

INVITED REVIEW

Heterogeneity in IgG-CD16 signaling in infectious disease outcomes*

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Abstract

In this review, we discuss how IgG antibodies can modulate inflammatory signaling during viral infections with a focus on CD16a-mediated functions. We describe the structural heterogeneity of IgG antibody ligands, including subclass and glycosylation that impact binding by and downstream activity of CD16a, as well as the heterogeneity of CD16a itself, including allele and expression density. While inflammation is a mechanism required for immune homeostasis and resolution of acute infections, we focus here on two infectious diseases that are driven by pathogenic inflammatory responses during infection. Specifically, we review and discuss the evolving body of literature showing that afucosylated IgG immune complex signaling through CD16a contributes to the overwhelming inflammatory response that is central to the pathogenesis of severe forms of dengue disease and coronavirus disease 2019 (COVID-19).

KEYWORDS

COVID-19, dengue disease, effector functions, Fc afucosylation, IgG1 antibodies

1 | INTRODUCTION

Immunoglobulin G (IgG) antibodies provide a dominant form of defense against infectious diseases. IgGs are composed of two distinct structural domains that enable coupling of the recognition of pathogens to cellular responses including both adaptive and innate immune functions. This is achieved through direct binding of pathogens by the IgG Fab domain to form antigen-IgG immune complexes. These immune complexes, in turn, direct the inflammatory response elicited during an infection through interactions with Fc

gamma receptors (FcγRs) on immune cells. FcγRs are expressed on a variety of immune cells and serve as conduits for crosstalk between the adaptive and innate arms of the immune system (Figure 1A). Depending on the cell type and the specific FcγRs engaged, FcγRs transduce signaling that can escalate or limit the degree of the inflammatory response to an ongoing infection.

Viral infections are controlled through a combination of adaptive and innate pathways, including virus neutralization, IgG Fc-mediated effector/inflammatory cellular functions and innate antiviral pathways such as the Type I interferon system.^{1,2} Upon viral infection,

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IgG-mediated effector control is initiated when viral particles are bound by reactive antibodies to form high-valence immune complexes that stabilize the otherwise low-affinity interactions between IgG Fc and Fc γ R-expressing cells. Some viral immune complexes may be neutralizing, thus preventing direct infection of host cells by viral particles. In the absence of neutralizing antibodies, immune complexes form that can both trigger Fc γ R-mediated functions and remain infectious. Neutralizing and non-neutralizing viral immune complexes as well as IgG-bound viral debris may activate Fc γ R-expressing host cells to initiate cellular processes that facilitate the resolution of infections. Clearance of immune complexes formed from viral particles and debris is critical for Fc γ R-driven homeostatic mechanisms and persistence of immune complexes can drive prolonged activation of Fc γ R-expressing cells leading to inflammatory sequelae and disease.³⁻⁵ Once control of an infection is established and the viral load wanes, decreasing amounts of viral antigen are available to form immune complexes and perpetuate the inflammatory response. Consequently, antibody-dependent immune activation and inflammation decrease as the antigen is cleared and immune homeostasis is restored.

In this review, we discuss how IgGs can modulate inflammatory signaling during viral infections with a focus on CD16a-mediated functions. While inflammation is a mechanism required for immune homeostasis and resolution of acute infections, we focus here on two infectious diseases that are driven by pathogenic inflammatory responses during infection. Specifically, we review and discuss the evolving body of literature showing that afucosylated IgG immune complex signaling through CD16a contributes to the overwhelming inflammatory response that is central to the pathogenesis of severe forms of dengue disease and coronavirus disease 2019 (COVID-19).

2 | MECHANISMS GOVERNING HETEROGENEITY IN ANTIBODY EFFECTOR FUNCTIONS IN VIVO

IgG antibody effector functions are determined by the ratio of activating to inhibitory (A/I) signaling transduced through Fc γ Rs on the surface of immune cells following their engagement by immune complexes. The A/I signaling ratio, first articulated by Ravetch and colleagues, governs the maturation of effector cells and is a major determinant of protection mediated by IgG antibodies.⁶⁻⁹ A/I signal transduction is impacted by variables within both host IgG Fc and Fc γ R repertoires. Studies in the last decade have revealed a tremendous amount of heterogeneity in human IgG Fc domain repertoires.^{10,11} These observations lead to the broader hypothesis that antibody heterogeneity is a dominant driver of divergent effector responses during infection.¹² The cumulative work on this topic shows that specific IgG Fc domain repertoires are predictors of clinical outcomes in some human diseases, yet it also shows that IgG repertoires, alone, do not clearly predict outcomes in many diseases where antibodies are thought to have a critical role in immunity. This invites the hypothesis that the less well studied heterogeneity on the other end of the signaling axis – Fc γ R expression and Fc γ R-mediated effector cell functionality – may be an equally, or more important, driver of clinical outcomes that depend on antibody effector function.¹³ Fc γ R heterogeneity might be particularly relevant to the increased risk for many infectious diseases that is associated with some demographic and health features such as advanced age, obesity, and diabetes, where effector cells are known to be different from healthy adults in distribution and/or functionality.¹⁴⁻¹⁸ Studies characterizing IgG Fc repertoires and

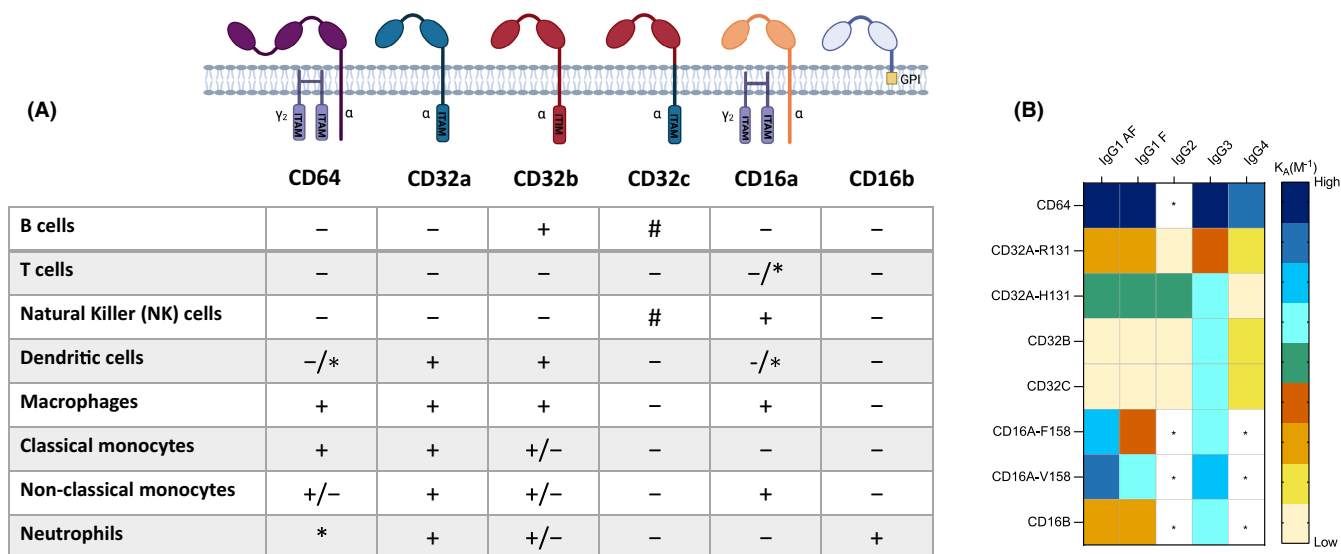


FIGURE 1 (A) Type I Fc γ receptors (Fc γ R) expression pattern on human white blood cell subsets. + indicates constitutive expression; - indicates no expression; +/- indicates no or low expression; * indicates inducible expression; */- indicates low or inducible expression; # indicates expression depending on FCGR2C allelic status. Classical monocytes are defined as CD14 highCD16 - and non-classical monocytes are defined as CD14 dimCD16 ++. (B) Binding affinities (association constant of binding [K_A]) of four IgG subclasses (IgG1, IgG2, IgG3 and IgG4) to the various type I Fc γ R. No color and * indicates that binding was either negligible or not detected to that Fc γ R. IgG1 AF denotes afucosylated IgG1, whereas IgG1 F is the core fucosylated IgG

effector cells from matched donors are needed to truly address the role of antibody effector function in human immunity.

2.1 | Regulation of A/I signaling by IgG Fc domain structure

Fc γ R affinity for a particular IgG antibody is determined in large part by the structural aspects of the Fc domain (Figure 2). The human IgG isotype is comprised of four subclasses: IgG1, IgG2, IgG3, and IgG4 with decreasing abundance from IgG1-4. Each subclass contains a structurally distinct Fc domain, which has additional structural heterogeneity arising from IgG allotypes,²⁰ and variable Fc glycosylation.²¹ IgG subclass and Fc glycoforms are clear modulators of Fc-Fc γ R interactions while the role of specific allotypes is less understood.

Fc glycosylation introduces even greater structural and functional diversity to the Fc domain repertoire. All IgG subclasses contain a highly conserved core N-linked glycosylation site at Asn-297 within the CH2 domain of each heavy chain. The core N-glycan structure is composed of four N-acetylglucosamine (GlcNac) and three mannose residues arranged in a biantennary formation (Figure 2). As the IgG1 glycoprotein travels through the endoplasmic reticulum and Golgi apparatus, the core N-glycan structure can be modified by the addition of other sugar residues such as core fucose linked to the innermost GlcNac, an additional bisecting GlcNac linked to the central mannose, as well as galactose and sialic acid residues that can be added to either or both antennae. Not all modifications are wholly independent; for example, sialic acid can only be added to glycans that contain galactose. Nonetheless, the many possible combinations of these modifications contribute to a variety of complex glycans at Asn-297. The presence or absence of particular residues within these glycans impacts the binding affinity between IgG and Type I and Type II Fc γ Rs.¹² At present, our understanding of the functional diversity conferred on IgG by various Fc glycoforms is largely limited to the most abundant subclass, IgG1. For IgG1, a major regulator of inflammatory Fc γ R signaling is core fucosylation of the Fc glycan that regulates interactions with the activating Fc γ R, CD16, as reviewed in greater detail below (Figure 1B). Sialylation of the IgG1 Fc confers the ability of immune complexes to signal through Type II Fc γ Rs.²² The activity of Type II Fc γ Rs has been demonstrated

largely *in vivo* where they have been observed to mediate diverse functions via interactions with sialylated immune complexes. For example, sialylated IgG ICs promote the anti-inflammatory activity of therapeutic IVIG in context of autoimmune diseases through the engagement of DC-SIGN/SIGN-R1²³⁻²⁶ and CD23-mediated modulation of B cell selection during adaptive responses.^{11,27} There remains much to learn about the molecular regulation of IgG Fc glycosylation but sex and age are known to correlate with specific patterns of glycosylation, as well as factors that correlate with geographic location; whether these are heritable or not, is not yet clear.²⁸⁻³⁰

2.2 | Regulation of A/I signaling by Fc γ Rs

There are six human Type I Fc γ Rs including Fc γ R11c (CD32c), which is expressed by a minority of individuals.^{31,32} Type I Fc γ Rs are made up of both activating and inhibitory receptors as determined by their signaling through immunoreceptor tyrosine-based activation (ITAM) or inhibition (ITIM) motifs, respectively. Type I receptors are activating, except for Fc γ R11b (CD32b), which is the sole inhibitory receptor. The extracellular domains of the Fc γ R alpha chains are variable in their affinities for IgG Fc domains. Fc γ R1 (CD64) is the only high affinity receptor that can bind to monomeric IgG. Co-expression patterns of Fc γ R on immune cells shape the direction and magnitude of Fc-mediated immune activity (Figure 1A,B).

High-avidity, productive interactions between Fcs within immune complexes and Fc γ Rs on innate cells trigger cell-type specific signaling cascades that culminate in diverse cellular responses, shaping the innate and adaptive immune landscape. Fc γ R-mediated activation of innate immune cells leads to a gamut of cellular responses ranging from those often modeled in *in vivo* assays such as antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP), to the induction of more complex immune programs including cytotoxic degranulation, proinflammatory cytokine and chemokine production, macrophage polarization, enhancement of antigen presentation by APCs, maturation of DCs and maintaining immune homeostasis.^{20,33-35} Despite the distinct effector roles of the different cellular subsets, all of them signal through ITAM or ITIM motifs via pathways that are fairly conserved and are well reviewed elsewhere.^{36,37} These motifs are present either in the cytoplasmic tail of the Fc receptors themselves (CD32a,b,c) or on non-covalently

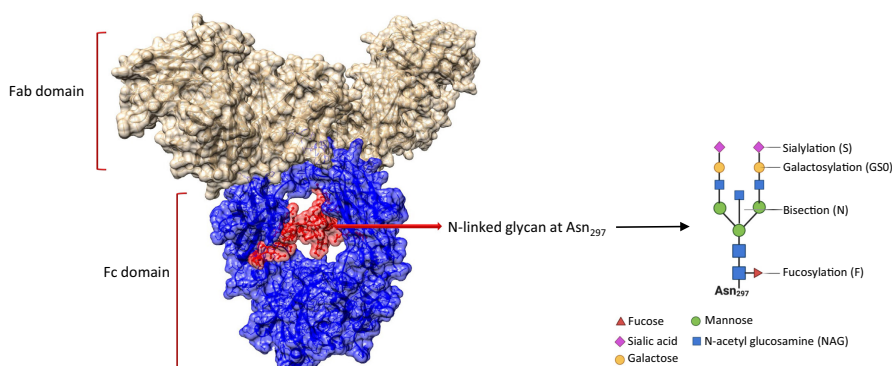


FIGURE 2 IgG glycoprotein and N-linked glycan. IgG domain architecture (PDB 1HZH¹⁹) and cartoon representation of IgG1 Fc glycans

associated ITAM-containing adaptor proteins Fc ϵ R1 γ (FcR γ) or CD3 ζ (Figure 1A).

Multivalent Fc-Fc γ R engagement on the cell surface leads to clustering of the Fc γ Rs and CD45 phosphatase exclusion on the plasma membrane, enabling the activation of the Lyn and/or Fyn and Hgr Src-family kinases, that then phosphorylate conserved tyrosines (Tyr) in the cytoplasmic ITAM motifs. Phosphorylation of the two Tyrs creates docking sites for Syk kinases through their SH2 domains and leads to activation of these Syk kinases. Subsequently, activated Syk then phosphorylates a plethora of downstream adaptor molecules that propagate further signaling cascades involving phospholipase C-gamma (PLC γ), phosphoinositide 3-kinase (PI3K), calcineurin, and Vav-1 pathways.³⁷⁻⁴³ Many of these pathways co-regulate or cooperate in the performance of cell-specific immune activities. To counterbalance activating signaling through ITAM motifs, most of the innate cell subsets co-express an inhibitory Fc receptor that signals via the conserved ITIM in its cytoplasmic domain. When activating and inhibitory Fc γ Rs are simultaneously cross-linked and co-cluster, the ITAM-phosphorylating Src kinases also phosphorylate the conserved Tyrs of proximal ITIMs. ITIM phosphorylation promotes non-covalent binding of tyrosine phosphatases SHP-1 and SHP2 and the inositol phosphatases SHIP-1 and SHIP2 via the SH2 domains of the phosphatases. These phosphatases subsequently dephosphorylate the Tyr phosphates on proximal ITAMs and other adaptor molecules, thereby tempering ITAM signaling and any associated immune activation.^{44,45}

Multiple factors contribute to the heterogeneity of Fc γ R repertoires including single nucleotide polymorphisms (SNP), copy number variation (CNV), and glycosylation patterns of Fc γ Rs.^{21,31,46,47} SNPs in the Fc γ R genes modulate binding affinity to IgG Fc. There are two major CD32a allotypes, R131 and H131 where H131 exhibits greater binding affinity for IgG1 and IgG2.³¹ CD16a similarly has two well characterized allotypes, F158 and V158 where the less frequent V158 allotype exhibits higher affinity for IgG compared to F158.^{31,48-52} While the CD16a allele expressed correlates with the clinical response to some therapeutic anti-tumor monoclonal antibodies,⁵³⁻⁵⁶ there is limited data supporting a correlation between either CD16 variants and clinical outcomes in infectious diseases. Recent studies suggest that apart from allelic variations, Fc γ R expression levels can also be highly heterogeneous amongst individuals, possibly explained in part by CNV, and may be a previously underappreciated determinant of antibody signaling outcomes.^{13,46,47,57,58} The glycan repertoire of Fc γ Rs is also highly heterogeneous owing to the variability in the number of N-glycosylation sites on the Fc γ Rs and an array of alternative glycoforms that can exist at each site.⁵⁹ Structural and biophysical data have shown that the presence or absence of certain glycans at particular amino acid positions of these receptors impacts CD16a binding to IgG, bringing into focus the role of Fc γ R N-glycans in shaping effector immune functions.⁶⁰⁻⁶⁴ Elegant profiling studies of Fc γ Rs from primary human NK cells and monocytes have shown that receptor glycosylation profiles vary amongst different immune cells and are also governed by the Fc γ R allotype of an individual.^{60,65-68} More studies are required to gain an understanding of this heterogeneity of the glycan profiles of Fc γ R

and whether they change upon certain activating/inhibitory signals, or between healthy and diseased states.

3 | AFUCOSYLATED IGG1-CD16A SIGNALING AXIS

A major focus of this review is the abundance of recent data that show how heterogeneity within the IgG and Fc γ R repertoires, and specifically in the afucosylated IgG1-CD16a signaling axis, impacts the course of some infectious diseases. Specifically, the proinflammatory IgG Fc glycoform lacking a core fucose, here termed "afucosylated", is enriched on IgG1 in people who progress to severe forms of dengue disease and COVID-19, the disease caused by SARS-CoV-2.⁶⁹⁻⁷⁴ Afucosylated IgG1 has high affinity for the activating Fc γ R, CD16a, which mediates diverse inflammatory functions that likely only partly overlap in these two very different viral infectious diseases (Figure 1B).

3.1 | IgG1 afucosylation

In healthy adults and children, IgG1 antibodies are largely modified by fucosylated Fc glycoforms with afucosylated glycoforms generally comprising <10% of the IgG population.²⁹ An increased abundance of afucosylated IgG1 has been observed in a number of clinical settings including fetal/neonatal alloimmunity, acute dengue disease, in people with, or who will develop, severe COVID-19, and in malaria.⁶⁹⁻⁷⁶ Increased afucosylated glycoforms have also been shown in association with bulk IgG glycans, including glycans on the Fab and Fc from all IgG subclasses, in HIV elite controllers and latent tuberculosis patients.^{77,78} The genetic or environmental factors that contribute to afucosylated IgG1 production are not yet understood.

3.2 | CD16a

Expression of the receptor for afucosylated IgG1, CD16a, is mostly limited to natural killer (NK) cells, monocytes, and macrophages.⁷⁹ Recent intriguing studies also suggest that very low levels of CD16a may be expressed on neutrophils and induced on CD4+ and/or CD8+ T cell subsets in disease states such as autoimmunity and chronic infection.^{47,80-84} Within the monocyte lineage, CD16a expression is limited to the non-classical and intermediate subsets, while the more abundant classical monocyte subset does not express CD16a (Figure 1A).⁸⁵ Historically, it has been unclear whether these three monocyte subsets represent distinct developmental trajectories or are part of one linear trajectory, an implication of the latter being that CD16a is a marker of monocyte maturity, similar to NK cells.⁸⁶ More recent studies on CD16a strongly support one linear trajectory in which CD16a expression is acquired as CD16a- classical monocytes give rise to CD16a+ non-classical monocytes through the transitional CD16a+ intermediate subset.⁸⁷⁻⁹⁰ Unlike monocytes, a majority of macrophages, including alveolar macrophage, express relatively high levels of CD16a.⁹¹

Functionally, CD16a is the major cytotoxic receptor but also mediates maturation and activation of CD16a-expressing cells. This modulates autocrine and paracrine immunity, impacting antigen presentation and inflammatory processes including cytokine release and chemotactic cell migration.^{74,79}

4 | PATHOGENIC ROLE OF AFUCOSYLATED IGG1-CD16 SIGNALING IN SEVERE DENGUE DISEASE AND COVID-19

There are multiple viral and host-derived factors that determine the trajectory of disease following dengue virus or SARS-CoV-2 infections, yet severe disease caused by these viruses is fundamentally the result of inadequate pre-existing immunity together with an inflammatory host response during infection that damages tissues and disrupts critical physiologic processes. Dengue viruses and SARS-CoV-2 are similar in that they are both enveloped, positive-sense, single-stranded RNA viruses; however, dengue viruses are mosquito-borne and can infect cells in a broad range of organs while SARS-CoV-2 is a respiratory virus with a majority of replication thought to take place in the upper and lower respiratory tracts. While hyperinflammation is common to severe forms of disease caused by dengue viruses and SARS-CoV-2, severe dengue disease and severe COVID-19 are distinct and result from very different pathological inflammatory processes. That both diseases are likely mediated in part by an aberrant afucosylated IgG1-CD16a signaling speaks to the diversity of functions that can arise through engaging this effector pathway.

4.1 | Afucosylated IgG1 in dengue disease

There are four serotypes of dengue viruses (DENV), DENV1-4 and primary infection with one serotype elicits antibodies that are broadly reactive, but that do not neutralize other DENV serotypes. The vast majority of DENV infections result in a subclinical or mild clinical phenotype but a subset of individual's progress to more severe disease. The most severe dengue disease forms, dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS), are characterized by sequelae including thrombocytopenia and plasma leakage due to increased capillary permeability, in some cases resulting in shock (DSS).⁹² A majority of DHF/DSS occurs in secondary DENV infections when non-neutralizing, reactive IgGs are present that modulate the level of infection and the cellular response to infection, through "extrinsic" and "intrinsic" antibody-dependent enhancement (ADE) mechanisms, respectively.^{93,94} An aggressive inflammatory response including elevated production of interferon (IFN)-gamma, tumor necrosis factor (TNF)- α , interleukin (IL)-2, IL-6, IL-8, and vascular endothelial growth factor (VEGF)⁹⁵ likely contribute to capillary permeability and other sequelae of severe disease. These cytokines are thought to derive from virus-infected monocytes, dendritic cells, platelets, mast cells and T cell subsets. Inherent

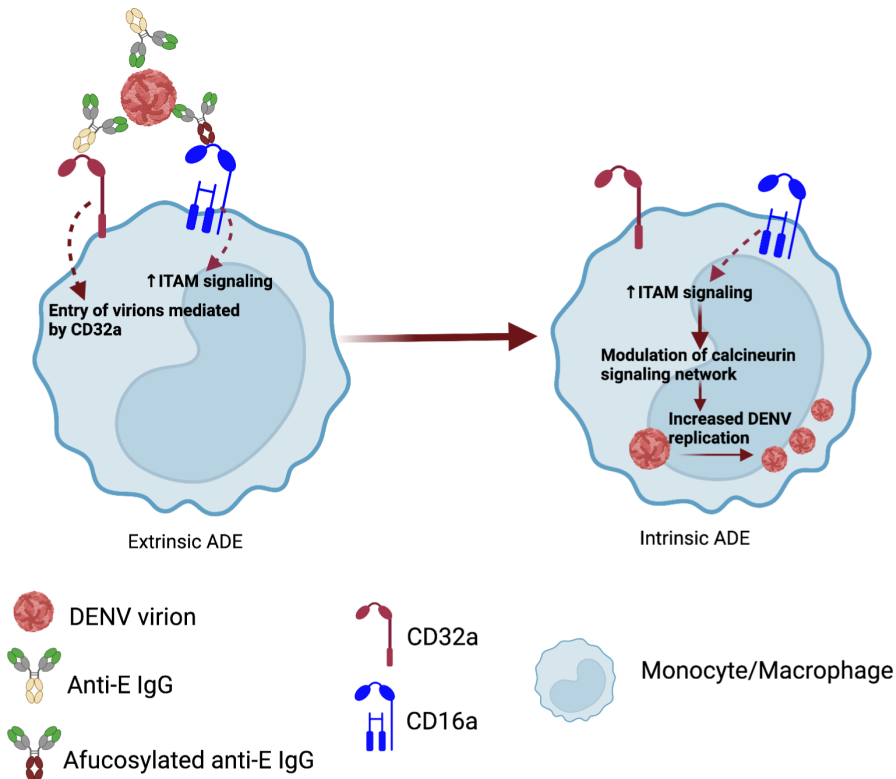
cellular differences have been associated with cytokine production in both protective and pathologic inflammatory responses during infection.⁹⁶

Although the presence of reactive, non-neutralizing IgG is a risk factor for progression to severe disease, it has been appreciated for several decades that the presence of non-neutralizing IgG alone does not predict the development of DHF/DSS. This suggests that only specific IgG repertoires, and potentially specific Fc γ Rs, mediate ADE of disease. Recent studies have begun to resolve this important topic in dengue disease pathogenesis. In 2017, our group and others studied a cohort of patients with secondary DENV infection with mild or severe disease. They observed that patients who progressed to DHF/DSS produced anti-DENV IgG1 with significantly elevated Fc afucosylation over those that did not develop severe disease.⁶⁹ An increased frequency of anti-DENV IgG1 relative to IgG2 was also associated with severe disease. Further, afucosylated IgG from patients with severe disease could activate platelets and trigger platelet loss in an Fc γ R-dependent manner, *in vivo*, consistent with thrombocytopenia characteristic of severe dengue. The identification of afucosylated anti-DENV IgG1 as a biomarker of severe dengue disease shed light on why only a subset of DENV patients with anti-DENV IgGs develop severe disease.

In 2020, we further showed that elevated afucosylated anti-DENV IgG1 in maternal circulation predicted the development of clinically significant disease in their infants during primary dengue infection.⁷⁰ This was presumably due to transplacental transfer of the maternal IgG, rendering infants susceptible to ADE of disease during primary infections. This study reproduced our earlier finding that afucosylated anti-DENV IgGs predicted dengue disease severity. As afucosylated IgG1 has increased affinity for CD16a, the identification of IgG1 afucosylation as a correlate of dengue disease severity implicates CD16a in the pathophysiology of ADE. We assessed the requirement for CD16a in antibody-dependent DENV infections using engineered human monocytic cell lines (U937) that express different combinations of CD16a and the other low-affinity activating Fc γ R, CD32a⁷⁰. This work showed that CD16a and CD32a had distinct roles in ADE of dengue infection. CD32a mediated the majority of entry by DENV immune complexes (extrinsic ADE), while the presence of CD16a did not modify the ability of DENV immune complexes to enter cells. However, in the presence of both CD16a and CD32a, infection was enhanced through a mechanism that required functional CD16a signaling. Thus, CD16a-mediated ITAM signaling triggered intrinsic ADE of DENV infection in a mechanism that was shown to require the calcineurin signaling network. Together these data support a functional role for afucosylated anti-DENV IgG1 in driving severe DENV disease though increased infection in and ITAM signaling of CD16a expressing monocytes (Figure 3). In addition to modulating viral infection in CD16a-expressing cells, afucosylated DENV immune complexes likely contribute more broadly to the hyperinflammatory sequelae in severe dengue disease.

In 2021 Bournazos et al. again demonstrated afucosylated IgG1 as a correlate of severe disease and provided significant additional insights into the kinetics of afucosylated IgG1 production in less

FIGURE 3 Model for antibody-dependent enhancement (ADE) in dengue infection and the roles of activating type I Fc γ receptors (Fc γ R) and afucosylated IgG. Viral attachment and entry occurs mostly by CD32a mediated endocytosis after interactions with anti-E IgG1:dengue ICs. Additionally, high affinity interactions between afucosylated anti-E IgG1:dengue ICs and CD16a promotes increased ITAM signaling. ITAM signaling triggers downstream calcium flux and activates the calcineurin signaling network. Specific calcineurin inhibitors prevented Fc γ R-dependent dengue virus infection



severe cases of DENV infection. Using matched patient plasma, Bournazos et al. showed that the abundance of afucosylated IgG1 increases during convalescence after mild primary infection and separately in response to secondary infection.⁷¹ These two independent increases in afucosylated IgG1 drove a generally higher abundance of afucosylated IgG1 in patients with secondary DENV. The timing of these increases in afucosylated IgG1 strongly suggests that production of afucosylated IgG1 is part of the host response to DENV infection. However, only a subset of DENV patients exhibited increased afucosylated IgG1, indicating that some individuals may be more predisposed to produce antibodies with this modification than others. Further investigation into the regulation and mechanism of afucosylated anti-dengue IgG1 production and activity are required to understand the role of these antibodies in DENV disease pathogenesis.

4.2 | Afucosylated IgG1 in severe COVID-19

As with dengue virus infections, the vast majority of infections with SARS-CoV-2 result in subclinical or very mild disease. Severe COVID-19 arises in part from aberrant activation of cells in the myeloid compartment, triggering a hyperinflammatory cascade.^{97,98} High concentrations of proinflammatory cytokines, particularly IL-6 and TNF α are strongly associated with severe disease and poor prognosis.⁹⁹ Further, unlike other respiratory viral infections, patients with severe COVID-19 benefit from steroid treatment and other medications targeting inflammatory pathways, highlighting the underlying hyperinflammatory pathophysiology of this disease.

In 2021, our group described a proinflammatory feature of IgG that correlated with severe COVID-19: afucosylated IgG1.⁷² While this was a familiar correlate of disease severity from dengue disease studies, this finding was unexpected given the distinct pathophysiologies of severe COVID-19 and dengue disease. Afucosylated IgG1 antibodies against the SARS-CoV-2 receptor-binding domain (RBD) were more abundant in hospitalized COVID-19 patients compared to both adult COVID-19 outpatients and seropositive children. This increased abundance of afucosylated IgG1 was predominantly observed in hospitalized male patients from two independent cohorts but did not correlate with age. IgG subclass differences amongst the various groups were also probed as they are known modulators Fc-Fc γ R interactions. A significant increase in anti-RBD IgG3 antibody level was observed in patients admitted to the intensive care unit (ICU).⁷² The IgG3 subclass and afucosylated IgG1 are among the most inflammatory IgG structures due to their higher affinities to activating Fc γ Rs, particularly CD16a (Figure 1B). Thus, the Fc signature observed in hospitalized COVID-19 patients was highly proinflammatory, and IgG from patients with elevated afucosylation (>20%) had 5–6 fold higher affinity to recombinant CD16a as compared to patient IgG that exhibited low afucosylation (<10%). Enhanced binding to CD16a translated to higher inflammatory potential as evidenced by robust production of IL-6, TNF α , and other proinflammatory factors by healthy donor monocytes stimulated with ICs composed of afucosylated IgG and SARS-CoV-2 spike trimer in vitro. A subsequent study by Larsen et al. reproduced the finding of enriched afucosylated IgG1 in sera of severe COVID-19 patients and proposed an interesting hypothesis that Fc fucosylation is regulated by whether an antigen is presented to the B cell in a membrane-bound or soluble form.⁷³ Findings from Hoepel

et al. reinforced the proinflammatory capability of afucosylated ICs as they demonstrated that even homeostasis-promoting human alveolar macrophage could be thoroughly reprogrammed toward a proinflammatory phenotype by stimulation with afucosylated immune complexes.¹⁰⁰

While these studies established that afucosylated IgGs were present in severe COVID-19, it was not clear whether this was a cause or effect of severe disease. In either case, afucosylated immune complexes might modulate disease severity, yet clarifying the timing of production was important to determining whether Fc afucosylation was an early biomarker of risk in SARS-CoV-2 infections. To assess whether elevated afucosylated IgG1 preceded progression to severe symptoms, samples were studied from two independent, longitudinal cohorts of mild COVID-19 patients, a subset of whom would go on to develop more severe disease. From this analysis, in 2022, our group further reported that elevated IgG1 afucosylation, together with absent or low early neutralizing antibodies was an early predictor of risk for disease progression in mild COVID-19 patients.⁷⁴ Receiver operating curve (ROC) analysis showed that, individually, neutralizing titers and IgG afucosylation were only moderate predictors of disease progression; however, combining the two features enabled robust predictive capacity to identify mild COVID-19 patients who were at risk for developing severe symptoms ("progressors").⁷⁴ We also observed that ASCs, specifically plasmablasts from "progressors" expressed significantly less of the enzyme α -1,6-fucosyltransferase (FUT8) that is responsible for core fucosylation, consistent with increased afucosylated IgG1 in this group.⁷⁴

Dysregulation in innate immune effector cell subsets has been shown to correlate with COVID-19 disease severity.¹⁰¹⁻¹⁰⁴ As IgG-mediated, inflammatory effector cell activity is a function of both cell frequency and Fc γ R co-expression by these cells, we went on to perform an in-depth profiling of peripheral innate immune cells from "progressors" and non-progressor controls to quantify both cell frequency and Fc γ R expression. We observed that monocyte distribution within peripheral blood immune cells (PBMCs) was distinct in "progressors" prior to the development of more severe symptoms. Specifically, "progressors" had an increased frequency of CD16a-expressing immune cells, non-classical and intermediate monocytes. In addition to being more frequent, non-classical and intermediate monocytes from "progressors" expressed more surface copies of CD16a protein. Other activating and inhibitory Fc γ Rs were not distinct between "progressors" and controls. ROC analysis showed that, separate from the non-neutralizing, afucosylated IgG1 correlate of progression to more severe COVID-19, CD16a expression alone within the peripheral myeloid cell compartment was also strongly predictive of disease progression.⁷⁴ Together, these data link the afucosylated IgG1-CD16a axis to the development of worsening symptoms in COVID-19.

Although the recruitment of monocytes and other innate immune cells to sites of infection and inflammation is fundamentally a homeostatic immune mechanism, autopsies from patients with COVID-19 revealed extreme infiltration of lungs thought to result

in poor gas exchange, and co-localization of viral RNA with myeloid cell infiltrates, likely demonstrating an overwhelming inflammatory response to viral antigens.^{105,106} To study how afucosylated immune complexes might modulate inflammation in the lung, specifically, our group developed a mouse model enabling assessment of human immune complex-mediated lung inflammation. Administration of human afucosylated immune complexes into the lungs of Fc γ R-humanized mice¹⁰⁷ promoted a rapid and robust inflammatory response characterized by neutrophil and monocyte infiltration, as well as the production of numerous proinflammatory soluble factors including IL-6, TNF α , and myelo-attractive chemokines.⁷⁴ This inflammatory response to afucosylated immune complexes was CD16a dependent and was not present in mice that received fucosylated immune complexes. The absence of virus and infection in this mouse model allows for the characterization of the inflammatory potential of afucosylated IgG1, as well as other human IgG subclasses and glycoforms, unconfounded by viral and antiviral activity alike. While the humanized mouse model used to study how antibody signaling impacts the lung is not a model of COVID-19 pathogenesis, the inflammatory response within the lung in response to afucosylated IgG1 resembles the inflammatory state of the lungs of severe COVID-19 patients, namely a persistence of neutrophils and inflammatory monocytes or monocyte-derived macrophages (Figure 4).¹⁰⁸⁻¹¹² These data collectively demonstrate that the enhanced afucosylated IgG1-CD16a signaling axis observed in COVID-19 "progressors" can promote a rapid and robust inflammatory response in vivo that exhibits similarities to findings in severe COVID-19.

5 | CONCLUDING REMARKS AND OUTSTANDING QUESTIONS

The molecular regulation of IgG1 Fc glycosylation, particularly Fc fucosylation is incompletely understood. Although a number of studies have identified genes other than *Fut8* that might be involved in core fucosylation, including *Ikzf1*, *Ikzf3*, *Hnf1a*, *Hnf4a*, and less enigmatic genes involved in fucose biosynthesis, the details of how these genes might participate in the regulation of FUT8 expression in ASCs and the production of afucosylated IgG1 have yet to be fully described.¹¹³⁻¹¹⁶ Sex and age are also known to correlate with IgG Fc glycoforms²⁸⁻³⁰ and exogenous delivery of sex hormones can impact Fc glycosylation.^{117,118} A greater understanding of how and why afucosylated IgG1 antibodies are produced in healthy and disease contexts could lead to the development of therapeutic interventions to modulate IgG afucosylation for clinical benefit.

Another key question in regulation of human antibody effector function is the extent to which heterogeneity in Fc γ Rs, as opposed to IgG Fcs, impacts disease outcomes. Is Fc γ R repertoire heterogeneity a more, less, or equivalently important variable relative to Fc heterogeneity in driving antibody-mediated disease outcomes (both positive and negative outcomes)? As demonstrated in vivo, the inflammatory potential of afucosylated IgG1 in the lung was mediated almost entirely by CD16a. Further, humans with mild COVID-19

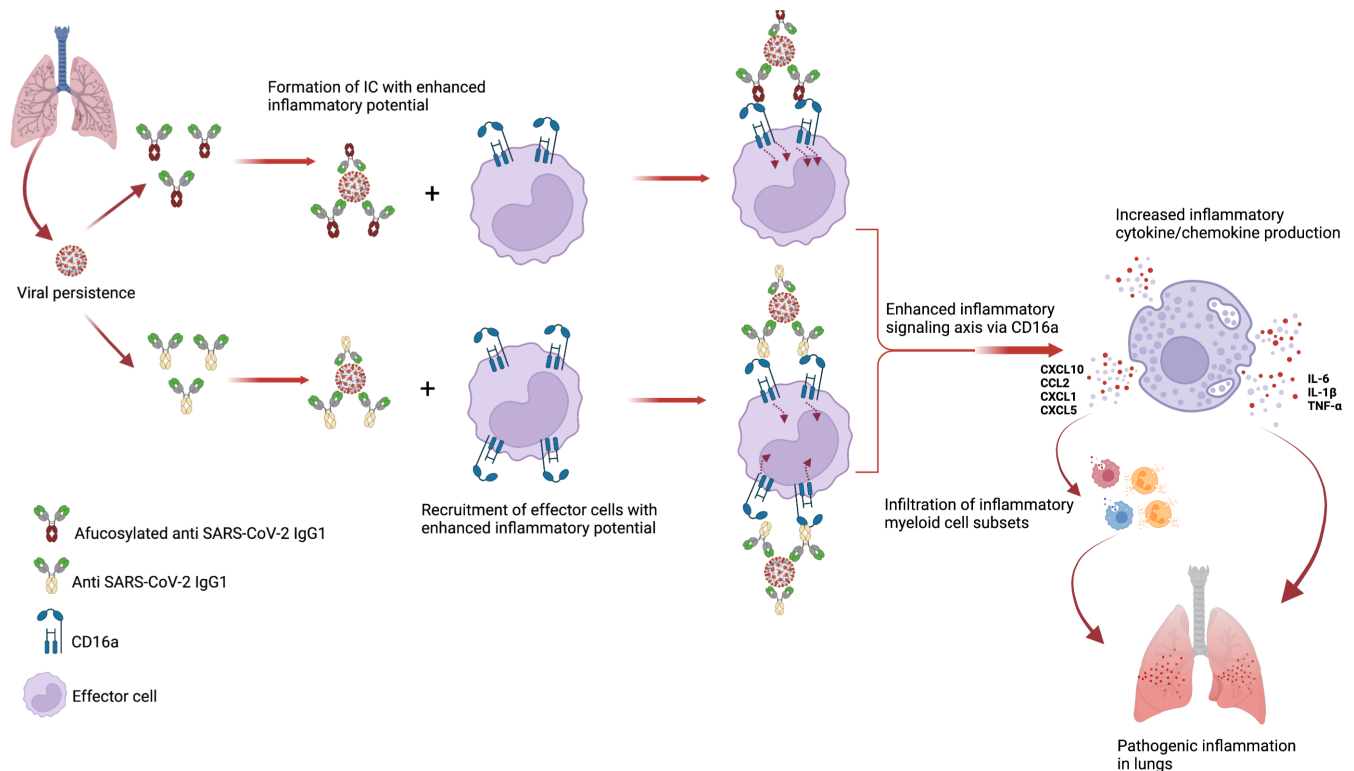


FIGURE 4 Model for immunopathology of severe COVID-19 mediated by enhanced CD16a:IgG signaling axis. Enhanced signaling via the ITAM domain of CD16a, which in turn leads to increased proinflammatory cytokine production in lung of severe COVID-19 patients might be achieved through either formation of ICs with persisting viral antigens and afucosylated anti-SARS-CoV-2 IgG1, which have higher affinity for CD16a on effector cells or by recruitment of effector cells, which have an increased expression of CD16a on their surface and thus an increased inflammatory signaling potential. Thus, proinflammatory elements on both sides of the receptor-ligand axis might contribute to more severe disease

who progressed to more severe disease had increased expression of CD16a on myeloid cells and this early cellular feature alone predicted progression to more severe disease.⁷⁴ These observations point to the question of how CD16a protein expression is regulated within the myeloid compartment. Additional identification of the viral or host-derived factors responsible for eliciting classical monocyte differentiation or maturation into CD16a-expressing non-classical monocytes might also clarify the distinct monocyte response observed in patients who progress to severe COVID-19. Structural heterogeneity of CD16a imparted by genetics and glycosylation, as well as heterogeneity of CD16a expression by immune effector cells are likely co-determinants of both protective and hyperinflammatory antibody signaling outcomes in infectious disease and are deserving of further investigation. Ideally, studies would characterize both human FcγR-expressing effector cells and IgG antibodies from matched donors to clarify how heterogeneity in antibody signaling contributes to infectious disease outcomes.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing not applicable - no new data generated, or the article describes entirely theoretical research.

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