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The Role of IgG3 in Infectious Diseases

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Abstract

Immunoglobulin IgG3 comprises only a minor fraction of IgG in human plasma 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-mediated phagocytosis or antibody-mediated cellular cytotoxicity. 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 antibody half-life and excessive pro-inflammatory activation. Understanding the molecular and functional properties of IgG3 may facilitate the development of improved antibody-based immunotherapies and vaccines against infectious diseases.

Human IgG3 a highly potent Immunoglobulin

Antibodies (Ab) 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 (Ig), a growing number of recent studies have highlighted IgG3 as critical for the control and/or protection of a range of pathogens, including viral (eg HIV [1-5]), bacterial (eg *Neisseria* Spp [6-8]) and parasitic (eg. *Plasmodium* Spp. [9-13]) pathogens. This review will describe the unique characteristics of human IgG3 that contribute to its potent anti-pathogenic functions including enhanced neutralization, FcγR affinity and Fc-mediate activity. Furthermore, we will discuss how the unique properties of IgG3 can be harnessed for future preventative and therapeutic manipulations and highlight the major obstacles and outstanding questions that are pertinent.

Human IgG3: a unique but understudied 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%) [14]. The amino acid sequences of the human IgG subclasses are >95% homologous in the constant domains of their heavy chains (Fig.1). IgG3 has a distinct amino acid composition and structure to the **'hinge region'** (see Glossary) between C_H1 and C_H2 [15]. The flexibility of the hinge region decreases in the order IgG3>IgG1>IgG4>IgG2 [16]. Further, IgG3 has an extended hinge region (Fig. 1 + Tab. 1), consisting of up to 62 amino acids, forming a poly-proline double helix consisting of 11 disulfide bridges (compared to two in hinge regions of IgG1 and IgG4 and four in hinge region of IgG2) [17]. This is the result of duplications of a hinge exon, encoded by one single exon in IgG1, IgG2, and IgG4, but up to 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 [18]. 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 IgG3's high potential to activate effector functions [17, 18].

It is important to note that the structure and enhanced functionality of human IgG3, is unique compared to most animal models, including murine and macaque species, which do not have an equivalent analogue among its IgG subclasses [19-21]. This is particularly relevant for non-human primate models, where macaque spp. are commonly used for pre-human clinical trials and may historically have contributed to the why the importance of human IgG3 is understudied in comparison to other IgG subclasses.

Allotypes of IgG3

Allotypes are **polymorphisms** in the constant regions of Ig heavy and light chains (Fig. 1b). IgG3 is the most polymorphic subclass with thirteen Gm allotypes termed G3m, which is thought to be due to its higher rapid evolutionary diversification compared to other subclasses [17]. G3m allotypes are inherited in different combinations or G3m alleles (encoded by one or several *IGHG3* alleles) shared among individuals within populations (Tab. 2). Several G3m allotypes consist of a combination of multiple amino acids, which lead to changes of their tertiary protein structures, with all known G3m allotypes located within the C_H2 and C_H3 domains[22].

IgG3 allotypic variations can have structural and functional consequences, such as a shorter hinge regions and extended **half-life** compared to other allotypes [23, 24]. In addition, polymorphisms in the C_H3 domain affect the C_H3–C_H3 interdomain interactions [25], with potential consequences for **C1q** binding in **complement activation** [25, 26]. 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 [27].

Glycosylation of IgG3

Glycosylation is a post-translational modification of IgG Abs, which can be regulated by a range of B-cell stimuli, including environmental factors, such as stress or diseases, cytokines 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 [28]. Glycosylation is an inherent mechanism of Ab diversification, on top of **V(D)J recombination**, **somatic hypermutation** (SHM) and **class switch recombination** (CSR), and thereby contributes 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 (Fig. 1c). The glycans at this N-glycosylation site can influence Ab stability [30], binding to **Fcγ-receptors (FcγRs)** and complement [31], consequently modulating effector functions, such as **complement-dependent cytotoxicity (CDC)** and **Ab-dependent cell cytotoxicity (ADCC)** [32-36]. For instance, it has been shown that monoclonal IgG3 Abs expressed

from α1,6-fucosyltransferase gene knockout Chinese hamster ovary cell lines, which lack the ability to attach fucose to asparagine 297 of antibodies, have increased binding to FcγRIIIa, which enhances CDC and ADCC [26, 35]. Furthermore, a high degree of agalactosylation has been associated with disease progression in various infectious diseases, including HIV, Hepatitis B, Leishmaniasis and active Mycobacterium Tuberculosis (TB) [37-40], while sialic acid has been linked to anti-inflammatory activities in autoimmune diseases such as rheumatoid arthritis [41, 42]. A second N-glycosylation site in heavy and light chain variable regions V_H and V_L have been observed in approximately 15-25% of all serum IgG, which can contribute to Ab stability [43], and also can modulate antigen binding, as demonstrated by a recent study by van de Bovenkamp et al., where presence of Fab glycans could increase Fab binging affinity up to two-fold [29].

Unique to IgG3, is the presence of O-glycosylation sites in the hinge region [44]. Approximately 10% of IgG3 derived from polyclonal serum samples and ~13% of monoclonal IgG3 Abs have been observed to contain O-glycans [44]. Each IgG3 can contain up to three O-glycans at threonine residues at triple repeat regions within the hinge [44]. Indeed, the IgG3 hinge region has a high degree of surface accessibility associated with O-glycosylation [45]. Surface accessibility may be responsible for the slightly lower degree of O-linked glycosylation observed in recombinant G3m15 IgG3 with a shorter hinge region than other IgG3 allotypes [44]. Although the function of O-glycosylation is still not fully understood, the hinge structure has been hypothesized to be able to protect the Ig from proteolytic cleavage, and might also help to maintain the extended conformation and flexibility of IgG3 hinge regions, though this has yet to be demonstrated [44]. 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 [17, 31]. IgG3 is the most functional subclass, closely followed by IgG1, due to its superior affinity to FcγR [46]. However, IgG2 also plays an important role in targeting polysaccharides and is commonly induced during bacterial infections [47], while IgG4 is often induced in response to allergens [48] (Tab. 1).

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** [49, 50]. IgG3 binds with a higher affinity to C1q compared to other IgG subclasses (Fig. 2) [51]. While this activity is thought to be linked to IgG3's increased flexibility, the interaction between C1q and IgG3 is not dependent on the length of its hinge region, as shown by studies, where engineered IgG3 with shorter IgG1 or IgG4 hinge regions exhibited even greater binding affinity for C1q than wild-type IgG3 [26, 44]. Instead Ab-dependent complement-mediated lysis studies have shown that one amino acid (Lys 322) in the human IgG3 C_H2 domain is critical for C1q interactions and enhanced CDC [52]. Nevertheless, the importance of IgG3 in the lectin pathway and the relevance of IgG3 glycosylation on CDC is still unknown.

151 ***FcγR binding***

152 Abs forming complexes with antigens, termed opsonization, can initiate important cell-
153 based functions such as: ADCC, **Ab-dependent phagocytosis (ADP)**, **Ab-**
154 **dependent cellular inhibition (ADCI)**, **Ab-dependent cellular viral inhibition**
155 **(ADCVI)**, inducing the respiratory burst, triggering the release of pro- and anti-
156 inflammatory mediators and enzymes, as well as modulating antigen presentation and
157 the clearance of pathogenic complexes (Fig. 2) [46, 53]. The efficiency of monomeric
158 IgG3 binding to FcγRIIa, FcγRIIIa, and FcγRIIIb on effector cells such as neutrophils,
159 monocytes, macrophages or natural killer (NK) is even higher than of monomeric IgG1
160 (Tab. 1) [23]. Thus, the interaction of IgG3 with FcγRs on these effector cells has been
161 increasingly recognized as a critical immune response against a range of infectious
162 diseases including viruses, bacteria and parasitic pathogens [1, 34, 39, 54-56].

163

164 ***FcRn binding***

165 The interaction between IgG and **neonatal Fc receptors (FcRn)** is important for IgG
166 transplacental passage of IgG Abs and Ab half-life in humans [57, 58]. FcRn binds to
167 IgG at an acidic pH in the endosomes of vascular endothelial cells and recycles it back
168 to circulation by dissociating at physiological pH [59]. FcRn-mediated transport of IgG3
169 is inhibited with the presence of IgG1 due to intracellular competition [24]. Therefore,
170 the unbound IgG3 is degraded and not returned into circulation, which in part may
171 explain its shorter half-life in most individuals [24]. However, IgG3 affinity to FcRn is
172 modified by an amino acid substitution at position 435, where IgG3 has an arginine
173 instead of the histidine found in all other subclasses [24]. The R435 side chain stays
174 positively charged at physiological pH and can still maintain ionic interactions with
175 FcRn. This contributes to strong binding of IgG3 to FcRn at physiological pH, thus

leading to endosomal degradation [59]. In contrast, individuals with certain allotypes (Tab.2), such as G3m15 and G3m16, have a histidine at position 435. This substitution makes their Ab half-life comparable to the half-life of IgG1 [24]. 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 [60]. 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 pro-inflammatory responses which can cause collateral damage and over stimulation. Indeed, it has been hypothesized that the arginine substitution, which reduces the half-life of IgG3, may function to limit the potential for excessive activation and inflammatory responses, due to its more rapid clearance from circulation compared to other less inflammatory subclasses [9]. Lastly, R/H435 allotypes are relevant for infectious diseases, as discussed below.

IgG3 responses to pathogens

Generation of IgG3 during various infection types

IgG3 is often one of the earliest subclasses to be elicited against protein antigens upon infection [3, 61-63], due to its genetic locus positioning among Ig heavy constant chains (*IGHC*) in humans [64, 65]. Sequentially, IgG3 is the first *IGHG* gene located within the locus, followed by IgG1 *(Gene order as follows: IGHG3, IGHG1, IGHA1, IGHG2, IGHG4, IGHE, and IGHA2* from 5' to 3' [64, 65]); thus IgG3 responses are often closely followed by IgG1 responses. Early IgG3 responses may be particularly beneficial for the rapid clearance of pathogens that express high levels of proteins, especially viruses, specific bacteria and parasites (discussed below), due to its potent Fc effector functions [59, 61]. Chronic infections, such as chronic HIV, in contrast, are

often associated with a reduction in IgG3 in sera [3, 63], likely due to repeated antigen stimulation and subsequent CSR to 'downstream' IgG subclasses or isotypes, which are comparatively less pro-inflammatory, due to their weaker affinity to FcγRs [23, 65]. CSR of human Ig to IgG3 *in vitro* is predominantly modulated by cytokines, in particular IL-4, IL-10 and IL-21 [66-69]. However the exact signals that modulate CSR in humans during various infections *in vivo* are not fully defined. Moreover, similar cytokines have also been linked with IgG1 CSR *in vitro* (IgG3 and IgG1 are the first two genes in the *IGHC* loci), which might potentially explain why IgG3 and IgG1 responses are often linked during infection [66-69]. Likely due to stepwise CSR events of IgG3 to 'downstream' IgG subclasses, analysis of clonally related Ig sequences have identified less SHM within IgG3 compared to class-switched Ig subclasses, especially IgG4 [65].

IgG3 in Viral Infections

Virus-specific IgG3 appears early during infection, while IgG1 progressively dominates the responses in most infections [46]. However, recent HIV vaccine studies suggest that despite being of low titer, IgG3 Abs are potent effectors of vaccine-induced humoral immune responses [2, 4, 5]. In the RV144 Phase III vaccine trial (http://clinicaltrials.gov NCT00223080), the only human HIV 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) [4]. Accordingly, the depletion of IgG3 from RV144 plasma samples resulted in decreased Fc-mediated effector activities *in vitro* including significant decreases in ADCC, ADP and the loss of Fc effector polyfunctionality, compared to undepleted samples [5].

During early HIV infection, functional Ab responses decline drastically with HIV disease progression, which is correlated with waning IgG3 Ab concentrations [3, 61, 63]. In HIV-1 neutralization studies, polyclonal IgG3 Abs are more potent than any other subclass [70], with increased length of the hinge region enhancing neutralization potency and significantly improved phagocytosis and **trogocytosis** activity *in vitro* compared to IgG1 [71]. Moreover, Env-binding monoclonal IgG3 Abs are more effective at internalizing viral particles compared to Env-binding IgG1 of the same specificity [72]. 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 individuals, but not aviremic individuals, observed reduced sensitivity to B cell receptor stimulation compared to their $IgG3^-IgM^+$ counterparts [73]. This modulation of IgM^+ B-cell stimulation was reported to occur through multiple mechanisms including, direct co-localization of IgG3 with IgM, along with interactions with FcRIIb (inhibitory FcR), C1q and **C-reactive protein** [73]. IgG3 binding to the IgM-B-cell receptors could also arise in the setting of persistent HIV viremia and contribute to refractory IgM^+ B-cell stimulation during chronic infection [73]. Furthermore, a recent HIV cohort study also reported that IgG Fc polyfunctionality (ADCC, ADCP and antibody dependant trogocytosis) and greater subclass diversity (i.e. induction of all IgG subclasses and not IgG3 alone) were associated with the development of **broadly neutralizing Abs (bNAbs)** compared to HIV-individuals that failed to develop bNAbs [74]. Further studies are needed to determine the advantages and disadvantages of IgG3 responses during HIV infections, as well as any putative therapeutic applications, especially in context of balancing chronic B-cell regulation, against the potential for

eliciting highly functional IgG3 Abs with more potent anti-viral Fc effector functions and improved bNAb responses.

IgG3-mediated responses play a role in an array of other viral infections. One study found that Chikungunya virus-specific responses are dominated by neutralizing IgG3 Abs, which developed rapidly in patients with high viremia [75]. In contrast, pre-existing IgG in patients, mainly IgG1 and IgG3, against severe Dengue virus (DENV) can form virus-Ab complexes with a different DENV serotype, promoting infection which can lead to complications, such as dengue hemorrhagic fever and dengue shock syndrome [62, 76, 77]. This cross-serotype activity is referred to as Ab-dependent enhancement (ADE), that is theorized to be due to higher antigen **avidity** [34], activation of complement [62], and FcγR binding [78, 79] compared to same serotype responses. IgG3 responses might also be detrimental in this scenario, because it bears the greatest potential to activate complement and initiate FcγR uptake by effector immune cells relative to any other subclass [23, 51]. In a recent study, among **monoclonal Abs (mAbs)** recovered from survivors of Ebola virus infections, IgG3 Abs induced high-level ADE and FcγR blocking experiments with THP-1 cells, a monocytic cell line, showed that blocking of FcγRIII eliminated IgG3-mediated ADE completely, while blocking of FcγRI or FcγRII reduced it significantly [80].

Bacterial Infections

The IgG subclass distribution in anti-bacterial responses is more heterogeneous than those produced in anti-viral responses due to the diversity of bacteria epitopes [46]. Limited studies have explored the importance of IgG3 in bacterial infections; however, a key anti-bacterial property of human IgG3 Abs is their ability to induce CDC (Fig. 2) [16]. Protection against the invasive *Neisseria meningitidis* has been found to depend

on the recognition of bacterial surface antigens by Abs, activation of complement, and degradation of bacteria by **bacteriolysis**. Human IgG3 displayed higher bacteriolysis activity than IgG1 *in vitro* when the target antigen (FHbp) was sparsely expressed on wildtype bacteria (group B strain H44/76-WT) compared to a mutant bacteria that expressed three-fold more surface target antigen [8]. Additionally, this study showed that recombinant IgG3 mAbs, with shorter hinge regions, such as IgG3 G3m15, performed better than other IgG3 allotype mAbs, irrespective of Ab specificity, in enhancing complement-mediated bactericidal activity against *N. meningitidis* [8]. By contrast, the long hinge region of non-G3m15 IgG3 seems to dampen the high CDC capability of IgG3 Fc through an unknown mechanism in *N. meningitidis* infections [8]. It is therefore possible that IgG3 Abs with a shorter hinge region might be more effective in clearing *N. meningitidis* infections, and potentially other bacterial pathogens, but this remains to be further tested.

IgG3 are also potent mediators of opsonophagocytosis of bacterial pathogens. 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 then IgG2 [81]. Similar results have been observed with *N. meningitidis* infections, where chimeric IgG3, isolated from plasma of vaccinees immunized with a meningococcal vaccine, is highly efficient in mediating phagocytosis after opsonisation, when using polymorphonuclear leucocytes as effector cells in the presence of human complement [6, 7].

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

300 the predominant human Ab subclasses formed against a *Mycobacterium tuberculosis*
301 (*Mtb*) infection [82]. They stimulate the release of tumor necrosis factor (TNF) from
302 primary monocytes [83], enhance bacterial uptake by macrophages and form
303 complement immune complexes, which remain elevated during active TB, and
304 furthermore, have been associated with increased disease severity [84]. As a result,
305 excessive IgG1 and IgG3 subclasses might potentially cause an excessive pro-
306 inflammatory response, which in the case of active TB may be more harmful than
307 protective [84]. This argues for a complex role of IgG3 in bacterial infections including
308 both bacterial uptake and clearance and in C1q immune complex formation.
309 Consequently, this illustrates the need for extensive and robust studies that examine
310 the role of IgG3 Abs in relevant bacterial infections.
311
312 ***Parasitic Infections***
313 The predominant IgG subclass produced in response to parasites like *Plasmodium*
314 *falciparum* are IgG1 and IgG3 [85]. IgG3 is linked to protective immunity against
315 malaria [11, 12, 85, 86], due to opsonisation of malaria-infected erythrocytes and
316 promotion of Ab-dependent effector functions [10, 13, 87, 88]. In addition, they can
317 induce complement deposition at the merozoite stage of the parasite, thus mediating
318 inhibition of erythrocyte invasion [89-91]. IgG3-induced CDC is also associated with
319 anti-plasmodium sporozoite immunity in children [92]. In regards to *Plasmodium*
320 *vivax* merozoites, the predominant Abs produced are also IgG1 and IgG3 [93, 94].
321 Moreover, Abs against the classical α-Gal (Galα1-3Galβ1-4GlcNAc-R) epitopes have
322 correlated with protection against *Plasmodium* spp. infections, reducing malaria
323 transmission by *Anopheles* mosquitoes [95]. In addition, higher α-Gal IgG3, but also

IgG4 and IgM are associated with protection against clinical malaria in contrast to what has been previously observed for other protein antigens [96].

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 [97] and children with these allotypes have been reported to be better protected against **uncomplicated malaria** than non-carriers [98]. However, other studies have suggested that G3m but not Km allotypes might influence host susceptibility to malaria infection together with the Ab profile of the donors [99, 100]. Individuals with the G3m6 allotype had a higher incidence of uncomplicated malaria than non-carriers, due to low baseline IgG3 concentrations and high baseline of non-cytophilic subclasses [99]. Of note, G3m6 is predominantly found in African populations, possibly as a result of differential evolutionary selection caused by infectious diseases such as malaria [100]. 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 trans-placental transfer and half-life of malaria-specific IgG3 in young infants compared with non-carriers 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 [101]. More research on IgG3 polymorphism frequencies in endemic populations may aid in the identification of potential selective advantages and disadvantages of these IgG3 polymorphisms [9].

Potential of IgG3 for therapeutic applications

Current state of play

By the end of 2018, there were approximately 80 approved therapeutic Abs (thAbs) on the market [102]. However, only three monoclonal thAbs are currently licensed for the treatment of infectious diseases (Palivizumab, a humanized IgG1 mAb against the respiratory syncytial virus (RSV) [103]; Raxibacumab, a humanized IgG1 mAb against *Bacillus anthracis* [104]; and Ibalizumab, a humanized IgG4 mAb, which inhibits HIV from entering host cells [105]). Currently, a limited number of IgG-based thAbs are in late-stage clinical studies for infectious indications [102, 106, 107]. Almost all thAbs to date have human IgG1 or IgG4 Fc domains, and there are presently no approved IgG3 thAbs [102]. Different reasons for this include : i) its extensive hinge region, which can contribute to increased proteolysis *in vitro* [108]; ii) the multiple IgG3 allotypes that exist across populations, including allo-immunity to foreign allotype polymorphisms [60], and iii) the reduced half-life of specific IgG3 allotypes [24]. Given the significance of IgG3 responses against natural infections and IgG's unique structural and functional characteristics, 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 [24]. In a mouse model of *Streptococcus pneumoniae* infection, IgG3-H435

374 demonstrated significantly better protection against *S. pneumoniae* than IgG1 and
375 IgG3-R435, indicating that IgG3-H435 might be considered when devising future
376 improved therapeutic approaches to treat infections *in vivo,* that aim to increase IgG3
377 serum longevity [24].
378
379 In the past, differences in allotypes of a thAb were thought to potentially contribute to
380 therapeutic resistance due to the recognition of “non-self” Fc protein structures,
381 potentially by enhancing clearance of the thAb or in the worst-case scenario, by
382 becoming immunogenic and precipitating severe adverse reactions [109]. However,
383 studies have now demonstrated that allotypic differences between IgG1 thAbs do not
384 appear to produce novel T-cell epitopes in mismatched individuals, or induce ‘non-
385 self’ anti-allotypic Abs and are unlikely to represent a significant risk factor for induction
386 of immunogenicity [110, 111], but instead can improve the half-life of thAbs via FcRn
387 binding [112]. Further studies, especially for IgG3 allotypes which have significantly
388 greater polymorphisms and structural changes than IgG1, are required before any
389 allotype contributions to anti-thAb responses can be disregarded.
390
391 **Engineering therapeutic IgG3 Abs with enhanced functional properties**
392 A major advantage of thAbs, is their ability to promote Fc effector functions most
393 relevant for clearance and control of a targeted pathogen [113, 114]. Mounting
394 evidence has identified key mutations in the IgG1 Fc domain of thAbs that can
395 enhance the affinity to FcγR or complement [113, 114]. However, as previously
396 discussed, specific allotypes and structural properties of IgG3 Abs can enhance Fc
397 effector functions, in a manner that is superior to that of IgG1. One study showed that
398 a longer hinge region of IgG3 variants improved the Fc effector function (including

ADCP and antibody dependent cellular trogocytosis) and neutralization potency of a broadly neutralizing anti-HIV Ab (CAP256-VRC26) compared to IgG1 *in vitro* [71]. 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 [26]. 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 [115, 116]. Bispecific and polyspecific IgG3, with its extended flexibility and reach in its Fab arms might potentially contribute to enhanced neutralization and avidity to pathogens relative to the other subclasses [17, 70, 71, 75]. 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 effective thAbs to 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 straight forward 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 [14]. 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, 117]. This highlights the need to rigorously pursue current successful vaccines that can induce durable long lasting protection, and which elicit high IgG3 concentrations; indeed solid examples include the need to understand the mechanisms that lead to long lasting IgG3 serum Abs in response to tetanus and meningococcal vaccines [6, 118].

Another example is that of the non-protective HIV vaccine trial (VAX003), where higher IgG3 was induced at two months (into a six-month vaccination protocol) compared to the moderately protective HIV RV144 trial at the same time point. However, the IgG Abs elicited in VAX003 at two months were less functional than IgG3 elicited by RV144 [5]. The reason for this is still unclear, though 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 their 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 over-activation. For instance, a recent study suggested that excessive IgG3 might lead to negative regulation of TLM B-cells, resulting in refractory BCR signalling, as seen in chronic HIV-infected individuals [73]. Similarly over induction of IgG3 effector functions, such as ADCC have been reported to increase the severity of disease in case of DENV [119] and have been suggested to be associated with excessive

449 inflammatory responses in active TB [120].

450

451 A further challenge is the dearth of suitable animal models for studying IgG3 Abs and
452 their interaction with FcγRs, which are vital for understanding the role of IgG3 in the
453 pathogenesis, protection and vaccination against a range of infectious diseases.
454 Although there are significant differences between human and murine Abs in terms of
455 structures, capacity to activate complement [19] as well as between FcγR structures
456 and functions [121]. Thus, humanized mice which bear genetically engineered human
457 Igs [122] and human FcγRs [123]might help overcome some of these limitations .
458 Similarly, non-human primates harbour different IgG subclass binding properties
459 compared to humans [124]. This creates an obvious difficulty in translating immune
460 response findings in animal models to predicting human vaccination results, or thAb
461 trials [20]. Thus, Acknowledging the strengths and limitations of animal models and
462 working towards improving these may potentiate our understanding of IgG3
463 responses in infectious diseases, and better harness the possible benefits of IgG3-
464 based pharmaceuticals.

465

466 ## Concluding remarks

467 In summary, the critical role of IgG3 in the control and/or protection against a range of
468 infectious diseases is evident from the numerous studies highlighted herein [1-5, 9,
469 96, 125]. This ability is mainly due to the inherit unique molecular properties of IgG3
470 which can confer highly functional potent effector responses. Although numerous
471 questions remain (see outstanding questions), increasing our molecular
472 understanding of IgG3 and its functional properties will improve the engineering and

development of future next generation thAb and potentially expand our understanding of how to induce more protective IgG3-centric vaccines.

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Figure Legends:

Figure 1: Schematic overview of human IgG3 antibody. (a) IgG1-4 molecules consist of four polypeptide chains, composed of two identical γ heavy (green) chains of 50 kDa and two identical light (yellow) chains of 25 KDa, linked together by inter-chain disulfide bonds. Each heavy chain consists of an N-terminal variable domain (V_H) and three constant domains (C_H1, C_H2, C_H3). IgG molecules comprise similar-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, C_H1 domains and the light chains form the fragment antigen binding (Fab) regions [25]. 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 to the constant regions on the C_H2 and C_H3 domains. The correlations between the most common G3m allotypes and amino acids are shown. Positions in bold are according to the IMGT unique numbering for C domain [126] and in italics for Eu numbering [127].

(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 (dash line). The glycosylation patterns in healthy human serum often includes fucosylated glycoforms (red triangle) [128]. 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 [28, 38, 129].

Figure 2: Overview of human IgG3 properties and effector mechanisms. (Key Figure)

Green background: IgG3 is associated with better activity than other IgG subclasses, red background: IgG3 is associated with poorer outcomes compared to other IgG subclasses.

* allotype-dependent

† shown in HIV-infected individuals [73]

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525 **Tables**

526 **Table 1: Properties of human IgG subclasses.**

	IgG1	IgG2	**IgG3**	IgG4
Heavy chain type	gamma 1	gamma 2	**gamma 3**	gamma 4
Molecular mass (kD)	146	146	**170**	146
Amino acids in hinge region	15	12	**62***	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 level (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***	21
Placental transfer	+++	++	**++/+++***	++
Antibody response to				
Proteins	++	+/-	**++**	+/-
Polysaccharides	+	++	+/-	+/-
Allergens	+	(-)	**(-)**	++
Complement activation				
C1q binding	++	+	**+++**	+
Binding to FcγR				
FcγRI	+++	-	**+++**	+
FcγRIIa	++	+/-	**++**	+/-
FcγRIIb†/c	++	+/-	**++**	++
FcγRIIIa	+	+/-	**+++**	+/-
FcγRIIIb	+/-	+/-	**+**	-

FcRn (at pH <6.5)	+++	+++	**++/+++***	+++
Binding to Staphylococcus Protein A	++	++	*	+
Binding to Staphylococcus Protein G	++	++	**++**	++
* allotype-dependent † inhibitory				

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534 **Table 2: Nomenclature of the six most prevalent human IgG3 Gm allotypes**
535 **shared among individuals within populations.** Adapted from [10].
536

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)

Glossary

- **Antibody-dependent cell cytotoxicity (ADCC):** killing of an antibody-coated target cell by a cytotoxic effector cell through a non-phagocytic process.
- **Antibody-dependent cellular inhibition (ADCI):** a process, in which antibodies can inhibit *Plasmodium* growth in the presence of monocytes.
- **Antibody-dependent cellular viral inhibition (ADCVI):** Fcγ-receptor-mediated antiviral activity, which occurs when antibody bound to virus-infected target cells engages FcγR-bearing effector cells, such as natural killer cells, monocytes or macrophages.
- **Antibody-dependent phagocytosis (ADP):** the process by which innate immune phagocytic effector cells e.g. neutrophils, macrophages, monocytes ingest or engulf other cells, organisms or particles.
- **Allotype:** amino acid differences in the constant region of either the heavy chain or light chain within a subclass of antibodies.
- **Avidity:** the overall sum of binding strength of multiple affinities, often used to describe the accumulated strength of the two antibody **Fab** arms with its antigens.
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- **Bacteriolysis:** the destruction of bacterial cells, can often be mediated by antibodies.
- **Bispecific antibody:** recombinant protein that can simultaneously bind to two different types of antigens.
- **Broadly neutralizing antibody (bNAb):** special type of antibodies that can recognize and block many strains of a particular virus from entering healthy cells.
- **C-reactive protein:** a protein produced by the liver in response to inflammation or infection.
- **Class switch recombination:** a process by which proliferating B-cells rearrange the constant region genes in the immunoglobulin heavy chain locus to switch from expressing one class of immunoglobulin to another.
- **C1q:** a protein complex involved in the complement system, which is part of the innate immune system.
- **Complement activation:** part of the innate immune system, which is responsible for a quick and non-specific clearance of pathogens by attracting phagocytic cells.
- **Complement-dependent cytotoxicity (CDC):** C1q binds an antibody, which triggers the complement cascade, as a result of the classical pathway complement activation.
- **Envelope (Env) protein: a** protein expressed on the surface of enveloped viruses.
- **Fab region:** domain on an antibody that binds to antigens, involved in neutralization.

- **Fc region:** tail region of an antibody that determines innate immune effector function.
- **Fcγ-receptors (FcγRs):** are surface protein receptors, defined as either activating receptors (FcγRI, FcγRIIa/c, FcγRIII) or inhibitory receptor (FcγRIIb) as they elicit or inhibit immune functions.
- **Glycosylation:** a post-translational modification, whereby carbohydrates are attached to proteins (e.g. antibodies) through the aid of certain enzymes.
- **Half-life:** a measure of the mean survival time of a molecule to reduce to half its initial value.
- **Hinge region:** a flexible amino acid sequence in the central part of the heavy chains of the antibodies; can be linked by disulfide bonds.
- **Isotype switching:** the mechanism that changes the B-cell production of an immunoglobulin from one isotype to another.
- **Monoclonal antibody (mAb):** antibody produced by a single clone of cells.
- **Neonatal Fc receptors (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:** a process by which the pathogen is marked by an opsonin, e.g. an antibody, for ingestion and eliminated by the phagocytes.**Polymorphism:** the occurrence of different forms among the members of a population or colony, or in the life cycle of an individual organism**.**
- **Polyspecific antibody:** antibody, that can simultaneously bind to multiple types of antigens.
- **Somatic hypermutation (SHM)**: a process that diversifies B-cell receptors to better recognize antigens and produce antibodies against pathogens.

- **Toll-like receptors:** expressed on sentinel cells such as macrophages and dendritic cells, that recognize structurally conserved molecules derived from pathogens.
- **Trogocytosis:** the transfer of plasma membrane fragments from target cell to the effector cell.
- **Uncomplicated malaria:** symptomatic malaria without signs of severity or evidence of vital organ dysfunction.
- **V(D)J recombination:** a unique mechanism of genetic recombination that occurs only during the early stages of T- and B-cell maturation.
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References

1. Ackerman, M.E. et al. (2016) Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control. PLoS Pathog 12 (1), e1005315.
2. Chung, A.W. et al. (2015) Dissecting Polyclonal Vaccine-Induced Humoral Immunity against HIV Using Systems Serology. Cell 163 (4), 988-98.
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 (4), 443-50.
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 (6), 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 (228), 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, 388-96.
7. Vidarsson, G. et al. (2001) Activity of human IgG and IgA subclasses in immune defense against Neisseria meningitidis serogroup B. J Immunol 166 (10), 6250-6.
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 (8), 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 (10), 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 (6), 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 (3), 1165-74.
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 Medicine 12 (108).
14. Lefranc, G. and Lefranc, M.-P. (2001) The Immunoglobulin FactsBook, Academic Press.
15. Pumphrey, R.S.H. (1986) Computer models of the human immunoglobulins: Binding sites and molecular interactions. Immunol Today 7 (7-8), 206-11.
16. Carrasco, B. et al. (2001) Crystallohydrodynamics for solving the hydration problem for multi-domain proteins: open physiological conformations for human IgG. Biophys Chem 93 (2-3), 181-96.
17. Vidarsson, G. et al. (2014) IgG subclasses and allotypes: from structure to effector functions. Front Immunol 5 (520).
18. Roux, K.H. et al. (1997) Flexibility of human IgG subclasses. J Immunol 159 (7), 3372-82.
19. Bruhns, P. (2012) Properties of mouse and human IgG receptors and their contribution to disease models. Blood 14 (119), 5640-9.
20. Chan, Y.N. et al. (2016) IgG Binding Characteristics of Rhesus Macaque FcgammaR. J Immunol 197 (7), 2936-47.

21. Gaarder, P.I. and Natvig, J.B. (1974) Distribution of isotypic and allotypic human IgG antigens in non-human primates. J Immunol 113 (2), 635-44.
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, 635-80.
23. Bruhns, P. et al. (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113 (16), 3716-25.
24. 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 (599).
25. 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 (9), 6098-109.
26. Natsume, A. et al. (2008) Engineered antibodies of IgG1/IgG3 mixed isotype with enhanced cytotoxic activities. Cancer Res 68 (10), 3863-72.
27. Zwolak, A. et al. (2017) Rapid Purification of Human Bispecific Antibodies via Selective Modulation of Protein A Binding. Sci Rep 7 (1), 15521.
28. Mahan, A.E. et al. (2016) Antigen-Specific Antibody Glycosylation Is Regulated via Vaccination. PLoS Pathog 12, e1005456.
29. van de Bovenkamp, F.S. et al. (2018) Adaptive antibody diversification through N-linked glycosylation of the immunoglobulin variable region. PNAS 115 (8), 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 (12-13), 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 (8), 889-98.
32. Subedi, G.P. and Barb, A.W. (2015) The structural role of antibody N-glycosylation in receptor interactions. Structure 23 (9), 1573-83.
33. 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 (34), 10611-6.
34. Wang, T.T. et al. (2017) IgG antibodies to dengue enhanced for FcγRIIIA binding determine disease severity. Science 355 (6323), 369-8.
35. 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 (1-2), 151-60.
36. Chung, A.W. et al. (2014) Identification of antibody glycosylation structures that predict monoclonal antibody Fc-effector function. AIDS 28 (17), 2523-30.
37. Ho, C.H. et al. (2015) Aberrant Serum Immunoglobulin G Glycosylation in Chronic Hepatitis B Is Associated With Histological Liver Damage and Reversible by Antiviral Therapy. J Infect Dis 211 (1), 115-124.
38. Gardinassi, L.G. et al. (2014) Clinical Severity of Visceral Leishmaniasis Is Associated with Changes in Immunoglobulin G Fc N-Glycosylation. mBio 5 (6), e01844-14.
39. Lu, L.L. et al. (2016) A Functional Role for Antibodies in Tuberculosis. Cell 167, 433-43.
40. Vadrevu, S.K. et al. (2018) Frontline Science: Plasma and immunoglobulin G galactosylation associate with HIV persistence during antiretroviral therapy. J Leukoc Biol 104 (3), 461-471.
41. Quast, I. et al. (2015) Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity. J Clin Invest 125 (11), 4160-70.
42. Kaneko, Y. et al. (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313 (5787), 670-3.

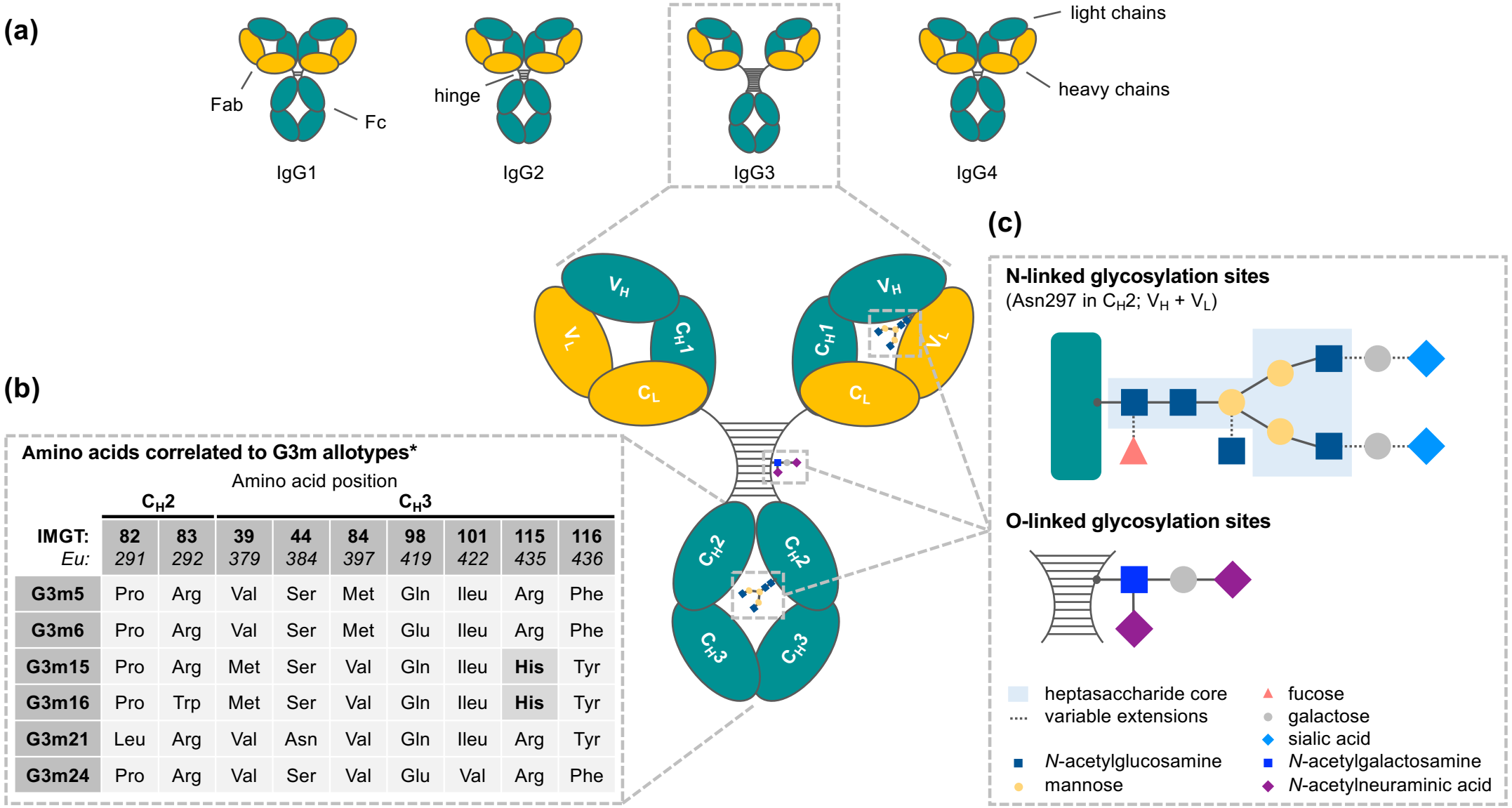
720 43. van de Bovenkamp, F.S. et al. (2018) Variable Domain N-linked glycans acquired During
721 antigen-specific immune responses can contribute to immunoglobulin G antibody stability.
722 Front Immunol 9.
723 44. Plomp, R. et al. (2015) Hinge-Region O-Glycosylation of Human Immunoglobulin G3
724 (IgG3). Mol Cell Proteomics 14 (5), 1373-84.
725 45. Julenius, K. et al. (2005) Prediction, conservation analysis, and structural characterization
726 of mammalian mucin-type O-glycosylation sites. Glycobiology 15 (2), 153-64.
727 46. Ferrante, A., L. et al. (1990) IgG subclass distribution of antibodies to bacterial and viral
728 antigens. Pediatr Infect Dis J 9 (16).
729 47. Schauer, U. et al. (2003) Levels of Antibodies Specific to Tetanus Toxoid, Haemophilus
730 influenzae Type b, and Pneumococcal Capsular Polysaccharide in Healthy Children and
731 Adults. Clin Diagn Lab Immunol 10 (2), 202-7.
732 48. Nouri-Aria, K.T. et al. (2004) Grass pollen immunotherapy induces mucosal and
733 peripheral IL-10 responses and blocking IgG activity. J Immunol 172 (5), 3252-9.
734 49. Arnold, J.N. et al. (2006) Mannan binding lectin and its interaction with
735 immunoglobulins in health and in disease. Immunol Lett 106, 103-10.
736 50. Malhotra, R. et al. (1995) Glycosylation changes of IgG associated with rheumatoid
737 arthritis can activate complement via the mannose-binding protein. Nat Med 1 (3), 237-43.
738 51. Lu, Y.L. et al. (2007) Solution conformation of wild-type and mutant IgG3 and IgG4
739 immunoglobulins using crystallohydrodynamics: Possible implications for complement
740 activation. Biophys J 93 (11), 3733-3744.
741 52. Thommesen, J.F. et al. (2000) Lysine 322 in the human IgG3 CH2 domain is crucial for
742 antibody dependent complement activation. Mol Immunol 37, 995-1004.
743 53. Kapur, R. et al. (2014) IgG-effector functions: "The Good, The Bad and The Ugly".
744 Immunol Lett 160 (2), 139-144.
745 54. McLean, M.R. et al. (2017) Dimeric Fc gamma Receptor Enzyme-Linked
746 Immunosorbent Assay To Study HIV-Specific Antibodies: A New Look into Breadth of Fc
747 gamma Receptor Antibodies Induced by the RV144 Vaccine Trial. J Immunol 199 (2), 816-
748 826.
749 55. Chung, A.W. et al. (2011) Activation of NK cells by ADCC antibodies and HIV disease
750 progression. J Acquir Immune Defic Syndr 58 (2), 127-31.
751 56. Joos, C. et al. (2015) Antibodies to Plasmodium falciparum merozoite surface protein-
752 1p19 malaria vaccine candidate induce antibody-dependent respiratory burst in human
753 neutrophils. Malar J 14, 409.
754 57. Firan, M. et al. (2001) The MHC class I-related receptor, FcRn, plays an essential role in
755 the maternofetal transfer of γ-globulin in humans. Int Immunol 13 (8), 993-1002.
756 58. Pyzik, M. et al. (2015) FcRn: The Architect Behind the Immune and Nonimmune
757 Functions of IgG and Albumin. J Immunol 194 (10), 4595-603.
758 59. Shah, I.S. et al. (2017) Structural characterization of the Man5 glycoform of human IgG3
759 Fc. Mol Immunol 92, 28-37.
760 60. Einarsdottir, H. et al. (2014) H435-containing immunoglobulin G3 allotypes are
761 transported efficiently across the human placenta: implications for alloantibody-mediated
762 diseases of the newborn. Transfusion 54 (3), 665-71.
763 61. Dugast, A.S. et al. (2014) Independent evolution of Fc- and Fab-mediated HIV-1-specific
764 antiviral antibody activity following acute infection. Eur J Immunol 44 (10), 2925-37.
765 62. Posadas-Mondragón, A. et al. (2017) Indices of anti-dengue immunoglobulin G
766 subclasses inadult Mexican patients with febrile and hemorrhagicdengue in the acute phase.
767 Mircobiol Immunol 61, 433-41.
768 63. Yates, N.L. et al. (2011) Multiple HIV-1-specific IgG3 responses decline during acute
769 HIV-1: implications for detection of incident HIV infection. AIDS 25 (17), 2089-97.
770 64. Horns, F. et al. (2016) Lineage tracing of human B cells reveals the in vivo landscape of
771 human antibody class switching. Elife 5.

65. 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.
66. 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 (9), 5154-7.
67. Malisan, F. et al. (1996) Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytes. J Exp Med 183 (3), 937-47.
68. 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 (2), 757-62.
69. 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 (5), 2318-28.
70. 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 (14), 6558-65.
71. Richardson, S. et al., IgG3 Hinge Length Enhances Neutralization Potency and Fc Effector Function of an HIV V2-specific Broadly Neutralizing Antibody, HIV Research for Prevention, Madrid, 2018.
72. Tay, M.Z. et al. (2016) Antibody-Mediated Internalization of Infectious HIV-1 Virions Differs among Antibody Isotypes and Subclasses. PLoS Pathog 12 (8), e1005817.
73. Kardava, L. et al. (2018) IgG3 regulates tissue-like memory B cells in HIV-infected individuals. Nat Immunol.
74. Richardson, S.I. et al. (2018) HIV-specific Fc effector function early in infection predicts the development of broadly neutralizing antibodies. PLoS Pathog 14 (4), e1006987.
75. 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 (7), 1147-54.
76. Syenina, A. et al. (2015) Dengue vascular leakage is augmented by mast cell degranulation mediated by immunoglobulin Fcγ receptors. eLife 4, e05291.
77. Rodrigo, W.W. et al. (2010) Dengue virus neutralization is modulated by IgG antibody subclass and Fcγ receptor subtype. Virology 394 (2), 175-82.
78. Ayala-Nunez, N.V. et al. (2016) How antibodies alter the cell entry pathway of dengue virus particles in macrophages. Sci Rep 6 (28768).
79. Wang, T.T. et al. (2017) IgG antibodies to dengue enhanced for FcgammaRIIIA binding determine disease severity. Science 355 (6323), 395-398.
80. Kuzmina, N.A. et al. (2018) Antibody-Dependent Enhancement of Ebola Virus Infection by Human Antibodies Isolated from Survivors. Cell Rep 24 (7), 1802-1815 e5.
81. 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 (1), 74-83.
82. 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 (1), 48-55.
83. 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 (3), 449-55.
84. Cai, Y. et al. (2014) Increased complement C1q level marks active disease in human tuberculosis. PLoS One 9 (3), 92340.
85. 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 (8), 50-60.

86. 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 (11), e320.
87. Mathiesen, L. et al. (2013) Maternofetal trans-placental transport of recombinant IgG antibodies lacking effector functions. Blood 122 (7), 1174-81.
88. 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 (3), 1143-52.
89. 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 (3), 580-90.
90. 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 (3), e9871.
91. Larsen, M.D. et al., Malaria parasite evasion of classical complement pathway European Meeting on Complement in Human Disease, 2017, Molecular Immunology, 2017, p. 159.
92. 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 (1), 61.
93. 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.
94. 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 (9), 1801-11.
95. Yilmaz, B. et al. (2014) Gut Microbiota Elicits a Protective Immune Response against Malaria Transmission. Cell 159 (6), 1277-89.
96. 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 (9999), DOI:10.1038/s41598-018-28325-w.
97. 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 (1), e53948.
98. 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 (12), 1892-5.
99. 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.
100. 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 (267-9).
101. 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).
102. Kaplon, H.K. and Reichert, J.M. (2018) Antibodies to watch in 2019. MAbs.
103. Synagis (palivizumab). Full prescribing information, MedImmune, LLC, Gaithersburg, MD, 2014.
104. Mazumdar, S. (2009) Raxibacumab. MAbs 1 (6), 531-8.
105. Weinheimer, S. et al., Ibalizumab susceptibility in patient HIV isolates resistant to antiretrovirals, 25th Conference on Retroviruses and Opportunistic Infections (CROI 2018), Boston, US, 2018.

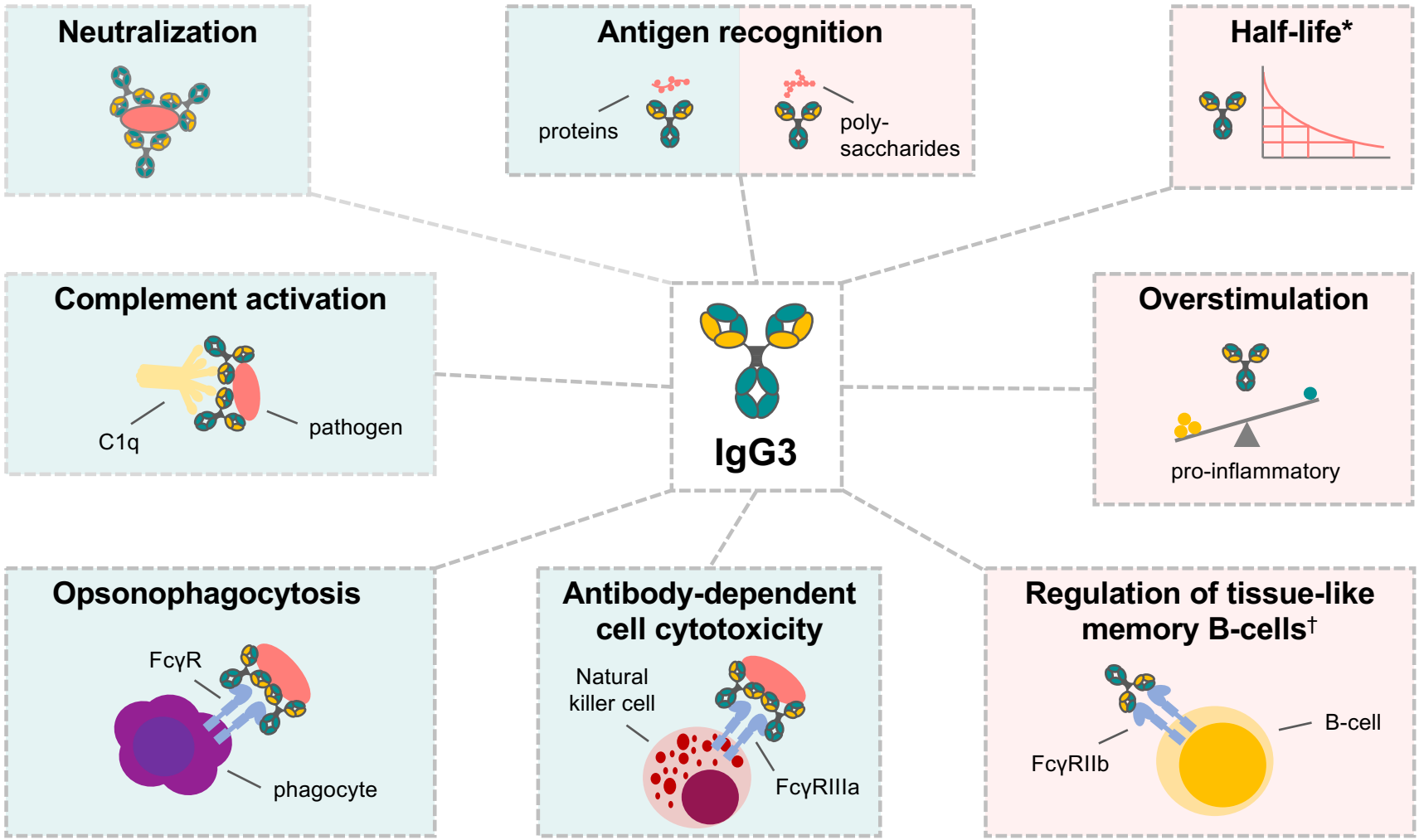
106. Wu, Y. et al. (2014) IMGT/mAb-DB: The IMGT® Database for Therapeutic Monoclonal Antibodies. (accessed 14/2/2018).
107. Desoubeaux, G. et al., Therapeutic antibodies and infectious diseases, Tours, France, November 20−22, 2012, mAbs, 2013, pp. 626-32.
108. Lakbub, J.C. et al. (2016) Disulfide Bond Characterization of Endogenous IgG3 Monoclonal Antibodies Using LC-MS: An Investigation of IgG3 Disulfide-mediated Isoforms. Anal Methods 8 (31), 6045-55.
109. Magdelaine-Beuzelin, C. et al. (2009) IgG1 heavy chain-coding gene polymorphism (G1m allotypes) and development of antibodies-to-infliximab. Pharmacogenet Genomics 19 (5), 383-7.
110. 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.
111. 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 (2), 253-63.
112. Ternant, D. et al. (2016) IgG1 Allotypes Influence the Pharmacokinetics of Therapeutic Monoclonal Antibodies through FcRn Binding. J Immunol 196 (2), 607-13.
113. Lazar, G.A. et al. (2006) Engineered antibody Fc variants with enhanced effector function. Proc Natl Acad Sci U S A 103 (11), 4005-10.
114. Moore, G.L. et al. (2010) Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. MAbs 2 (2), 181-9.
115. Huang, Y. et al. (2016) Engineered Bispecific Antibodies with Exquisite HIV-1-Neutralizing Activity. Cell 165 (7), 1621-31.
116. Mahlangu, J. et al. (2018) Emicizumab Prophylaxis in Patients Who Have Hemophilia A without Inhibitors. N Engl J Med 379, 811-22.
117. 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 (7), 531-537.
118. Cooper, P.J. et al. (1999) Human Onchocerciasis and Tetanus Vaccination: Impact on the Postvaccination Antitetanus Antibody Response. Infect Immun 67 (11), 5951-7.
119. Garcia, G. et al. (2006) Antibodies from patients with dengue viral infection mediate cellular cytotoxicity. J Clin Virol 37 (1), 53-7.
120. 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.
121. Nimmerjahn, F. et al. (2005) Fc gamma RIV: A novel FcR with distinct IgG subclass specificity. Immunity 23 (1), 41-51.
122. Murphy, A.J. et al. (2014) Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. PNAS 111 (14), 5153-5158.
123. Smith, P. et al. (2012) Mouse model recapitulating human Fcgamma receptor structural and functional diversity. Proc Natl Acad Sci U S A 109 (16), 6181-6.
124. Trist, H.M. et al. (2014) Polymorphisms and Interspecies Differences of the Activating and Inhibitory Fc γRII of Macaca nemestrina Influence the Binding of Human IgG Subclasses. J Immunol 192, 792-803.
125. 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 (4), 623-30.
126. 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 (3), 185-203.
127. Lefranc, M.P. et al. (2009) IMGT, the international ImMunoGeneTics information system. Nucleic Acids Res 37 (Database issue), D1006-12.

926 128. Plomp, R. et al. (2017) Subclass-specific IgG glycosylation is associated with markers
927 of inflammation and metabolic health. Scientific Reports 7.
928 129. Ackerman, M.E. et al. (2013) Natural variation in Fc glycosylation of HIV-specific
929 antibodies impacts antiviral activity. J. Clin. Investig 123, 2183-92.
930



Amino acids correlated to G3m allotypes*

	Amino acid position								
	C_H2		C_H3						
IMGT: *Eu:*	**82** *291*	**83** *292*	**39** *379*	**44** *384*	**84** *397*	**98** *419*	**101** *422*	**115** *435*	**116** *436*
G3m5	Pro	Arg	Val	Ser	Met	Gln	Ileu	Arg	Phe
G3m6	Pro	Arg	Val	Ser	Met	Glu	Ileu	Arg	Phe
G3m15	Pro	Arg	Met	Ser	Val	Gln	Ileu	**His**	Tyr
G3m16	Pro	Trp	Met	Ser	Val	Gln	Ileu	**His**	Tyr
G3m21	Leu	Arg	Val	Asn	Val	Gln	Ileu	Arg	Tyr
G3m24	Pro	Arg	Val	Ser	Val	Glu	Val	Arg	Phe


Neutralization
Antigen recognition
proteins
poly-
saccharides
Half-life*
Complement activation
C1q
pathogen
IgG3
Overstimulation
pro-inflammatory
Opsonophagocytosis
FcγR
phagocyte
Antibody-dependent
cell cytotoxicity
Natural
killer cell
FcγRIIIa
Regulation of tissue-like
memory B-cells†
FcγRIIb
B-cell