The Role of IgG3 in Infectious Diseases

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

**Department of Microbiology and Immunology, [†]Department of Medicine, both at The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia*

Correspondence: awchung@unimelb.edu.au

Keywords: IgG3, antibody, complement, ADCC, glycosylation, allotypes,

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 α 1,6-fucosyltransferase gene knockout Chinese hamster ovary cell lines, which lack the ability to attach fucose to asparagine 297 of antibodies, have increased binding to Fc γ RIIIa, 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 binding 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.

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)**, **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 (Fig. 2) [46, 53]. The efficiency of monomeric IgG3 binding to FcγRIIa, FcγRIIIa, and FcγRIIIb on effector cells such as neutrophils, monocytes, macrophages or natural killer (NK) is even higher than of monomeric IgG1 (Tab. 1) [23]. Thus, the interaction of IgG3 with FcγRs on these effector cells has been increasingly recognized as a critical immune response against a range of infectious diseases including viruses, bacteria and parasitic pathogens [1, 34, 39, 54-56].

FcRn binding

The interaction between IgG and **neonatal Fc receptors (FcRn)** is important for IgG transplacental passage of IgG Abs and Ab half-life in humans [57, 58]. 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 [59]. FcRn-mediated transport of IgG3 is inhibited with the presence of IgG1 due to intracellular competition [24]. Therefore, the unbound IgG3 is degraded and not returned into circulation, which in part may explain its shorter half-life in most individuals [24]. However, IgG3 affinity to FcRn is modified by an amino acid substitution at position 435, where IgG3 has an arginine instead of the histidine found in all other subclasses [24]. The R435 side chain stays positively charged at physiological pH and can still maintain ionic interactions with 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].

226 During early HIV infection, functional Ab responses decline drastically with HIV
227 disease progression, which is correlated with waning IgG3 Ab concentrations [3, 61,
228 63]. In HIV-1 neutralization studies, polyclonal IgG3 Abs are more potent than any
229 other subclass [70], with increased length of the hinge region enhancing neutralization
230 potency and significantly improved phagocytosis and **trogocytosis** activity *in vitro*
231 compared to IgG1 [71]. Moreover, Env-binding monoclonal IgG3 Abs are more
232 effective at internalizing viral particles compared to Env-binding IgG1 of the same
233 specificity [72]. Thus, the maintenance of IgG3 Abs could be useful for both prevention
234 of HIV infection as well as for antiviral control. However, a recent study also identified
235 IgG3 as a regulator of IgM⁺ tissue-like memory (TLM) B-cells, where IgG3⁺IgM⁺ TLM
236 B-cells from chronic HIV individuals, but not aviremic individuals, observed reduced
237 sensitivity to B cell receptor stimulation compared to their IgG3⁻IgM⁺ counterparts [73].
238 This modulation of IgM⁺ B-cell stimulation was reported to occur through multiple
239 mechanisms including, direct co-localization of IgG3 with IgM, along with interactions
240 with FcRIIb (inhibitory FcR), C1q and **C-reactive protein** [73]. IgG3 binding to the IgM-B-
241 cell receptors could also arise in the setting of persistent HIV viremia and contribute
242 to refractory IgM⁺ B-cell stimulation during chronic infection [73]. Furthermore, a recent
243 HIV cohort study also reported that IgG Fc polyfunctionality (ADCC, ADCP and
244 antibody dependant trogocytosis) and greater subclass diversity (i.e. induction of all
245 IgG subclasses and not IgG3 alone) were associated with the development of **broadly**
246 **neutralizing Abs (bNAbs)** compared to HIV-individuals that failed to develop bNAbs
247 [74]. Further studies are needed to determine the advantages and disadvantages of
248 IgG3 responses during HIV infections, as well as any putative therapeutic applications,
249 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

the predominant human Ab subclasses formed against a *Mycobacterium tuberculosis* (*Mtb*) infection [82]. They stimulate the release of tumor necrosis factor (TNF) from primary monocytes [83], enhance bacterial uptake by macrophages and form complement immune complexes, which remain elevated during active TB, and furthermore, have been associated with increased disease severity [84]. As a result, excessive IgG1 and IgG3 subclasses might potentially cause an excessive pro-inflammatory response, which in the case of active TB may be more harmful than protective [84]. 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 like *Plasmodium falciparum* are IgG1 and IgG3 [85]. IgG3 is linked to protective immunity against malaria [11, 12, 85, 86], due to opsonisation of malaria-infected erythrocytes and promotion of Ab-dependent effector functions [10, 13, 87, 88]. In addition, they can induce complement deposition at the merozoite stage of the parasite, thus mediating inhibition of erythrocyte invasion [89-91]. IgG3-induced CDC is also associated with anti-plasmodium sporozoite immunity in children [92]. In regards to *Plasmodium vivax* merozoites, the predominant Abs produced are also IgG1 and IgG3 [93, 94]. Moreover, Abs against the classical α -Gal (Gal α 1-3Gal β 1-4GlcNAc-R) epitopes have correlated with protection against *Plasmodium* spp. infections, reducing malaria transmission by *Anopheles* mosquitoes [95]. In addition, higher α -Gal IgG3, but also

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

326

327 IgG3 allotypes may also play a role in malaria susceptibility or protection. The
328 combination of homozygous G3m6 allotype and Km3 may protect against placental
329 malaria [97] and children with these allotypes have been reported to be better
330 protected against **uncomplicated malaria** than non-carriers [98]. However, other
331 studies have suggested that G3m but not Km allotypes might influence host
332 susceptibility to malaria infection together with the Ab profile of the donors [99, 100].
333 Individuals with the G3m6 allotype had a higher incidence of uncomplicated malaria
334 than non-carriers, due to low baseline IgG3 concentrations and high baseline of non-
335 cytophilic subclasses [99]. Of note, G3m6 is predominantly found in African
336 populations, possibly as a result of differential evolutionary selection caused by
337 infectious diseases such as malaria [100]. Evidently, further studies are needed to
338 validate any conclusions regarding general protection or susceptibility.

339

340 In a recent study in Beninese women, the H435-IgG3 polymorphism (i.e. allotypes
341 G3m15 and G3m16 which potentiate binding of IgG3 to FcRn) increased the trans-
342 placental transfer and half-life of malaria-specific IgG3 in young infants compared with
343 non-carriers and was associated with reduced risk of clinical malaria during infancy
344 [9]. The H435-IgG3 allele is most common in Africans (30%–60%), suggesting a
345 possible role for positive selection, given its relatively high frequency in malaria
346 endemic areas [9]. However, the G3m15 and G3m16 allotypes have also been
347 associated with increased susceptibility to the autoantibody-mediated disease
348 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

demonstrated significantly better protection against *S. pneumoniae* than IgG1 and IgG3-R435, indicating that IgG3-H435 might be considered when devising future improved therapeutic approaches to treat infections *in vivo*, that aim to increase IgG3 serum longevity [24].

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, potentially by enhancing clearance of the thAb or in the worst-case scenario, by becoming immunogenic and precipitating severe adverse reactions [109]. However, studies have now demonstrated that allotypic differences between IgG1 thAbs do not appear to produce novel T-cell epitopes in mismatched individuals, or induce ‘non-self’ anti-allotypic Abs and are unlikely to represent a significant risk factor for induction of immunogenicity [110, 111], but instead can improve the half-life of thAbs via FcRn binding [112]. Further studies, especially for IgG3 allotypes which have significantly greater polymorphisms and structural changes than IgG1, 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 [113, 114]. Mounting evidence has identified key mutations in the IgG1 Fc domain of thAbs that can enhance the affinity to FcγR or complement [113, 114]. 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 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

inflammatory responses in active TB [120].

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. Although there are significant differences between human and murine Abs in terms of structures, capacity to activate complement [19] as well as between FcγR structures and functions [121]. Thus, humanized mice which bear genetically engineered human Igs [122] and human FcγRs [123] might help overcome some of these limitations. Similarly, non-human primates harbour different IgG subclass binding properties compared to humans [124]. This creates an obvious difficulty in translating immune response findings in animal models to predicting human vaccination results, or thAb trials [20]. 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 the numerous studies highlighted herein [1-5, 9, 96, 125]. This ability is mainly due to the inherit unique molecular properties of IgG3 which can confer highly functional potent effector responses. Although numerous questions remain (see outstanding questions), increasing our molecular 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.

Acknowledgements

We thank Milla McClean and Ester Lopez for their assistance revising this manuscript. This work was supported by funding from the University of Melbourne (TD), National Health and Medical Research Council of Australia (NHMRC) (SJR, SJK, AWC), by the Australian Centre for Research Excellence in Malaria Elimination (ACREME) (SJR) and the American Foundation for AIDS Research (amfAR) Mathilde Krim Fellowship (AWC).

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]

523

524

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				

527

528

529

530

531

532

533

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

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 _H 3	European, North America, African and Asian
G3m6	G3m(c3)	G3m5, 6, 10, 11, 14, 26, 27	G3m6*	IGHG3*13	H-γ3 C _H 3	African
G3m15	G3m(s)	G3m10, 11, 13, 15, 27	G3m15*	IGHG3*17	H-γ3 C _H 3	Sub-Saharan with high frequencies in Khoisan
G3m16	G3m(t)	G3m10, 11, 13, 15, 16, 27	G3m16*	IGHG3*18, *19	H-γ3 C _H 2	most prevalent in North East Asians
G3m21	G3m(g1)	G3m21, 26, 27, 28	G3m21*	IGHG3*14, *15, *16	H-γ3 C _H 2	European, North American and Asian

G3m24	G3m(c5)	G3m5, 6, 11, 24, 26	G3m24*	IGHG3*03	H-γ3 C _H 3	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.
-

- 559 - **Bacteriolysis:** the destruction of bacterial cells, can often be mediated by
560 antibodies.
- 561 - **Bispecific antibody:** recombinant protein that can simultaneously bind to two
562 different types of antigens.
- 563 - **Broadly neutralizing antibody (bNAb):** special type of antibodies that can
564 recognize and block many strains of a particular virus from entering healthy
565 cells.
- 566 - **C-reactive protein:** a protein produced by the liver in response to inflammation
567 or infection.
- 568 - **Class switch recombination:** a process by which proliferating B-cells
569 rearrange the constant region genes in the immunoglobulin heavy chain
570 locus to switch from expressing one class of immunoglobulin to another.
- 571 - **C1q:** a protein complex involved in the complement system, which is part of the
572 innate immune system.
- 573 - **Complement activation:** part of the innate immune system, which is
574 responsible for a quick and non-specific clearance of pathogens by
575 attracting phagocytic cells.
- 576 - **Complement-dependent cytotoxicity (CDC):** C1q binds an antibody, which
577 triggers the complement cascade, as a result of the classical pathway
578 complement activation.
- 579 - **Envelope (Env) protein:** a protein expressed on the surface of enveloped
580 viruses.
- 581 - **Fab region:** domain on an antibody that binds to antigens, involved in
582 neutralization.

- **Fc region:** tail region of an antibody that determines innate immune effector function.
- **Fcy-receptors (FcyRs):** are surface protein receptors, defined as either activating receptors (FcyRI, FcyRIIa/c, FcyRIII) or inhibitory receptor (FcyRIIb) 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.

- 608 - **Toll-like receptors:** expressed on sentinel cells such as macrophages and
609 dendritic cells, that recognize structurally conserved molecules derived from
610 pathogens.
- 611 - **Trogocytosis:** the transfer of plasma membrane fragments from target cell
612 to the effector cell.
- 613 - **Uncomplicated malaria:** symptomatic malaria without signs of severity or
614 evidence of vital organ dysfunction.
- 615 - **V(D)J recombination:** a unique mechanism of genetic recombination that
616 occurs only during the early stages of T- and B-cell maturation.
- 617 -
618
619

620 **References**

- 621 1. Ackerman, M.E. et al. (2016) Polyfunctional HIV-Specific Antibody Responses Are
622 Associated with Spontaneous HIV Control. *PLoS Pathog* 12 (1), e1005315.
- 623 2. Chung, A.W. et al. (2015) Dissecting Polyclonal Vaccine-Induced Humoral Immunity
624 against HIV Using Systems Serology. *Cell* 163 (4), 988-98.
- 625 3. Sadanand, S. et al. (2018) Temporal variation in HIV-specific IgG subclass antibodies
626 during acute infection differentiates spontaneous controllers from chronic progressors. *AIDS*
627 32 (4), 443-50.
- 628 4. Yates, N.L. et al. (2014) Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1
629 infection risk and declines soon after vaccination. *Sci Transl Med* 19 (6), 228.
- 630 5. Chung, A.W. et al. (2014) Polyfunctional Fc-effector profiles mediated by IgG subclass
631 selection distinguish RV144 and VAX003 vaccines. *Sci Transl Med* 6 (228), 228ra38.
- 632 6. Aase, A. et al. (1998) Opsonophagocytic and Bactericidal Activity Mediated by Purified
633 IgG Subclass Antibodies After Vaccination with the Norwegian Group B Meningococcal
634 Vaccine. *Scand J Immunol* 47, 388-96.
- 635 7. Vidarsson, G. et al. (2001) Activity of human IgG and IgA subclasses in immune defense
636 against *Neisseria meningitidis* serogroup B. *J Immunol* 166 (10), 6250-6.
- 637 8. Giuntini, S. et al. (2016) Human IgG1, IgG3, and IgG3 Hinge-Truncated Mutants Show
638 Different Protection Capabilities against Meningococci Depending on the Target Antigen and
639 Epitope Specificity. *Clin Vaccine Immunol* 23 (8), 698-706.
- 640 9. Dechavanne, C. et al. (2017) Associations between an IgG3 polymorphism in the binding
641 domain for FcRn, transplacental transfer of malaria-specific IgG3, and protection against
642 *Plasmodium falciparum* malaria during infancy: A birth cohort study in Benin. *PLoS Med* 14
643 (10), e1002403.
- 644 10. Kana, I.H. et al. (2018) Cytophilic Antibodies Against Key *Plasmodium falciparum*
645 Blood Stage Antigens Contribute to Protection Against Clinical Malaria in a High
646 Transmission Region of Eastern India. *J Infect Dis* 218 (6), 956-965.
- 647 11. Stanisic, D.I. et al. (2009) Immunoglobulin G subclass-specific responses against
648 *Plasmodium falciparum* merozoite antigens are associated with control of parasitemia and
649 protection from symptomatic illness. *Infect Immun* 77 (3), 1165-74.
- 650 12. Weaver, R. et al. (2016) The association between naturally acquired IgG subclass specific
651 antibodies to the PfPRH5 invasion complex and protection from *Plasmodium falciparum*
652 malaria *Sci Rep* 6, 33094.
- 653 13. Osier, F.H.A. et al. (2014) Opsonic phagocytosis of *Plasmodium falciparum* merozoites:
654 mechanism in human immunity and a correlate of protection against malaria. *BMC Medicine*
655 12 (108).
- 656 14. Lefranc, G. and Lefranc, M.-P. (2001) *The Immunoglobulin FactsBook*, Academic Press.
- 657 15. Pumphrey, R.S.H. (1986) Computer models of the human immunoglobulins: Binding
658 sites and molecular interactions. *Immunol Today* 7 (7-8), 206-11.
- 659 16. Carrasco, B. et al. (2001) Crystallohydrodynamics for solving the hydration problem for
660 multi-domain proteins: open physiological conformations for human IgG. *Biophys Chem* 93
661 (2-3), 181-96.
- 662 17. Vidarsson, G. et al. (2014) IgG subclasses and allotypes: from structure to effector
663 functions. *Front Immunol* 5 (520).
- 664 18. Roux, K.H. et al. (1997) Flexibility of human IgG subclasses. *J Immunol* 159 (7), 3372-
665 82.
- 666 19. Bruhns, P. (2012) Properties of mouse and human IgG receptors and their contribution to
667 disease models. *Blood* 14 (119), 5640-9.
- 668 20. Chan, Y.N. et al. (2016) IgG Binding Characteristics of Rhesus Macaque FcγγR. *J*
669 *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 Fcγ 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. Rispen, 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 Fcγ 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.

43. 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.
44. Plomp, R. et al. (2015) Hinge-Region O-Glycosylation of Human Immunoglobulin G3 (IgG3). *Mol Cell Proteomics* 14 (5), 1373-84.
45. Julenius, K. et al. (2005) Prediction, conservation analysis, and structural characterization of mammalian mucin-type O-glycosylation sites. *Glycobiology* 15 (2), 153-64.
46. Ferrante, A., L. et al. (1990) IgG subclass distribution of antibodies to bacterial and viral antigens. *Pediatr Infect Dis J* 9 (16).
47. 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 (2), 202-7.
48. Nouri-Aria, K.T. et al. (2004) Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 172 (5), 3252-9.
49. Arnold, J.N. et al. (2006) Mannan binding lectin and its interaction with immunoglobulins in health and in disease. *Immunol Lett* 106, 103-10.
50. Malhotra, R. et al. (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat Med* 1 (3), 237-43.
51. 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 (11), 3733-3744.
52. 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.
53. Kapur, R. et al. (2014) IgG-effector functions: "The Good, The Bad and The Ugly". *Immunol Lett* 160 (2), 139-144.
54. 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 (2), 816-826.
55. Chung, A.W. et al. (2011) Activation of NK cells by ADCC antibodies and HIV disease progression. *J Acquir Immune Defic Syndr* 58 (2), 127-31.
56. Joos, C. et al. (2015) Antibodies to Plasmodium falciparum merozoite surface protein-1p19 malaria vaccine candidate induce antibody-dependent respiratory burst in human neutrophils. *Malar J* 14, 409.
57. 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 (8), 993-1002.
58. Pyzik, M. et al. (2015) FcRn: The Architect Behind the Immune and Nonimmune Functions of IgG and Albumin. *J Immunol* 194 (10), 4595-603.
59. Shah, I.S. et al. (2017) Structural characterization of the Man5 glycoform of human IgG3 Fc. *Mol Immunol* 92, 28-37.
60. 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 (3), 665-71.
61. 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 (10), 2925-37.
62. 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. *Microbiol Immunol* 61, 433-41.
63. 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 (17), 2089-97.
64. Horns, F. et al. (2016) Lineage tracing of human B cells reveals the in vivo landscape of human antibody class switching. *Elife* 5.

772 65. Kitaura, K. et al. (2017) Different Somatic Hypermutation Levels among Antibody
773 Subclasses Disclosed by a New Next-Generation Sequencing-Based Antibody Repertoire
774 Analysis. *Front Immunol* 8, 389.

775 66. Pene, J. et al. (2004) Cutting edge: IL-21 is a switch factor for the production of IgG1 and
776 IgG3 by human B cells. *J Immunol* 172 (9), 5154-7.

777 67. Malisan, F. et al. (1996) Interleukin-10 induces immunoglobulin G isotype switch
778 recombination in human CD40-activated naive B lymphocytes. *J Exp Med* 183 (3), 937-47.

779 68. Briere, F. et al. (1994) Human interleukin 10 induces naive surface immunoglobulin D+
780 (sIgD+) B cells to secrete IgG1 and IgG3. *J Exp Med* 179 (2), 757-62.

781 69. Fujieda, S. et al. (1995) IL-4 plus CD40 monoclonal antibody induces human B cells
782 gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, and gamma 4, but
783 not gamma 2. *J Immunol* 155 (5), 2318-28.

784 70. Scharf, O. et al. (2001) Immunoglobulin G3 from Polyclonal Human Immunodeficiency
785 Virus (HIV) Immune Globulin Is More Potent than Other Subclasses in Neutralizing HIV
786 Type 1. *J Virol* 75 (14), 6558-65.

787 71. Richardson, S. et al., IgG3 Hinge Length Enhances Neutralization Potency and Fc
788 Effector Function of an HIV V2-specific Broadly Neutralizing Antibody, HIV Research for
789 Prevention, Madrid, 2018.

790 72. Tay, M.Z. et al. (2016) Antibody-Mediated Internalization of Infectious HIV-1 Virions
791 Differs among Antibody Isotypes and Subclasses. *PLoS Pathog* 12 (8), e1005817.

792 73. Kardava, L. et al. (2018) IgG3 regulates tissue-like memory B cells in HIV-infected
793 individuals. *Nat Immunol*.

794 74. Richardson, S.I. et al. (2018) HIV-specific Fc effector function early in infection predicts
795 the development of broadly neutralizing antibodies. *PLoS Pathog* 14 (4), e1006987.

796 75. Kam, Y.-W. et al. (2012) Early Appearance of Neutralizing Immunoglobulin G3
797 Antibodies is Associated with Chikungunya Virus Clearance and Long-term Clinical
798 Protection. *J Infect Dis* 205 (7), 1147-54.

799 76. Syenina, A. et al. (2015) Dengue vascular leakage is augmented by mast cell
800 degranulation mediated by immunoglobulin Fcγ receptors. *eLife* 4, e05291.

801 77. Rodrigo, W.W. et al. (2010) Dengue virus neutralization is modulated by IgG antibody
802 subclass and Fcγ receptor subtype. *Virology* 394 (2), 175-82.

803 78. Ayala-Nunez, N.V. et al. (2016) How antibodies alter the cell entry pathway of dengue
804 virus particles in macrophages. *Sci Rep* 6 (28768).

805 79. Wang, T.T. et al. (2017) IgG antibodies to dengue enhanced for FcγRIIIA binding
806 determine disease severity. *Science* 355 (6323), 395-398.

807 80. Kuzmina, N.A. et al. (2018) Antibody-Dependent Enhancement of Ebola Virus Infection
808 by Human Antibodies Isolated from Survivors. *Cell Rep* 24 (7), 1802-1815 e5.

809 81. Goh, Y.S. et al. (2011) Human IgG isotypes and activating Fcγ receptors in the
810 interaction of *Salmonella enterica* serovar Typhimurium with phagocytic cells. *Immunology*
811 133 (1), 74-83.

812 82. Sousa, A.O. et al. (1998) IgG subclass distribution of antibody responses to protein and
813 polysaccharide mycobacterial antigens in leprosy and tuberculosis patients. *Clin Exp*
814 *Immunol* 111 (1), 48-55.

815 83. Hussain, R. et al. (2000) PPD-specific IgG1 antibody subclass upregulate tumour necrosis
816 factor expression in PPD-stimulated monocytes: possible link with disease pathogenesis in
817 tuberculosis. *Clin Exp Immunol* 119 (3), 449-55.

818 84. Cai, Y. et al. (2014) Increased complement C1q level marks active disease in human
819 tuberculosis. *PLoS One* 9 (3), 92340.

820 85. Richards, J.S. et al. (2010) Association between naturally acquired antibodies to
821 erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and
822 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-3 α (PvMSP-3 α) 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.

875 106. Wu, Y. et al. (2014) IMGT/mAb-DB: The IMGT® Database for Therapeutic
876 Monoclonal Antibodies. (accessed 14/2/2018).

877 107. Desoubeaux, G. et al., Therapeutic antibodies and infectious diseases, Tours, France,
878 November 20–22, 2012, mAbs, 2013, pp. 626-32.

879 108. Lakbub, J.C. et al. (2016) Disulfide Bond Characterization of Endogenous IgG3
880 Monoclonal Antibodies Using LC-MS: An Investigation of IgG3 Disulfide-mediated
881 Isoforms. *Anal Methods* 8 (31), 6045-55.

882 109. Magdelaine-Beuzelin, C. et al. (2009) IgG1 heavy chain-coding gene polymorphism
883 (G1m allotypes) and development of antibodies-to-infliximab. *Pharmacogenet Genomics* 19
884 (5), 383-7.

885 110. Bartelds, G.M. et al. (2010) Surprising negative association between IgG1 allotype
886 disparity and anti-adalimumab formation: a cohort study. *Arthritis Res Ther* 12, R221.

887 111. Webster, C.I. et al. (2016) A comparison of the ability of the human IgG1 allotypes
888 G1m3 and G1m1,17 to stimulate T-cell responses from allotype matched and mismatched
889 donors. *MABs* 8 (2), 253-63.

890 112. Ternant, D. et al. (2016) IgG1 Allotypes Influence the Pharmacokinetics of Therapeutic
891 Monoclonal Antibodies through FcRn Binding. *J Immunol* 196 (2), 607-13.

892 113. Lazar, G.A. et al. (2006) Engineered antibody Fc variants with enhanced effector
893 function. *Proc Natl Acad Sci U S A* 103 (11), 4005-10.

894 114. Moore, G.L. et al. (2010) Engineered Fc variant antibodies with enhanced ability to
895 recruit complement and mediate effector functions. *MABs* 2 (2), 181-9.

896 115. Huang, Y. et al. (2016) Engineered Bispecific Antibodies with Exquisite HIV-1-
897 Neutralizing Activity. *Cell* 165 (7), 1621-31.

898 116. Mahlangu, J. et al. (2018) Emicizumab Prophylaxis in Patients Who Have Hemophilia A
899 without Inhibitors. *N Engl J Med* 379, 811-22.

900 117. Robb, M.L. et al. (2012) Risk behaviour and time as covariates for efficacy of the HIV
901 vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the
902 Thai phase 3 efficacy trial RV 144. *Lancet Infect Dis* 12 (7), 531-537.

903 118. Cooper, P.J. et al. (1999) Human Onchocerciasis and Tetanus Vaccination: Impact on
904 the Postvaccination Antitetanus Antibody Response. *Infect Immun* 67 (11), 5951-7.

905 119. Garcia, G. et al. (2006) Antibodies from patients with dengue viral infection mediate
906 cellular cytotoxicity. *J Clin Virol* 37 (1), 53-7.

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

909 121. Nimmerjahn, F. et al. (2005) Fc gamma RIV: A novel FcR with distinct IgG subclass
910 specificity. *Immunity* 23 (1), 41-51.

911 122. Murphy, A.J. et al. (2014) Mice with megabase humanization of their immunoglobulin
912 genes generate antibodies as efficiently as normal mice. *PNAS* 111 (14), 5153-5158.

913 123. Smith, P. et al. (2012) Mouse model recapitulating human Fc gamma receptor structural
914 and functional diversity. *Proc Natl Acad Sci U S A* 109 (16), 6181-6.

915 124. Trist, H.M. et al. (2014) Polymorphisms and Interspecies Differences of the Activating
916 and Inhibitory Fc γRII of Macaca nemestrina Influence the Binding of Human IgG
917 Subclasses. *J Immunol* 192, 792-803.

918 125. Kana, I.H. et al. (2017) Naturally acquired antibodies target the glutamate-rich protein
919 on intact merozoites and predict protection against febrile malaria. *J Infect Dis* 215 (4), 623-
920 30.

921 126. Lefranc, M.P. et al. (2005) IMGT unique numbering for immunoglobulin and T cell
922 receptor constant domains and Ig superfamily C-like domains. *Dev Comp Immunol* 29 (3),
923 185-203.

924 127. Lefranc, M.P. et al. (2009) IMGT, the international ImMunoGeneTics information
925 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

(a)

(b)

Amino acids correlated to G3m allotypes*

Amino acid position

	C _H 2					C _H 3				
	82	83	39	44	84	98	101	115	116	
IMGT:	291	292	379	384	397	419	422	435	436	
Eu:	291	292	379	384	397	419	422	435	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	

(c)

N-linked glycosylation sites

(Asn297 in C_H2; V_H + V_L)

O-linked glycosylation sites

heptasaccharide core
 variable extensions
 N-acetylglucosamine
 mannose
 fucose
 galactose
 sialic acid
 N-acetylgalactosamine
 N-acetylneuraminic acid

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Damelang, T;Rogerson, SJ;Kent, SJ;Chung, AW

Title:

Role of IgG3 in Infectious Diseases

Date:

2019-03-01

Citation:

Damelang, T., Rogerson, S. J., Kent, S. J. & Chung, A. W. (2019). Role of IgG3 in Infectious Diseases. *TRENDS IN IMMUNOLOGY*, 40 (3), pp.197-211. <https://doi.org/10.1016/j.it.2019.01.005>.

Persistent Link:

<http://hdl.handle.net/11343/284299>