e.g., Abs) via certain enzymes.

Half-life: measure of the mean survival time of a molecule to reduce in half, its initial value (e.g., concentration).

Hinge region: flexible amino acid sequence in the central part of the heavy chains of Abs; can be linked by disulfide bonds.

Isotype switching: mechanism changing the B cell production of an immunoglobulin from one isotype to another.

Monoclonal antibody (mAb): Ab produced by a single clone of cells.

Neonatal Fc receptor (FcRn): MHC class I like molecule that functions to protect IgG from catabolism, mediates transport of IgG across epithelial cells, and is involved in antigen presentation.

Opsonophagocytosis: process by which the pathogen is marked by an opsonin, for example, an Ab, for ingestion, and eliminated by phagocytes.

Polymorphism: occurrence of different genetic forms among the members of a population or colony, or in the lifecycle of an individual organism.

Polyspecific antibody: can simultaneously bind to multiple types of antigens.

degraded and not returned into circulation, which in part may explain its shorter half-life in most individuals [23]. However, IgG3 affinity to FcRn is modified by an amino acid substitution at position 435, where IgG3 harbors an arginine instead of the histidine found in all other subclasses [23]. The R435 side chain stays positively charged at physiological pH and can maintain ionic interactions with FcRn [54]. This contributes to strong binding of IgG3 to FcRn at physiological pH, leading to endosomal degradation [54]. By contrast, individuals with certain allotypes (Table 2), such as G3m15 and G3m16, harbor a histidine at position 435. This substitution makes their Ab half-life comparable with that of IgG1 [23]. In fact, this means the half-life of G3m15/16 is longer than that of R435 IgG3, and maternal–fetal H435 IgG3 transport is similar to that of IgG1 [55]. This highlights the importance of histidine residues on overall binding affinity to FcRn. It must be cautioned that due to the potent ability of IgG3 to trigger effector functions, an important balance is likely required to prevent excessive proinflammatory responses, which can cause collateral damage and over stimulation. Indeed, this arginine substitution – reducing the half-life of IgG3 – has been hypothesized to limit the potential for excessive activation and inflammation, due to its more rapid clearance from circulation compared with other less inflammatory subclasses [9]. Lastly, R/H435 allotypes are relevant for infectious diseases, as discussed below.

Somatic hypermutation (SHM): process that diversifies BCRs to improve antigen recognition; a high frequency of point mutations are generated within variable regions of expressed Igs; Abs with higher-affinity variants are thus generated.
Toll-like receptors: expressed on sentinel cells such as macrophages and dendritic cells; recognize structurally conserved molecules derived from pathogens.
Trogocytosis: transfer of plasma membrane fragments from target cells to effector cells.
Uncomplicated malaria: symptomatic malaria without signs of severity or evidence of vital organ dysfunction.
V(D)J recombination: mechanism of genetic recombination that occurs only during the early stages of T and B cell maturation, generating unique BCRs; leads to the generation of Ab diversity.

IgG3 Responses to Pathogens

Generation of IgG3 during Various Infections

IgG3 is often one of the earliest subclasses to be elicited against protein antigens upon infection [3,56–58], due to its genetic locus positioning among immunoglobulin heavy constant chains (*IGHC*) in humans [59,60]. IgG3 is the first *IGHG* gene located within the locus, sequentially followed by IgG1 (gene order as follows: *IGHG3*, *IGHG1*, *IGHA1*, *IGHG2*, *IGHG4*, *IGHE*, *and IGHA2* from 5′ to 3′ [59,60]); thus, IgG3 responses are often closely followed by IgG1 responses [61–64]. With potent Fc effector functions [54,56], early IgG3 responses may be particularly beneficial for the rapid clearance of pathogens expressing high concentrations of proteins, especially viruses, and specific bacteria and parasites expressing protein antigens (discussed below). In contrast, chronic infections such as HIV-1, are often associated with reduced serum IgG3 [3,58], likely due to repeated antigen stimulation and subsequent CSR to downstream, comparatively less proinflammatory IgG subclasses or isotypes, with weaker affinity to FcγRs [49,60]. CSR of human immunoglobulin to IgG3 *in vitro*, is predominantly modulated by cytokines, in particular interleukin (IL)-4, IL-10, and IL-21 [61–64]. However, the exact signals modulating CSR in humans during various infections *in vivo* are not fully defined. Moreover, similar cytokines have also been linked to IgG1 CSR *in vitro* (IgG3 and IgG1 proteins encoded by the first two genes in the *IGHC* locus), which might potentially explain why IgG3 and IgG1 responses are often linked during infection [61–64]. Perhaps because of stepwise CSR events of IgG3 to downstream IgG subclasses, the analysis of clonally related immunoglobulin sequences have identified less SHM within IgG3 sequences, compared with other class-switched immunoglobulin subclasses (especially IgG4) [60].

IgG3 in Viral Infections

Virus-specific IgG3 appears early during infection, while IgG1 progressively dominates the responses in most infections [41]. However, recent HIV vaccine studies suggest that despite being of low titers, IgG3 Abs are potent effectors of vaccine-induced humoral immune responses [2,4,5]. In the RV144 vaccine trial (http://clinicaltrials.gov NCT00223080), the only human HIV-1 vaccine trial to achieve partial efficacy, high IgG3 binding to the V1–V2 loops of the HIV-1 **envelope (Env) protein** correlated with a lower risk of HIV-1 infection (i.e., increased vaccine efficacy) relative to controls [4]. Accordingly, depletion of IgG3 from RV144 plasma samples resulted in decreased Fc-mediated effector activities *in vitro* – including significant

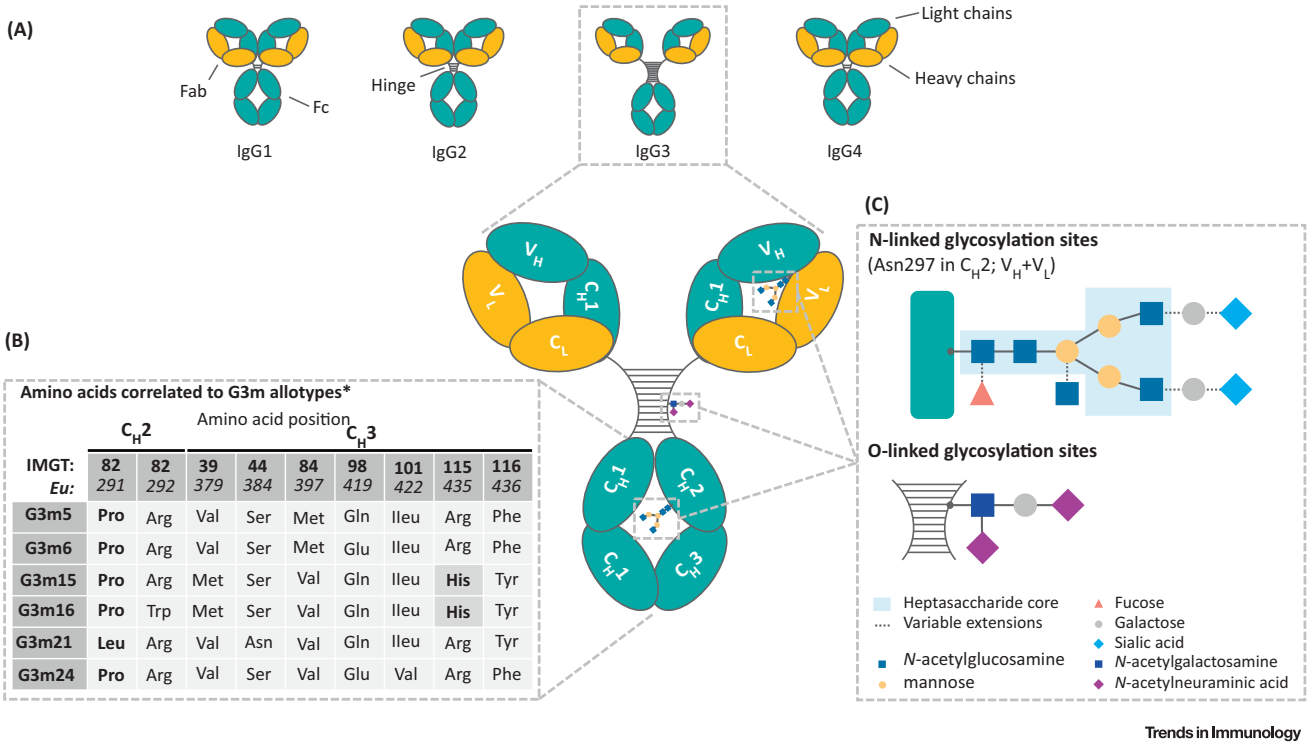

Figure 1. Schematic Overview of the Human IgG3 Antibody. (A) IgG1–4 molecules consist of four polypeptide chains, composed of two identical γheavy (green) chains of 50 kDa and two identical light (yellow) chains of 25 KDa, linked together by interchain disulfide bonds. Each heavy chain consists of an N-terminal variable domain (V_H) and three constant domains (C_H1, C_H2, and C_H3). IgG molecules comprise similarly sized globular portions joined by a flexible stretch of polypeptide chain between C_H1 and C_H2, known as the hinge region. The V_H and C_H1 domains and the light chains form the fragment antigen binding (Fab) regions. The lower hinge region and the C_H2/C_H3 domains form the fragment crystalline (Fc) region, which interacts with effector molecules and cells. (B) Allotypes of IgG3 are polymorphisms of the constant regions on C_H2 and C_H3 domains. The correlations between the most common G3 m allotypes and amino acids are shown. Positions in bold are according to the IMGT unique numbering for C domain [120] and in italics for Eu numbering [121]. (C) Within the C_H2 region is one N-glycosylation site containing carbohydrate groups attached to asparagine 297. The glycan has a heptasaccharide core (blue background block) and variable extensions, such as fucose, galactose and/or sialic acid (broken line). The glycosylation patterns in healthy human serum often include fucosylated glycoforms (red triangle). Additional N-glycosylation sites have been reported in the V_H and V_L of the variable region and IgG3-specific O-glycosylation sites in the hinge region. Glycosylation of IgG varies with age, gender, disease state, infection, and vaccination, reflecting the dynamic processes that can alter antibody effector function as responses to infectious agents [27,28].

decreases in ADCC, ADP, and the loss of Fc effector polyfunctionality – compared with undepleted samples [5].

During early HIV-1 infection, functional Ab responses decline drastically with HIV disease progression, which is correlated with waning IgG3 Ab concentrations [3,56,58]. In HIV-1 neutralization studies, polyclonal IgG3 Abs are more potent than any other subclass [65], with increased length of the hinge region enhancing neutralization potency and significantly improved phagocytosis and **trogocytosis** activity *in vitro* compared with IgG1 [66]. Moreover, Env-binding monoclonal IgG3 Abs are more effective at internalizing viral particles compared with Env-binding IgG1 of the same specificity [67]. Thus, the maintenance of IgG3 Abs could be useful for both prevention of HIV infection as well as for antiviral control. However, a recent study also identified IgG3 as a regulator of IgM^+ tissue-like memory (TLM) B cells, where $IgG3^+IgM^+$ TLM B cells from chronic HIV-1 infected individuals, but not aviremic individuals, exhibited reduced sensitivity to B cell receptor (BCR) stimulation compared with their $IgG3^-IgM^+$ counterparts [68]. This modulation of IgM^+ B cell stimulation was reported to occur through multiple mechanisms including, direct colocalization of IgG3 with IgM, along with

Table 1. Properties of Human IgG Subclasses

	IgG1	IgG2	**IgG3**	IgG4
Heavy chain type	γ1	γ2	**γ3**	γ4
Molecular mass (kDa)	146	146	**170**	146
Amino acids in hinge region	15	12	**62**[a]	12
Disulfide bonds in hinge region	2	4	**11**	2
Number of allotypes	4(+2)	1	**13(+2)**	0
Classical allotypes	G1m1, 2, 3, 17	G2m23	**G3m5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28**	–
Surnumerary allotypes	G1m27, 28	–	**G3m27, 28**	–
Mean adult serum concentration (g/l)	6.98	3.8	**0.51**	0.56
Proportion of total IgG (%)	43–75	16–48	**1.7–7.5**	0.8–11.7
Half-life (days)	21	21	**7/ ~ 21**[a]	21
Placental transfer	+++	++	**++/+++**[a]	++
Antibody response to				
Proteins	++	+/–	**++**	+/–
Polysaccharides	+	++	+/–	+/–
Allergens	+	(–)	**(–)**	++
Complement activation				
C1q binding	++	+	**+++**	+
Binding to FcγR				
FcγRI	+++	–	**+++**	+
FcγRIIa	++	+/–	**++**	+/–
FcγRIIb[b]/c	++	+/–	**++**	++
FcγRIIIa	+	+/–	**+++**	+/–
FcγRIIIb	+/–	+/–	**+**	–
FcRn (pH <6.5)	+++	+++	**++/+++**[a]	+++
Binding to *Staphylococcus* Protein A	++	++	[a]	+
Binding to *Staphylococcus* Protein G	++	++	**++**	++

[a]Allotype-dependent.
[b]Inhibitory.

interactions with FcRIIb (inhibitory FcR), C1q and **C-reactive protein** [68]. IgG3 binding to IgM–BCRs could also arise in the setting of persistent HIV viremia and contribute to refractory IgM^+ B cell stimulation during chronic infection [68]. Furthermore, a recent cohort study of HIV-1-infected individuals also reported that IgG Fc polyfunctionality (ADCC, ADCP, and Ab-dependent trogocytosis), along with greater subclass diversity (i.e., induction of all IgG subclasses in addition to IgG3) were associated with the development of **broadly neutralizing Abs (bNAbs)** compared with HIV-1-infected individuals that failed to develop bNAbs [69]. This suggested that induction of a diversity of IgG subclasses, and not IgG3 alone, may be required for the development of bNAbs [69]. Further studies are needed to determine the advantages and disadvantages of IgG3 responses during HIV infection, as well as any putative therapeutic applications, especially in the context of balancing chronic B cell regulation against the feasibility of eliciting highly functional IgG3 Abs with more potent antiviral Fc effector functions and improved bNAb responses.

Table 2. Nomenclature of the Six Most Prevalent Human IgG3 Gm Allotypes Shared Among Individuals within Populations[a]

Allotype (WHO nomenclature)	Allotype (previous designation)	Alleles (WHO/IUIS nomenclature)	Simplified form	*IGHG3* genes (IMGT nomenclature)	Ig heavy domain chain	Prevalence in ethnic groups
G3m5	G3m(b1)	G3m5, 10, 11, 13, 14, 26, 27	G3m5*	IGHG3*01, *05, *06, *07, *09, *10, *11, *12	H-γ3 C_H3	European, North America, African and Asian
G3m6	G3m(c3)	G3m5, 6, 10, 11, 14, 26, 27	G3m6*	IGHG3*13	H-γ3 C_H3	African
G3m15	G3m(s)	G3m10, 11, 13, 15, 27	G3m15*	IGHG3*17	H-γ3 C_H3	Sub-Saharan with high frequencies in Khoisan
G3m16	G3m(t)	G3m10, 11, 13, 15, 16, 27	G3m16*	IGHG3*18, *19	H-γ3 C_H2	Most prevalent in North East Asians
G3m21	G3m(g1)	G3m21, 26, 27, 28	G3m21*	IGHG3*14, *15, *16	H-γ3 C_H2	European, North American and Asian
G3m24	G3m(c5)	G3m5, 6, 11, 24, 26	G3m24*	IGHG3*03	H-γ3 C_H3	African (including North Africa)

[a]Adapted from [10].

IgG3-mediated responses play a role in an array of other viral infections. One study found that Chikungunya-virus-specific responses were dominated by neutralizing IgG3 Abs, which developed rapidly in patients with high viremia [70]. In contrast, pre-existing IgG in patients, mainly IgG1 and IgG3, against severe dengue virus (DENV) infection, could form virus–Ab complexes with a different DENV serotype, promoting infection, potentially leading to complications such as dengue hemorrhagic fever and dengue shock syndrome [57,71,72]. This cross-serotype activity is referred to as Ab-dependent enhancement (ADE), theorized to be due to higher antigen **avidity** [33], activation of complement [57], and FcγR binding [73,74] compared with same-serotype responses. IgG3 responses might also be detrimental in this scenario because IgG3 bears the greatest potential to activate complement and initiate FcγR uptake by effector immune cells relative to any other subclass [46,49]. Accordingly, in a recent study examining monoclonal Abs recovered from survivors of Ebola virus infections, among the different subclasses, IgG3 Abs induced high-level ADE; furthermore, *in vitro* FcγR blocking experiments in THP-1 monocytic cell lines, showed that blockade of FcγRIII eliminated IgG3-mediated ADE completely, while blocking FcγRI or FcγRII reduced it significantly, relative to controls [75].

Bacterial Infections

IgG subclass distribution in antibacterial responses is more heterogeneous than produced in antiviral responses, due to the diversity of bacterial epitopes [41]. Limited studies have explored the importance of IgG3 in bacterial infections; however, a key antibacterial property of human IgG3 Abs is their ability to induce CDC (Figure 2) [19]. Protection against invasive *Neisseria meningitidis* has been found to depend on recognition of bacterial surface antigens by Abs, activation of complement, and degradation of bacteria by **bacteriolysis** [8]. In this study, human IgG3 exhibited higher bacteriolysis activity than IgG1 *in vitro* when the target antigen (FHbp) was sparsely expressed on wild-type bacteria (group B strain H44/76-WT) compared with mutant bacteria expressing threefold higher concentrations of surface target antigen [8]. Additionally, recombinant IgG3 mAbs with shorter hinge regions – such as IgG3 G3m15 – performed better than other IgG3 allotype mAbs in enhancing complement-mediated bactericidal activity against *N. meningitidis*, irrespective of Ab specificity [8]. By contrast, the long hinge region of non-G3m15 IgG3 seems to dampen the high CDC capability of IgG3 Fc in *N. meningitidis* infections via unknown mechanisms [8]. It is therefore possible that IgG3 Abs with

Key Figure

Overview of Human IgG3 Properties and Effector Mechanisms.

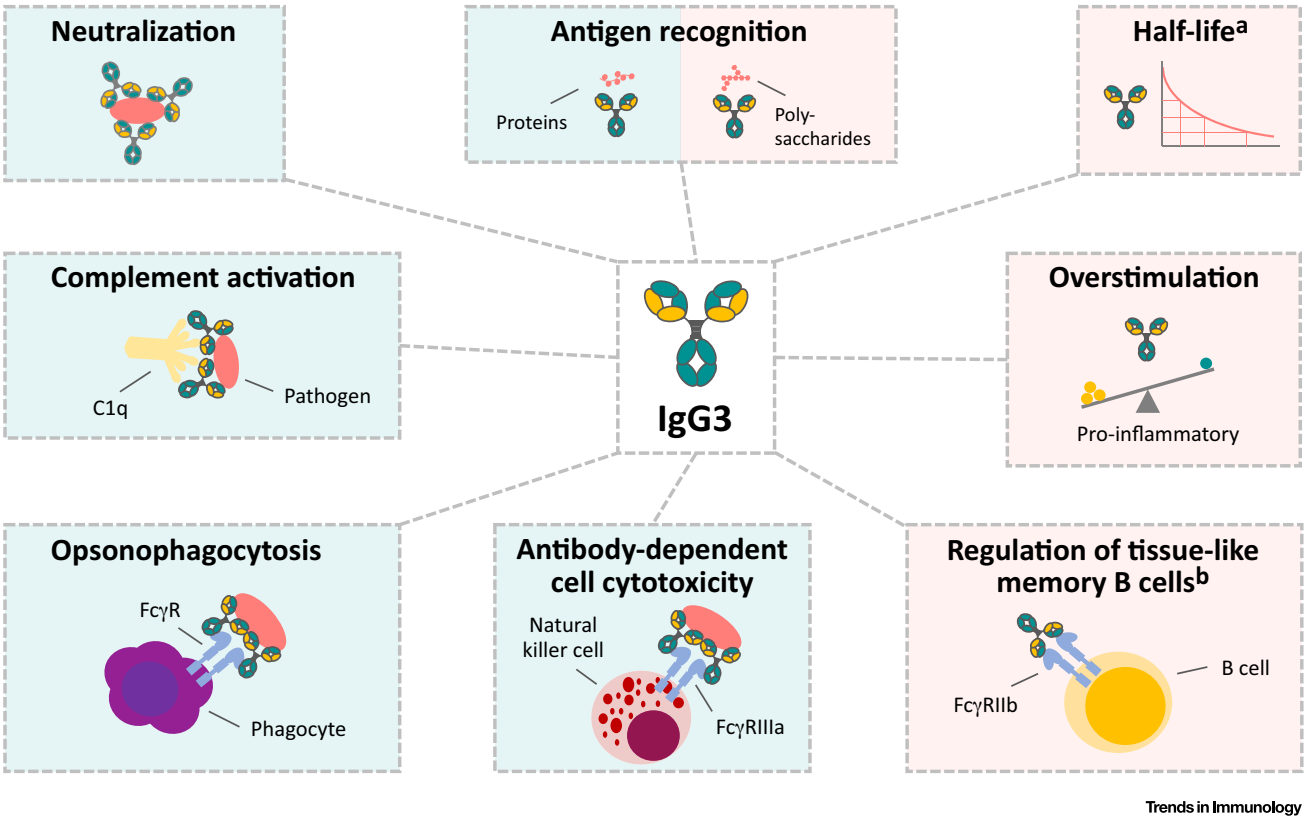

Figure 2. The schematic depicts the known properties and effector functions of human IgG3. Green background: IgG3 is associated with superior activity relative to other IgG subclasses; red background: IgG3 is associated with inferior outcomes compared with other IgG subclasses. [a]Allotype-dependent; [b]shown in HIV-1-infected individuals [68]. Abbreviation: FcγR, Fcγ receptor.

a shorter hinge region might be more effective in clearing *N. meningitidis* infections than long hinge region Abs are (and potentially other bacterial pathogens), but this remains to be further tested.

IgG3 Abs are also potent mediators of opsonophagocytosis of bacteria. The uptake of *Salmonella typhimurium* by human monocytic THP-1 cells *in vitro* is greatest when the bacteria are opsonized with human IgG3, followed by IgG1, IgG4, and then IgG2 [76]. Similar results have been observed with *N. meningitidis* infections, where chimeric IgG3, isolated from the plasma of individuals immunized with a meningococcal vaccine, is highly efficient in mediating phagocytosis following opsonization, and when using polymorphonuclear leukocytes as effector cells in the presence of human complement [67].

However, it should be cautioned that high expression of IgG3–complement immune complexes is not always advantageous to the host. For instance, IgG1 and IgG3 are the predominant

human Ab subclasses formed against *Mycobacterium tuberculosis (Mtb)* infections [77]. They stimulate the release of tumor necrosis factor (TNF) from primary monocytes [78], enhance bacterial uptake by macrophages, and form complement–immunoglobulin immune complexes, which remain elevated during active TB, and which furthermore, have been associated with increased disease severity, relative to other subclasses [79]. As a result, excessive IgG1 and IgG3 subclasses might potentially cause an excessive proinflammatory response, which in the case of active TB may be more harmful than protective [79]. This argues for a complex role of IgG3 in bacterial infections including both bacterial uptake and clearance and in C1q immune complex formation. Consequently, this illustrates the need for extensive and robust studies that examine the role of IgG3 Abs in relevant bacterial infections.

Parasitic Infections

The predominant IgG subclass produced in response to parasites such as *Plasmodium falciparum* are IgG1 and IgG3 [80]. IgG3 is linked to protective immunity against malaria [11,12,80,81], due to opsonization of malaria-infected erythrocytes and promotion of Ab-dependent effector functions [10,13,82,83]. In addition, they can induce complement deposition at the merozoite stage of the parasite, thus mediating inhibition of erythrocyte invasion [84–86]. IgG3-induced CDC has also been associated with antiplasmodium sporozoite immunity in children [87].

With regard to *Plasmodium vivax* merozoites, the predominant Abs produced are also IgG1 and IgG3 [88,89]. Moreover, Abs against the classical α-Gal (Galα1-3Galβ1-4GlcNAc-R) epitopes have being correlated with protection against *Plasmodium* spp. infections, reducing malaria transmission by *Anopheles* mosquitoes [90]. In addition, higher α-Gal IgG3, as well as IgG4 and IgM have been associated with protection against clinical malaria, unlike what has been previously observed for other protein antigens [91].

IgG3 allotypes may also play a role in malaria susceptibility or protection. The combination of homozygous G3m6 allotype and Km3 may protect against placental malaria [92] and children with these allotypes have been reported to be better protected against **uncomplicated malaria** than noncarriers are [93]. However, other studies have suggested that G3 m but not Km allotypes might influence host susceptibility to malaria infection together with the Ab profile of the donors [94,95]. Individuals with the G3m6 allotype presented with a higher incidence of uncomplicated malaria than noncarriers, due to low baseline IgG3 concentrations and high baseline concentrations of noncytophilic subclasses [94]. Of note, G3m6 is predominantly found in African populations; possibly as a result of differential evolutionary selection caused by infectious diseases such as malaria [95]. Evidently, further studies are needed to validate any conclusions regarding general protection or susceptibility.

In a recent study in Beninese women, the H435-IgG3 polymorphism (i.e., allotypes G3m15 and G3m16 which potentiate binding of IgG3 to FcRn) increased the transplacental transfer and half-life of malaria-specific IgG3 in young infants compared with noncarriers, and was associated with reduced risk of clinical malaria during infancy [9]. The H435-IgG3 allele is most common in Africans (30–60%), suggesting a possible role for positive selection, given its relatively high frequency in malaria endemic areas [9]. However, the G3m15 and G3m16 allotypes have also been associated with increased susceptibility to the autoantibody-mediated disease pemphigus vulgaris (PV), a severe blistering skin disorder, and thus, IgG3 might play a role in the pathogenesis of PV. Alternatively, there may be a genetic association between H435-IgG3 alleles and PV in Middle Eastern and North African countries [96]. More research on IgG3

polymorphism frequencies in endemic populations may aid in the identification of potential selective advantages and disadvantages of given IgG3 polymorphisms [9].

IgG3 Potential for Therapeutic Applications

Current State of Play

By the end of 2018, there were approximately 80 approved therapeutic Abs (thAbs) on the market [97]. However, only three monoclonal thAbs are currently licensed for the treatment of infectious diseases: palivizumab, a humanized IgG1 mAb against respiratory syncytial virus [98]; raxibacumab, a humanized IgG1 mAb against *Bacillus anthracis* [99]; and ibalizumab, a humanized IgG4 mAb, which inhibits HIV-1 from entering host cells [100]. Currently, a limited number of IgG-based thAbs are in late-stage clinical studies for infectious indications [97,101,102]. Almost all thAbs to date have human IgG1 or IgG4 Fc domains, and there are presently no approved IgG3 thAbs [97]. Different reasons for this include: (i) the extensive hinge region of IG3, which can contribute to increased proteolysis *in vitro* [103]; (ii) the multiple IgG3 allotypes that exist across populations, including allo-immunity to foreign allotype polymorphisms [55]; and (iii) the reduced half-life of specific IgG3 allotypes [23]. Given the significance of IgG3 responses against natural infections and the unique structural and functional characteristics of IgGs, we emphasize the need to consider IgG3 properties when developing and testing new thAbs. The shorter half-life of IgG3 can be overcome by focusing on allotypes such as G3m15 and G3m16 with H435, or glycosylation patterns that prolong Ab half-life [23]. In a mouse model of *Streptococcus pneumoniae* infection, IgG3-H435 demonstrated significantly better protection against *S. pneumoniae* than IgG1 and IgG3-R435 did, indicating that IgG3-H435 might be considered when devising future improved therapeutic approaches to treat infections *in vivo,* aiming to increase IgG3 serum longevity [23].

In the past, differences in allotypes of a thAb were thought to potentially contribute to therapeutic resistance due to the recognition of non-self Fc protein structures, perhaps by enhancing clearance of the thAb, or in the worst-case scenario, by becoming immunogenic and precipitating severe adverse reactions [104]. However, studies have now demonstrated that allotypic differences between IgG1 thAbs do not appear to produce novel T cell epitopes in mismatched individuals, or to induce non-self anti-allotypic Abs; thus, it is unlikely that they represent a significant risk factor in inducing immunogenicity [105,106], but instead, might improve the half-life of thAbs via FcRn binding [107]. Further studies, especially for IgG3 allotypes, which have significantly greater polymorphisms and structural changes than IgG1 has, are required before any allotype contributions to anti-thAb responses can be disregarded.

Engineering Therapeutic IgG3 Abs with Enhanced Functional Properties

A major advantage of thAbs, is their ability to promote Fc effector functions most relevant for clearance and control of a targeted pathogen [108,109]. Mounting evidence has identified key mutations in the IgG1 Fc domain of thAbs that can enhance the affinity to FcγR or complement [108,109]. However, as previously discussed, specific allotypes and structural properties of IgG3 Abs can enhance Fc effector functions, in a manner that is superior to that of IgG1. One study showed that a longer hinge region of IgG3 variants improved Fc effector function (including ADCP and Ab-dependent cellular trogocytosis) and neutralization potency of a broadly neutralizing anti-HIV Ab (CAP256-VRC26) compared with IgG1 *in vitro* [66]. Furthermore, the development of cross-IgG subclass variants has yielded promising results; namely, the combination of C_H1 regions of IgG1 with the C_H2 and C_H3 regions of IgG3 can also enhance CDC activity relative to IgG1 [25]. Thus, we posit that next-generation monoclonal thAbs should consider utilizing optimized Fc regions, including some of the best features of IgG3, which provide its greater molecular flexibility and stronger affinity to FcγR and C1q. Moreover, the

generation of **bispecific** and even **polyspecific** Abs is an exciting approach within the thAb community [110,111]. Bispecific and polyspecific IgG3, with their extended flexibility and reach in their Fab arms, might potentially contribute to enhanced neutralization and avidity to pathogens relative to the other subclasses [20,65,66,70]. Thus, utilizing IgG3 as a backbone for the design of polyspecific thAbs has the potential to enhance both the Fab and Fc functionalities. Clearly, a more detailed understanding of IgG3 molecular and functional properties may galvanize the engineering and development of approaches to yield effective thAbs that might treat a variety of conditions.

Outstanding Challenges and Limitations

The potential advantages and pathways forward for the development of IgG3 monoclonal thAbs against infectious diseases, while a steep learning curve, also appears to be relatively straightforward, as many of the technologies have already been developed to explore thAb with other subclass backbones, especially IgG1. By contrast, the steps towards the development of IgG3-centric vaccines are less clear. One area where many questions remain is to devise ways to elicit durable IgG3 concentrations in humans upon vaccination. IgG3 is only naturally ~8% of total plasma IgG [17]. Whether eliciting high IgG3 is possible, or even advisable, or whether adjuvants would be required to achieve this (and which), is presently unknown. The RV144 Phase III HIV vaccine clinical trial, which elicited protective IgG3, had a very short duration of protection within vaccinated individuals (60% protection at 1 year, as opposed to 31% by ~2.5 years) [4,5,112]. This highlights the need to rigorously pursue current successful vaccines that can induce durable long lasting protection, and which elicit high IgG3 concentrations; indeed examples include the need to understand the mechanisms that lead to long-lasting IgG3 serum Abs in response to tetanus and meningococcal vaccines [6,113].

Another example is that of the nonprotective HIV vaccine trial (VAX003), where higher IgG3 serum concentrations were induced at 2 months (into a 6-month vaccination protocol) compared with the moderately protective HIV RV144 trial, at the same time point [5]. However, IgG Abs elicited in VAX003 at 2 months were less functional than IgG3 Abs elicited by RV144 [5]. The reason for this is still unclear, although the authors hypothesize that the role of IgG3 glycosylation and IgG subclass balance may be essential [5]. Thus, this highlights another understudied area of research; namely, the importance of IgG3-specific N- and O-glycosylation, and its modulation by vaccination. Future studies should also investigate whether glycans exert variable effects on different allotypes.

Another unknown dynamic is the balance between IgG3 Ab-mediated activation and overactivation. For instance, a recent study suggested that excessive IgG3 might lead to negative regulation of TLM B cells, resulting in refractory BCR signaling, as seen in chronic HIV-1-infected individuals [68]. Similarly, overinduction of IgG3 effector functions, such as ADCC, have been reported to increase the severity of disease in the case of DENV infection [114] and might be associated with excessive inflammatory responses in active TB [115]. Future studies are warranted to better dissect the dynamics of IgG3 responses during a variety of infections.

A further challenge is the dearth of suitable animal models for studying IgG3 Abs and their interaction with FcγRs, which are vital for understanding the role of IgG3 in the pathogenesis, protection, and vaccination against a range of infectious diseases. In addition, there are significant differences between human and murine Abs in terms of structure, and capacity to activate complement [14], as well as between FcγRs in terms of structure and function [116]. Thus, humanized mice which bear genetically engineered human IgGs [117] and human FcγRs [118] might help overcome some of these limitations. Similarly, non-human primates harbor

different IgG subclass binding properties compared with humans [16]. This creates an obvious difficulty in translating immune response findings in animal models and predicting outcomes in human vaccination or thAb trials [15]. Thus, acknowledging the strengths and limitations of animal models and working towards improving these may potentiate our understanding of IgG3 responses in infectious diseases, and better harness the possible benefits of IgG3-based pharmaceuticals.

Concluding Remarks

In summary, the critical role of IgG3 in the control and/or protection against a range of infectious diseases is evident from numerous studies highlighted herein [1–5,9,91,119]. This ability is mainly due to the inherit unique molecular properties of IgG3 that can confer highly functional potent effector responses. Although numerous questions remain (see Outstanding Questions), increasing our molecular understanding of IgG3 and its functional properties may improve the engineering and development of future next generation thAbs and by expanding our understanding, potentially inform approaches to induce protective IgG3-centric vaccines.

Outstanding Questions

Do glycans and allotypes have variable effects on IgG3 effector functions? A detailed understanding of the glycosylation patterns and allotypes of IgG3 Abs and their interactions with FcγRs and/or complement complexes may be key to uncovering novel pathways of Ab-mediated and/or complement activation.

Could IgG3 be utilized in the development of next generation thAbs? While there are currently no IgG3 thAbs, IgG3, especially IgG3-H435, with prolonged half-life, may be useful for protection against infections that involve enhanced Fc effector responses, complement activation, and neutralization. However, whether alloimmune responses may be generated to foreign IgG3 allotypes is unclear.

How can a balance between IgG3 Ab-mediated activation and overactivation be established? When is there too much IgG3? IgG3 is the most potent IgG subclass, but an overactivation of effector function can cause severe pathogenesis in certain diseases and potentially worsen outcomes. Understanding the appropriate balance for optimal protective immunity needs to be further explored and may be pathogen specific.

How can vaccines induce durable long-lasting IgG3 responses? An improved understanding of how successful vaccines induce durable IgG3 responses, for example, tetanus and meningococcal vaccines, may help us to elicit actual protective, and long-lasting IgG3 concentrations via vaccination.

Acknowledgments

We thank Milla McClean and Ester Lopez for their assistance revising this manuscript. This work was supported by funding from the University of Melbourne (T.D.), National Health and Medical Research Council of Australia (NHMRC) (S.J.R., S.J.K., A.W.C.), by the Australian Centre for Research Excellence in Malaria Elimination (ACREME) (S.J.R.) and the American Foundation for AIDS Research (amfAR) Mathilde Krim Fellowship (A.W.C.).

References

1. Ackerman, M.E. *et al.* (2016) Polyfunctional HIV-specific antibody responses are associated with spontaneous HIV control. *PLoS Pathog.* 12, e1005315
2. Chung, A.W. *et al.* (2015) Dissecting polyclonal vaccine-induced humoral immunity against HIV using systems serology. *Cell* 163, 988–998
3. Sadanand, S. *et al.* (2018) Temporal variation in HIV-specific IgG subclass antibodies during acute infection differentiates spontaneous controllers from chronic progressors. *AIDS* 32, 443–450
4. Yates, N.L. *et al.* (2014) Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci. Transl. Med.* 19, 228
5. Chung, A.W. *et al.* (2014) Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci. Transl. Med.* 6, 228ra38
6. Aase, A. *et al.* (1998) Opsonophagocytic and bactericidal activity mediated by purified IgG subclass antibodies after vaccination with the Norwegian group B meningococcal vaccine. *Scand. J. Immunol.* 47, 388–396
7. Vidarsson, G. *et al.* (2001) Activity of human IgG and IgA subclasses in immune defense against *Neisseria meningitidis* serogroup B. *J. Immunol.* 166, 6250–6656
8. Giuntini, S. *et al.* (2016) Human IgG1, IgG3, and IgG3 hinge-truncated mutants show different protection capabilities against meningococci depending on the target antigen and epitope specificity. *Clin. Vaccine Immunol.* 23, 698–706
9. Dechavanne, C. *et al.* (2017) Associations between an IgG3 polymorphism in the binding domain for FcRn, transplacental transfer of malaria-specific IgG3, and protection against *Plasmodium falciparum* malaria during infancy: a birth cohort study in Benin. *PLoS Med.* 14, e1002403
10. Kana, I.H. *et al.* (2018) Cytophilic antibodies against key *Plasmodium falciparum* blood stage antigens contribute to protection against clinical malaria in a high transmission region of Eastern India. *J. Infect. Dis.* 218, 956–965
11. Stanisic, D.I. *et al.* (2009) Immunoglobulin G subclass-specific responses against *Plasmodium falciparum* merozoite antigens are associated with control of parasitemia and protection from symptomatic illness. *Infect. Immun.* 77, 1165–1174
12. Weaver, R. *et al.* (2016) The association between naturally acquired IgG subclass specific antibodies to the PfRH5 invasion complex and protection from *Plasmodium falciparum* malaria. *Sci. Rep.* 6, 33094
13. Osier, F.H.A. *et al.* (2014) Opsonic phagocytosis of *Plasmodium falciparum* merozoites: mechanism in human immunity and a correlate of protection against malaria. *BMC Med.* 12, 108
14. Bruhns, P. (2012) Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 14, 5640–5649
15. Chan, Y.N. *et al.* (2016) IgG binding characteristics of rhesus macaque FcgammaR. *J. Immunol.* 197, 2936–2947
16. Trist, H.M. *et al.* (2014) Polymorphisms and interspecies differences of the activating and inhibitory Fc (RII of *Macaca nemestrina* influence the binding of human IgG subclasses. *J. Immunol.* 192, 792–803
17. Lefranc, G. and Lefranc, M.-P. (2001) *The Immunoglobulin FactsBook,* Academic Press
18. Pumphrey, R.S.H. (1986) Computer models of the human immunoglobulins: Binding sites and molecular interactions. *Immunol. Today* 7, 206–211
19. Carrasco, B. *et al.* (2001) Crystallohydrodynamics for solving the hydration problem for multi-domain proteins: open physiological conformations for human IgG. *Biophys. Chem.* 93, 181–196
20. Vidarsson, G. *et al.* (2014) IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* 5
21. Roux, K.H. *et al.* (1997) Flexibility of human IgG subclasses. *J. Immunol.* 159, 3372–3382
22. Lefranc, M.-P. and Lefranc, G. (2012) Human Gm, Km and Am allotypes and their molecular characterization: a remarkable demonstration of polymorphism. *Methods Mol. Biol.* 882, 635–680
23. Stapleton, N.M. *et al.* (2011) Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. *Nat. Commun.* 2, Article number 599

24. Rispens, T. *et al.* (2014) Dynamics of inter-heavy chain interactions in human immunoglobulin G (IgG) subclasses studied by kinetic Fab arm exchange. *J. Biol. Chem.* 289, 6098–6109
25. Natsume, A. *et al.* (2008) Engineered antibodies of IgG1/IgG3 mixed isotype with enhanced cytotoxic activities. *Cancer Res.* 68, 3863–3872
26. Van Loghem, E. *et al.* (1982) Staphylococcal protein A and human IgG subclasses and allotypes. *Scand. J. Immunol.* 15, 275–278
27. Mahan, A.E. *et al.* (2016) Antigen-specific antibody glycosylation is regulated via vaccination. *PLoS Pathog.* 12, e1005456
28. Gardinassi, L.G. *et al.* (2014) Clinical severity of visceral leishmaniasis is associated with changes in immunoglobulin G Fc N-glycosylation. *mBio* 5, e01844-14
29. van de Bovenkamp, F.S. *et al.* (2018) Adaptive antibody diversification through N-linked glycosylation of the immunoglobulin variable region. *PNAS* 115, 1901–1906
30. Mimura, Y. *et al.* (2000) The influence of glycosylation on the thermal stability and effector function expression of human IgG1-Fc: properties of a series of truncated glycoforms. *Mol. Immunol.* 37, 697–706
31. Lee, C.-H. *et al.* (2017) IgG Fc domains that bind C1q but not effector Fcg receptors delineate the importance of complement-mediated effector functions. *Nat. Immunol.* 18, 889–898
32. Lin, C.W. *et al.* (2015) A common glycan structure on immunoglobulin G for enhancement of effector functions. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10611–10616
33. Wang, T.T. *et al.* (2017) IgG antibodies to dengue enhanced for Fc(RIIIA binding determine disease severity. *Science* 355, 368–369
34. Niwa, R. *et al.* (2005) IgG subclass-independent improvement of antibody-dependent cellular cytotoxicity by fucose removal from Asn297-linked oligosaccharides. *J. Immunol. Methods* 306, 151–160
35. Chung, A.W. *et al.* (2014) Identification of antibody glycosylation structures that predict monoclonal antibody Fc-effector function. *AIDS* 28, 2523–2530
36. Ackerman, M.E. *et al.* (2013) Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. *J. Clin. Investig.* 123, 2183–2192
37. Kaneko, Y. *et al.* (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 313, 670–673
38. van de Bovenkamp, F.S. *et al.* (2018) Variable domain N-linked glycans acquired during antigen-specific immune responses can contribute to immunoglobulin G antibody stability. *Front. Immunol.* 9, 740
39. Plomp, R. *et al.* (2015) Hinge-region O-glycosylation of human immunoglobulin G3 (IgG3). *Mol. Cell. Proteomics* 14, 1373–1384
40. Julenius, K. *et al.* (2005) Prediction, conservation analysis, and structural characterization of mammalian mucin-type O-glycosylation sites. *Glycobiology* 15, 153–164
41. Ferrante, A.L. *et al.* (1990) IgG subclass distribution of antibodies to bacterial and viral antigens. *Pediatr. Infect. Dis. J.* 9
42. Schauer, U. *et al.* (2003) Levels of antibodies specific to tetanus toxoid, *Haemophilus influenzae* type b, and Pneumococcal capsular polysaccharide in healthy children and adults. *Clin. Diagn. Lab. Immunol.* 10, 202–207
43. Nouri-Aria, K.T. *et al.* (2004) Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J. Immunol.* 172, 3252–3259
44. Arnold, J.N. *et al.* (2006) Mannan binding lectin and its interaction with immunoglobulins in health and in disease. *Immunol. Lett.* 106, 103–110
45. Malhotra, R. *et al.* (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat. Med.* 1, 237–243
46. Lu, Y.L. *et al.* (2007) Solution conformation of wild-type and mutant IgG3 and IgG4 immunoglobulins using crystallohydrodynamics: possible implications for complement activation. *Biophys. J.* 93, 3733–3744
47. Thommesen, J.F. *et al.* (2000) Lysine 322 in the human IgG3 CH2 domain is crucial for antibody dependent complement activation. *Mol. Immunol.* 37, 995–1004
48. Kapur, R. *et al.* (2014) IgG-effector functions: 'The Good, The Bad and The Ugly'. *Immunol. Lett.* 160, 139–144
49. Bruhns, P. *et al.* (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* 113, 3716–3725
50. Lu, L.L. *et al.* (2016) A functional role for antibodies in tuberculosis. *Cell* 167, 433–443
51. McLean, M.R. *et al.* (2017) Dimeric Fc gamma receptor enzyme-linked immunosorbent assay to study HIV-specific antibodies: a new look into breadth of Fc gamma receptor antibodies induced by the RV144 Vaccine Trial. *J. Immunol.* 199, 816–826
52. Chung, A.W. *et al.* (2011) Activation of NK cells by ADCC antibodies and HIV disease progression. *J. Acquir. Immune Defic. Syndr.* 58, 127–131
53. Firan, M. *et al.* (2001) The MHC class I-related receptor, FcRn, plays an essential role in the maternofetal transfer of γ-globulin in humans. *Int. Immunol.* 13, 993–1002
54. Shah, I.S. *et al.* (2017) Structural characterization of the Man5 glycoform of human IgG3 Fc. *Mol. Immunol.* 92, 28–37
55. Einarsdottir, H. *et al.* (2014) H435-containing immunoglobulin G3 allotypes are transported efficiently across the human placenta: implications for alloantibody-mediated diseases of the newborn. *Transfusion* 54, 665–671
56. Dugast, A.S. *et al.* (2014) Independent evolution of Fc- and Fab-mediated HIV-1-specific antiviral antibody activity following acute infection. *Eur. J. Immunol.* 44, 2925–2937
57. Posadas-Mondragón, A. *et al.* (2017) Indices of anti-dengue immunoglobulin G subclasses in adult Mexican patients with febrile and hemorrhagic dengue in the acute phase. *Mircobiol. Immunol.* 61, 433–441
58. Yates, N.L. *et al.* (2011) Multiple HIV-1-specific IgG3 responses decline during acute HIV-1: implications for detection of incident HIV infection. *AIDS* 25, 2089–2097
59. Horns, F. *et al.* (2016) Lineage tracing of human B cells reveals the *in vivo* landscape of human antibody class switching. *eLife* 5, e16578
60. Kitaura, K. *et al.* (2017) Different somatic hypermutation levels among antibody subclasses disclosed by a new next-generation sequencing-based antibody repertoire analysis. *Front. Immunol.* 8, 389
61. Pene, J. *et al.* (2004) Cutting edge: IL-21 is a switch factor for the production of IgG1 and IgG3 by human B cells. *J. Immunol.* 172, 5154–5157
62. Malisan, F. *et al.* (1996) Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytes. *J. Exp. Med.* 183, 937–947
63. Briere, F. *et al.* (1994) Human interleukin 10 induces naive surface immunoglobulin D+ (sIgD+) B cells to secrete IgG1 and IgG3. *J. Exp. Med.* 179, 757–762
64. Fujieda, S. *et al.* (1995) IL-4 plus CD40 monoclonal antibody induces human B cells gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, and gamma 4, but not gamma 2. *J. Immunol.* 155, 2318–2328
65. Scharf, O. *et al.* (2001) Immunoglobulin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. *J. Virol.* 75, 6558–6565
66. Richardson, S. *et al.* (2018) *(2018) IgG3 hinge length enhances neutralization potency and Fc effector function of an HIV V2-specific broadly neutralizing antibody,* HIV Research for Prevention, (Madrid)
67. Tay, M.Z. *et al.* (2016) Antibody-mediated internalization of infectious HIV-1 virions differs among antibody isotypes and subclasses. *PLoS Pathog.* 12, e1005817

68. Kardava, L. *et al.* (2018) IgG3 regulates tissue-like memory B cells in HIV-infected individuals. *Nat. Immunol.* 19, 1001–1012
69. Richardson, S.I. *et al.* (2018) HIV-specific Fc effector function early in infection predicts the development of broadly neutralizing antibodies. *PLoS Pathog.* 14, e1006987
70. Kam, Y.-W. *et al.* (2012) Early appearance of neutralizing immunoglobulin G3 antibodies is associated with Chikungunya virus clearance and long-term clinical protection. *J. Infect. Dis.* 205, 1147–1154
71. Syenina, A. *et al.* (2015) Dengue vascular leakage is augmented by mast cell degranulation mediated by immunoglobulin Fcγ receptors. *eLife* 4, e05291
72. Rodrigo, W.W. *et al.* (2010) Dengue virus neutralization is modulated by IgG antibody subclass and Fcγ receptor subtype. *Virology* 394, 175–182
73. Ayala-Nunez, N.V. *et al.* (2016) How antibodies alter the cell entry pathway of dengue virus particles in macrophages. *Sci. Rep.* 6, 28768
74. Wang, T.T. *et al.* (2017) IgG antibodies to dengue enhanced for FcgammaRIIIA binding determine disease severity. *Science* 355, 395–398
75. Kuzmina, N.A. *et al.* (2018) Antibody-dependent enhancement of Ebola virus infection by human antibodies isolated from survivors. *Cell Rep.* 24, 1802–1815 e5
76. Goh, Y.S. *et al.* (2011) Human IgG isotypes and activating Fcγ receptors in the interaction of *Salmonella enterica* serovar Typhimurium with phagocytic cells. *Immunology* 133, 74–83
77. Sousa, A.O. *et al.* (1998) IgG subclass distribution of antibody responses to protein and polysaccharide mycobacterial antigens in leprosy and tuberculosis patients. *Clin. Exp. Immunol.* 111, 48–55
78. Hussain, R. *et al.* (2000) PPD-specific IgG1 antibody subclass upregulate tumour necrosis factor expression in PPD-stimulated monocytes: possible link with disease pathogenesis in tuberculosis. *Clin. Exp. Immunol.* 119, 449–455
79. Cai, Y. *et al.* (2014) Increased complement C1q level marks active disease in human tuberculosis. *PLoS One* 9, 92340
80. Richards, J.S. *et al.* (2010) Association between naturally acquired antibodies to erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and high-density parasitemia. *Clin. Infect. Dis.* 51, 50–60
81. Roussilhon, C. *et al.* (2007) Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. *PLoS Med.* 4, e320
82. Mathiesen, L. *et al.* (2013) Maternofetal trans-placental transport of recombinant IgG antibodies lacking effector functions. *Blood* 122, 1174–1181
83. Stubbs, J. *et al.* (2011) Strain-transcending Fc-dependent killing of *Plasmodium falciparum* by merozoite surface protein 2 allele-specific human antibodies. *Infect. Immun.* 79, 1143–1152
84. Boyle, M.J. *et al.* (2015) Human antibodies fix complement to inhibit *Plasmodium falciparum* invasion of erythrocytes and are associated with protection against malaria. *Immunity* 42, 580–590
85. Joos, C. *et al.* (2010) Clinical protection from falciparum malaria correlates with neutrophil respiratory bursts induced by merozoites opsonized with human serum antibodies. *PLoS One* 5, e9871
86. Larsen, M.D. *et al.* (2017) Malaria parasite evasion of classical complement pathway attack. *Mol. Immunol.* 89, 159
87. Kurtovic, L. *et al.* (2018) Human antibodies activate complement against Plasmodium falciparum sporozoites, and are associated with protection against malaria in children. *BMC Med.* 16, 61
88. Fernandez-Becerra, C. *et al.* (2010) Naturally-acquired humoral immune responses against the N- and C-termini of the *Plasmodium vivax* MSP1 protein in endemic regions of Brazil and Papua New Guinea using a multiplex assay. *Malar. J.* 9
89. Lima-Junior, J.C. *et al.* (2011) B cell epitope mapping and characterization of naturally acquired antibodies to the *Plasmodium vivax* merozoite surface protein-3alpha (PvMSP-3alpha) in malaria exposed individuals from Brazilian Amazon. *Vaccine* 29, 1801–1811
90. Yilmaz, B. *et al.* (2014) Gut microbiota elicits a protective immune response against malaria transmission. *Cell* 159, 1277–1289
91. Aguilar, R. *et al.* (2018) Antibody responses to α-Gal in African children vary with age and site and are associated with malaria protection. *Sci. Rep.* 8, http://dx.doi.org/10.1038/s41598-018-28325-w Article number 9999
92. Iriemenam, N.C. *et al.* (2013) Association between immunoglobulin GM and KM genotypes and placental malaria in HIV-1 negative and positive women in Western Kenya. *PLoS One* 8, e53948
93. Migot-Nabias, F. *et al.* (2008) Imbalanced distribution of GM immunoglobulin allotypes according to the clinical presentation of *Plasmodium falciparum* malaria in Beninese children. *J. Infect. Dis.* 198, 1892–1895
94. Giha, H.A. *et al.* (2009) Antigen-specific influence of GM/KM allotypes on IgG isotypes and association of GM allotypes with susceptibility to *Plasmodium falciparum* malaria. *Malar J.* 8, 306
95. Pandey, J.P. *et al.* (2007) Significant differences in GM allotype frequencies between two sympatric tribes with markedly differential susceptibility to malaria. *Parasite Immunol.* 29, 267–269
96. Recke, A. *et al.* (2018) The p.arg435his variation of IgG3 with high affinity to FcRn is associated with susceptibility for pemphigus vulgaris – analysis of four different ethnic cohorts. *Front. Immunol.* 9, 1788
97. Kaplon, H.K. and Reichert, J.M. (2018) *Antibodies to watch in 2019,* MAbs
98. Synagis (palivizumab). Full prescribing information, MedImmune, LLC, Gaithersburg, MD, 2014.
99. Mazumdar, S. (2009) Raxibacumab. *MAbs* 1, 531–538
100. Weinheimer, S. *et al.* (2018) Ibalizumab susceptibility in patient HIV isolates resistant to antiretrovirals. *25th Conference on Retroviruses and Opportunistic Infections (CROI 2018), Boston, USA*
101. Poiron, C. *et al.* (2010) IMGT/mAb-DB: The IMGT® database for therapeutic monoclonal antibodies. http://www.imgt.org/IMGTposters/SFI2010_IMGTmAb-DB.pdf
102. Desoubeaux, G. *et al.* (2013) Therapeutic antibodies and infectious diseases. Tours, France, November 20–22, 2012. *MAbs* 5, 626–632
103. Lakbub, J.C. *et al.* (2016) Disulfide bond characterization of endogenous IgG3 monoclonal antibodies using LC-MS: an investigation of IgG3 disulfide-mediated isoforms. *Anal. Methods* 8, 6045–6055
104. Magdelaine-Beuzelin, C. *et al.* (2009) IgG1 heavy chain-coding gene polymorphism (G1m allotypes) and development of anti-bodies-to-infliximab. *Pharmacogenet. Genomics* 19, 383–387
105. Bartelds, G.M. *et al.* (2010) Surprising negative association between IgG1 allotype disparity and anti-adalimumab formation: a cohort study. *Arthritis Res. Ther.* 12, R221
106. Webster, C.I. *et al.* (2016) A comparison of the ability of the human IgG1 allotypes G1m3 and G1m1,17 to stimulate T-cell responses from allotype matched and mismatched donors. *MAbs* 8, 253–263
107. Ternant, D. *et al.* (2016) IgG1 allotypes influence the pharmacokinetics of therapeutic monoclonal antibodies through FcRn binding. *J. Immunol.* 196, 607–613
108. Lazar, G.A. *et al.* (2006) Engineered antibody Fc variants with enhanced effector function. *Proc. Natl. Acad. Sci. U. S. A.* 103, 4005–4010
109. Moore, G.L. *et al.* (2010) Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. *MAbs* 2, 181–189
110. Huang, Y. *et al.* (2016) Engineered bispecific antibodies with exquisite HIV-1-neutralizing activity. *Cell* 165, 1621–1631

111. Mahlangu, J. *et al.* (2018) Emicizumab prophylaxis in patients who have hemophilia A without inhibitors. *N. Engl. J. Med.* 379, 811–822

112. Robb, M.L. *et al.* (2012) Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. *Lancet Infect. Dis.* 12, 531–537

113. Cooper, P.J. *et al.* (1999) Human onchocerciasis and tetanus vaccination: impact on the postvaccination antitetanus antibody response. *Infect. Immun.* 67, 5951–5957

114. Garcia, G. *et al.* (2006) Antibodies from patients with dengue viral infection mediate cellular cytotoxicity. *J. Clin. Virol.* 37, 53–57

115. de Araujo, L.S. *et al.* (2018) IgG subclasses' response to a set of mycobacterial antigens in different stages of *Mycobacterium tuberculosis* infection. *Tuberculosis* 108, 70–76

116. Nimmerjahn, F. *et al.* (2005) Fc gamma RIV: a novel FcR with distinct IgG subclass specificity. *Immunity* 23, 41–51

117. Murphy, A.J. *et al.* (2014) Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. *PNAS* 111, 5153–5158

118. Smith, P. *et al.* (2012) Mouse model recapitulating human Fcgamma receptor structural and functional diversity. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6181–6186

119. Kana, I.H. *et al.* (2017) Naturally acquired antibodies target the glutamate-rich protein on intact merozoites and predict protection against febrile malaria. *J. Infect. Dis.* 215, 623–630

120. Lefranc, M.P. *et al.* (2005) IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains. *Dev. Comp. Immunol.* 29, 185–203

121. Lefranc, M.P. *et al.* (2009) IMGT, the international ImMunoGeneTics information system. *Nucleic Acids Res* 37, D1006-12