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BASIC AND APPLIED SCIENCE (I LEWKOWICH, SECTION EDITOR)

Immunoglobulin Glycosylation Effects in Allergy and Immunity

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Abstract
Purpose of Review The aim of this review will be to familiarize the reader with the general area of antibody (Ab) glycosylation and to summarize the known functional roles of glycosylation and how glycan structure can contribute to various disease states with emphasis on allergic disease.
Recent Findings Both immunoglobulin (Ig) isotype and conserved Fc glycosylation sites often dictate the downstream activity of an Ab where complexity and degree of glycosylation contribute to its ability to bind Fc receptors (FcRs) and activate complement. Most information on the effects of glycosylation center on IgG in cancer therapy and autoimmunity. In cancer therapy, glycosylation modifications that enhance affinity for activating FcRs are utilized to facilitate immune-mediated tumor cell killing. In autoimmunity, disease severity has been linked to alterations in the presence, location, and composition of Fc glycans. Significantly less is understood about the role of glycosylation in the setting of allergy and asthma. However, recent data demonstrate that glycosylation of IgE at the asparagine-394 site of Cε3 is necessary for IgE interaction with the high affinity IgE receptor but, surprisingly, glycosylation has no effect on IgE interaction with its low-affinity lectin receptor, CD23.
Summary Variations in the specific glycoform may modulate the interaction of an Ig with its receptors. Significantly more is known about the functional effects of glycosylation of IgG than for other Ig isotypes. Thus, the role of glycosylation is much better understood in the areas of autoimmunity and cancer therapy, where IgG is the dominant isotype, than in the field of allergy, where IgE predominates. Further work is needed to fully understand the role of glycan variation in IgE and other Ig isotypes with regard to the inhibition or mediation of allergic disease.

Keywords Asthma · Immunoglobulin glycosylation · Sialylation · Allergy

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Introduction

Antibodies are key mediators of the humoral immune response interconnecting innate and adaptive immune responses. Major advances have been made in the past few decades to use the diverse antigen specificity and effector functions of antibodies as biomarkers for disease recognition and to engineer effective immunomodulatory antibodies for antigen-specific immunotherapies, e.g., cancer and autoimmune diseases [1–5].

Immunoglobulins are heterotetrameric glycoproteins comprised of two identical heavy chains (HC) and two identical light chains (LC) joined by at least one disulfide bridge. Each Ig can be divided into two antigen-binding fragments (Fab)

and a receptor-binding fragment (Fc, crystallizable fragment) that defines the immunoglobulin isotype (IgM, IgD, IgE, IgG, IgA). The HC gene undergoes rearrangement of the variable (V), diversity (D), and joining (J) segments, while LC gene recombination involves the V and J segments. This recombination is responsible for formation of the variable domains at the terminus of the Fab that comprise the antigen-binding sites. Together, these unique genetic sequences encode a set of highly diverse glycoproteins, resulting in a large Ab repertoire [6–8]. Diversity is further enhanced through the process of bridging these coding joins. Moreover, somatic hypermutations (SHM) upon T cell interactions, cytokine stimulation, and antigen encounter introduce point mutations in the variable region to produce greater antigen affinity for improved immune responses. Subsequently, class switch recombination (CSR) changes the Ig isotype and therefore modulates the effector functions elicited by the Fc region. Due to the interaction of Fab fragments and Fc fragments, antibodies efficiently neutralize and opsonize pathogenic surfaces, activate the complement cascade, and trigger phagocytosis and antibody-dependent cytotoxicity (ADCC) [9–12]. As mentioned above, specific immune effector functions are governed by the isotype and subclass of Ig binding to a specific antigen and are additionally altered by cytokine secretion [7, 13].

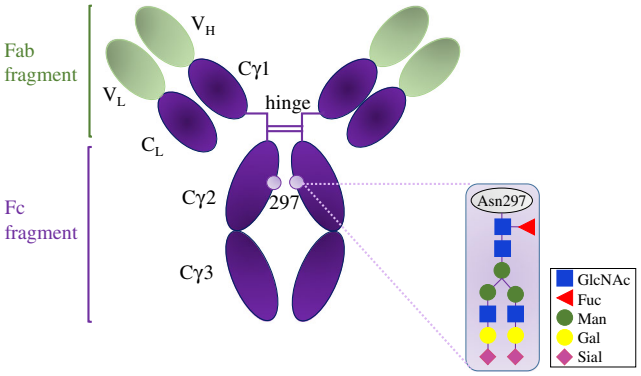

Fig. 1 Schematic of IgG domain structure and glycosylation. IgG is assembled from two identical heavy chains and two identical light chains. Each light chain consists of a variable domain (V_L) and a constant domain (C_L). Heavy chains are composed of a variable domain (V_H) and the constant domains Cγ1–Cγ3. Variable (green) domains form the antigen-binding region of the Fab fragment, while the constant domains (violet) exert effector functions via the Fc fragment. Cγ2 harbors a conserved N-linked biantennary complex glycosylation site at Asn297 (*light violet circles*). The core moiety consists of GlcNAc (*blue*) and branching mannose (*green*) terminated by two GlcNAc residues. The trimannosyl core structure can be modified by fucose (*red*), as well as by galactose (*Gal*) and sialic acid (*Sial*). The *inset* shows a fully sialylated, fucosylated biantennary N-glycan i.e., G2S2F). A bisecting GlcNac added on to the center mannose (not shown) has also been reported [15, 16]

IgG Subclasses and Their Binding to Fc Receptors

IgG is the immunoglobulin found in highest concentration and has the longest half-life in serum. It is subdivided in humans into four subclasses, IgG1, IgG2, IgG3, and IgG4, according to their abundance (IgG1 > IgG2 > IgG3 > IgG4). In mice, IgG is classified into the subclasses IgG1, IgG2a, IgG2b, and IgG3. The IgG HC is composed of four Ig domains, including a variable domain at the N-terminus followed by the constant domains Cγ1-3 (Fig. 1). The IgG LC is comprised of a variable domain and a constant domain. The Ab subclasses vary in their structural flexibility, determined primarily by the length and disulfide linkages present in the hinge region that connects the Fab and Fc regions. Amino acid differences in the Fc region of different IgG subclasses lead to distinct affinities for Fc receptors (FcRs), consequently leading to effector function specificity [5, 14].

IgG Abs elicit their effector functions, e.g., ADCC and complement-dependent cytotoxicity (CDC), by binding to FcRs via the Fc region. Here, one can differentiate between the classical type I Fc gamma receptors (FcγRs) and type II FcRs. Type I FcγRs, from the immunoglobulin superfamily receptor class, are canonical FcγRs that can either lead to activating intracellular signaling cascades (activating FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, FcγRIIIb) or to inhibitory immunomodulatory cellular responses by the only inhibitory FcγR, FcγRIIb, of this family. Activating FcγRs harbor an immunoreceptor tyrosine-based activation motif (ITAM) for signal transduction, while FcγRIIb transmits signal inhibition via immunoreceptor tyrosine-based inhibitory motif (ITIM). It has been recently shown that under certain conditions, ITAM receptors can also trigger inhibitory signaling through the inhibitory ITAM (ITAMi) mechanism [17]. C-type lectin receptors belong to the type II FcR, e.g., human DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN; murine orthologue: Icam-3 grabbing nonintegrin-related 1, SIGN-R1), Dectin-1, CD-23, and dendritic cell immunoreceptor (DCIR). Recent findings indicated that C type lectin receptors can participate in modulating immune responses [18•, 19–21, 22•, 23].

Human IgG1 and IgG3 have a higher affinity to activating FcγRs than to inhibitory FcγRIIb and thus display a high activating-to-inhibitory (*A*/*I*) ratio. This *A*/*I* ratio correlates with their potential to induce ADCC [13, 14, 20, 24]. On the contrary, human IgG4 has a low *A*/*I* ratio and was assumed to be a poor activator of complement, giving IgG4 the status of a blocking and neutralizing Ab. Intriguingly, recent findings were able to demonstrate that human IgG4 and murine IgG1, which structurally resembles human IgG4, activate complement by forming hexamers via non-covalent Fc-Fc interactions [25, 26]. Nevertheless, immunotherapies do lead to an increase in antigen-specific IgG1 and IgG4 in the early phase of treatment. Certain allergic diseases studied have shown the IgG increase correlates with symptom remission and increased quality of life [27–31]. Allergen-specific immunotherapies consequently lead to an immune response dominated by IgG4, which is assumed

to inhibit IgE-dependent hypersensitivities [30–32]. In addition, murine studies clearly demonstrated that IgG bound to allergen crosslinks allergen-specific IgE-linked FcεRI to the inhibitory FcγRIIb, thus intervening with effector cell activation (e.g., mast cells, basophils) [33–35].

IgG Fc Glycosylation Regulates Binding to Type I and II FcRs

The affinity for FcRs on effector cell surfaces is not only modulated by the IgG subclass but also by the conserved N-linked glycosylation site at the asparagine 297 (Asn297) in each of the Cγ2 regions [36, 37]. The glycans are masked in a hydrophobic pocket, stabilizing the Fc region and favoring an open conformation. Logically, loss of the conserved glycans removes the steric hindrance between the Cγ2 domains and allows them to collapse inward, which abolishes the FcγR binding site that spans the top of both Cγ2 domains [38].

The default IgG N-glycan is comprised of two N-acetylglucosamine (GlcNAc) and three mannose (Man) residues that form a "trimannosyl core" to which are linked two additional GlcNAc groups to form the biantennary branches. Further addition of galactose (Gal) can occur on each branch, as can terminal addition of a sialic acid to each Gal. This conserved structure can be additionally modified by a fucose at the core GlcNAc (Fig. 1). The standard shorthand nomenclature assumes a trimannosyl core with biantennary GlcNAc residues, named G0 (i.e., no galactose present); more complicated structures are described by the number of galactose and sialic acid residues present on the biantennary branches, followed by the potential fucose modification (e.g., G2S1 for a mono-sialylated, non-fucosylated N-glycan or G2S2F for fully sialylated and fucosylated). These variable modifications can lead to 32 different glycoforms at each Cγ2 region, providing additional diversification of the Ab repertoire [37, 38, 39•]. Protein crystallography studies demonstrate how glycosylation alters Fc conformation and explains how these glycans change the Ab's ability to bind either type I FcγRs or type II FcRs. IgG glycans bearing no terminal galactose and sialic acid at the biantennary core (i.e., G0 structures) stabilize an open Fc conformation that facilitate its interactions with type I FcγRs. On the contrary, galactosylated and sialylated glycans are larger (and negatively charged, in the case of sialylated N-glycans), which does not allow the approach of the two Cγ2 domains and leads to greater domain flexibility, in which the Fc region alternates between the closed and open conformation. These changes increase the affinity and favor the engagement of C type lectin receptors [18•, 22•, 40, 41•].

Biological Effects of IgG Fc Glycosylation

The location of the IgG N-glycans between the Cγ2 domains and near the site of FcγR binding means that even subtle alterations in glycosylation can have profound biological effects. For example, fucosylation at the glycan core modifies the ability of IgG to induce ADCC. IgG1 with low fucose content was characterized to induce ADCC with higher efficiency when compared with fucosylated IgG1 by engaging FcγRIIIa. Non-fucosylated IgG Abs are considered to be efficient in Ab therapies, e.g., in treating cancer. In particular, anti-tumor therapies aim to use IgG antibodies with high ADCC activity that can engage activating FcγRIIIa to induce tumor clearance [4, 5, 42–47]. The anti-tumor activity is sustained not only through FcγRIIIa activation but also through a secondary mechanism of FcγRIIa engagement that produces a long-term cellular immune response.

Intravenous immunoglobulin (IVIG), a treatment for inflammation and autoimmune diseases, consists of pooled total IgG from thousands of donors. In particular, the sialylated portion of IgG is thought to mediate immunomodulatory and anti-inflammatory properties. The α-2,6-linked sialic acid of the Fc N-glycan binds to the C-type lectin receptor DC-SIGN or the murine orthologue SIGN-R1 on splenic macrophages or dendritic cells, resulting in IL-33 secretion. IL-33 upregulates IL-4 production by basophils and results in increased FcγRIIb expression on macrophages and B cells. Increased surface expression of the inhibitory receptor FcγRIIb consequently increases the threshold for activation [48–50]. In mouse models of arthritis and epidermolysis bullosa acquisita (EBA), inhibitory FcγRIIb has an established role in improving disease symptoms with IVIG therapy [51]. There, the sialylated fraction of IVIG was demonstrated to ameliorate arthritis, while removal of sialic acid increased disease symptoms [39•]. Furthermore, prophylactic treatment with sialylated IgG suppressed auto-Ab depletion of platelets in the mouse model of immunothrombocytopenia (ITP) via SIGN-R1 [52]. Moreover, point mutations in the Fc region were introduced mimicking sialylation and the resulting Fc conformation. These mimicking Abs act like the natural sialylated IgG via type II FcRs (DC-SIGN/ SIGN-R1) and induced IL-33 production by macrophages, followed by increased frequencies of regulatory T (Treg) cells. Specifically, Abs mimicking sialylation led to amelioration of arthritis in the K/BxN-induced murine model and experimental autoimmune encephalomyelitis [53].

Recently, an additional C type lectin receptor for eliciting anti-inflammatory properties of the sialylated IVIG portion (sa-IVIG) was identified. Experiments with bone marrow-derived dendritic cells from WT and FcγR knockout mice demonstrated the inhibitory potential of sa-IVIG to be independent of FcγRs in the context of allergic airway disease (AAD) and allergic hyperresponsiveness (AHR). Instead, the DCIR on pulmonary CD11c+ dendritic cells (DC) internalizes sa-IVIG and promotes the upregulation of Treg cells, leading to symptomatic relief [22•].

Low levels of terminal Fc galactosylation were shown to correlate with disease onset and severity in rheumatoid

arthritis (RA) patients [37, 54, 55•, 56]. In RA, Fc glycosylation is an important biological marker for disease diagnosis. During pregnancy, RA patients benefit from tolerogenic conditions sustained for the fetus and an increase of galactosylation is observed. This increase in galactosylation supports disease amelioration temporarily but returns to baseline after child birth [57]. In a study aimed at identifying IgG glycan differences among inflammatory bowel disease (IBD) patients, it was observed that the level of IgG galactosylation was decreased in patients diagnosed with ulcerative colitis (UC) and Crohn's disease (CD). This suggests yet another example of how a lack of IgG galactosylation might contribute to inflammation and disease pathogenesis [58].

In addition to an increased affinity of non-glycosylated IgG to activating type I FcγR, the mannose-binding lectin (MBL) on macrophages and DCs binds partially deglycosylated IgG. This internalization of IgG results in antigen presentation to T cells and an increased propensity for acquiring autoimmune disorders [59]. Furthermore, it was shown that engagement of MBL by agalactosylated IgG can facilitate complement activation [56, 59, 60]. In the context of complement activation, galactosylated IgG1 was described to crosslink inhibitory FcγRIIb with the C-type lectin receptor Dectin-1. Cross linking induces intracellular signaling pathways, which inhibit C5aR-mediated cell activation [41•]. As mentioned above, recent studies suggest that IgG1 can form hexamers by non-covalent Fc-Fc interactions, and these IgG1 hexamers can efficiently induce CDC. However, sialylation of IgG1 reduces binding capacity to the component C1q of the classical complement pathway, thus decreasing IgG1's ability to induce CDC. Likewise, removal of terminal sialic acid exposes galactose of the N-linked Fc glycan, which results in CDC induction [25, 26, 61•].

Recently, it was demonstrated that both IgG1 and IgG2 subclasses of RA patients have reduced sialylation of the Fc due to a lack of galactosylation, which is necessary for covalent attachment of the terminal sialic acid residues. Furthermore, they describe an effective treatment scheme for CIA using a murine model in which artificially sialylated anti-type II collagen Abs specifically can control arthritogenicity [55•]. Moreover, sialylated IgG protects from bone destruction, e.g., in arthritis. In patients, increased bone volume correlates with sialylation levels of disease-specific auto-antibodies (i.e., anti-citrullinated protein antibodies) [55•, 62]. In accordance with this, glycoanalyses of RA and psoriatic arthritic patient IgG confirmed an increase of galactosylated IgG after anti-TNF therapy [63]. Also, in chronic inflammatory demyelinating polyneuropathy (CIDP), where autoreactive IgG damage the peripheral nervous system, disease remission correlated with increased abundance of sialylated IgG [61•].

In the mouse model of autoimmune hemolytic anemia (AIHA), murine non-galactosylated IgG1 showed a higher potency for erythrocyte depletion via activating FcγRIII, thereby exerting a higher pathogenicity. In comparison, Fc galactosylation of murine IgG1 reduced pathogenicity by engaging inhibitory FcγRIIb. The activity of murine low-galactosylated IgG1 was mediated by activating FcγR, as γ-chain knockout mice (no expression of activating FcγR) and FcγRIIIa knockout mice did not develop anemia. However, inhibitory FcγRIIb was not important for inhibition of IgG2a-dependent anemia. In fact, while IgG1 can induce disease without engaging the complement system, murine IgG2a acts via complement activation, demonstrated by an increased deposition of the major complement component C3 on erythrocytes. Moreover, IgG2a Fc galactosylation and sialylation cannot protect from AIHA onset [64, 65].

There is a well-characterized role for Ab glycosylation in both mounting and sustaining a tolerogenic response. Pro-inflammatory conditions and T cells, in particular T_H1 cells and T_H17 cells, induce low-glycosylated IgG Abs by down-regulating alpha-2,6-sialylatransferase in plasma cells, which correlated with severity of pro-inflammatory immune responses, e.g., delayed type hypersensitivity reaction (DTH) and allergic airway inflammation. In contrast, tolerogenic stimuli induce Treg cells, whereupon plasma cells are facilitated to upregulate alpha-2,6-sialyltransferase levels and, in turn, elevate the abundance of sialylated IgG [66]. Furthermore, T cell-independent stimuli result in plasma cells that produce immunosuppressive sialylated IgG Abs [67]. Specifically, sialylated IgG1 inhibited DTH and allergic airway inflammation. In addition, sialylated IgG was described to efficiently suppress B cell proliferation and DC maturation independently of inhibitory FcγRIIb [66]. IgG sialylation has also proven effective in the context of vaccination. It has been demonstrated that sialylated IgG, generated by influenza vaccination, binds the type II FcR, CD23, on B cells, leading to an upregulation of inhibitory FcγRIIb expression on B cells. As a result, the threshold for B cell activation was increased and led to selection of high affinity antigen-specific antibodies, thus increasing vaccination efficiency [21].

Studies of Fc glycosylation from antigen-specific antibodies of HIV patients clearly support the idea that specific antigens can regulate glycosylation. Moreover, vaccination modulated the glycoprofile of antigen-specific Abs despite initial differences [68]. Comparing the Fc glycans of HIV-untreated donors and donors receiving antiretroviral therapy revealed an improved antiviral activity, which correlated with an enhanced frequency of agalactosylated and afucosylated IgG [69]. Also, anti-tumor therapy aims to use IgG antibodies with a high ADCC initiation propensity in order to engage FcγRIIIa and induce tumor clearance. In this case, IgG is interacting with FcγRIIa on DCs for generation of a memory response. Furthermore, FcγRIV expression on CD11c+ DCs is crucial for vaccine efficiency [47].

Glycosylation Sites of IgE and Their Impact

IgE compared with IgG has an additional C epsilon (Cε) domain in place of a typical hinge region and is highly glycosylated, with six biantennary glycosylation sites in the Fab and Fc fragments (Asn140, Asn168, Asn218, Asn265, Asn371, Asn383) (Fig. 2). In addition, the glycosylation site on Asn394 of IgE Cε3 domain harbors an N-linked oligomannose-type glycan, which consists of GlcNAc and branching mannose residues (Fig. 2). The quantity and complexity of IgE glycans contribute to its unique structure and function, with ~12% of the total molecular mass contributed by glycans. IgE is the least abundant isotype in serum, which is compensated by its prolonged half-life when bound to the high affinity Fcε receptor I (FcεRI) on mast cells and basophils [38, 70–72]. Serum IgE leads to an upregulation of FcεRI expression on effector cells, thus modulating the threshold for cell activation [73].

IgE is a double-edged sword; though it is known to be involved in hypersensitivities and allergic reactions, IgE also plays a protective role in vivo. FcεRI is activated through a crosslinking mechanism at the surface of mast cells, basophils, and dendritic cells. When antigen binds to FcεRI-bound IgE, receptor crosslinking results in downstream signaling events that lead to a release of biologically active mediators, including histamine, proteases, and other lipid-derived mediators. This degranulation event drives an influx of immune cell mediators, vasodilatation, and smooth muscle contraction and can ultimately lead to anaphylactic shock [70, 74, 75]. Aside from hypersensitivity and allergy, IgE is also known to be beneficial for protection against parasites and venoms [76–79].

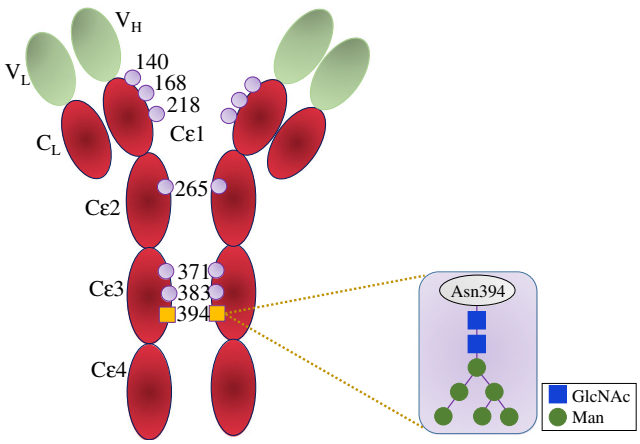

Fig. 2 IgE domain structure and glycosylation. IgE differs from IgG in that it lacks a typical hinge region but instead has an additional domain in each heavy chain, resulting in constant domains Cε1– Cε4. The constant domains contain six N-linked biantennary complex glycosylation sites (*light violet circles*) at Asn140, Asn168, Asn218, Asn265, Asn371, and Asn383. At Asn394, the conserved N-linked glycosylation site is of the oligomannose type (*yellow squares*). There GlcNAc (*light blue*) is N-linked to Asn394 followed by an additional GlcNAc, which can be modified via branching mannose (*green*)

In the last decade, studies focused on the impact of IgE glycosylation have led to confusing results. Earlier studies delineated that point mutations of Asn265 and Asn371, effectively deleting their respective glycosylation sites, did not alter the affinity of IgE for FcεRI nor did it affect the capacity of IgE to activate basophils [80]. Further investigation of CD23 interactions on B cells with recombinant ε-chain peptides showed no involvement of glycosylation [81]. One group of investigators report using IgE Fc chain fragments demonstrate that glycosylation at Cε3 is critical for proper protein synthesis and folding as well as for receptor binding, while another group emphasized the importance of the junction between Cε2 and Cε3 to be crucial for FcεRI binding [82, 83]. Thus, early studies suggest no role for glycosylation of IgE in its function during interaction with its two most common receptors. In contrast, a recent publication has shown that the conserved N-oligomannose site at Asn394 is crucial for IgE to maintain its structure and for its binding to FcεRI in vivo. In a series of elegant studies, Shade et al. demonstrated that eliminating Asn394 oligomannose reshaped secondary IgE structure and prevented mast cell degranulation and anaphylaxis in vivo [84•]. This newer information strongly supports a role for glycosylation effects on IgE, at least at one of its six glycosylation sites.

Glycosylation of IgA and Its Role in Disease

Preventing pathogen migration through the epithelial barrier, IgA is known for protection at mucosal sites. IgA is divided into two isotypes, IgA1 and IgA2, with IgA1 having a longer hinge region due to duplicated sequence of amino acids (Fig. 3a, b). This prolonged hinge region causes IgA1 to be more sensitive to bacterial proteases. Hence, IgA2 is the predominant IgA isotype in the mucosa, while IgA1 circulates in serum. Both IgA1 and IgA2 exist as monomer and as polymers. Polymeric IgA (primarily dimeric) is formed by disulfide bonds between the C-terminal tailpiece extension of IgA and the small J ("joining") chain protein (Fig. 3c) [85]. Monomeric IgA is predominantly present in serum, while polymeric IgA forms are transcytosed across mucosal epithelium into the mucosa by the polymeric Ig receptor (pIgR), during which the pIgR ectodomain is covalently linked to polymeric IgA and cleaved to form secretory IgA (SIgA) [86–92].

IgA1 has two N-glycosylation sites at Asn263 and Asn459 in the Fc region, while IgA2 harbors five N-glycosylation sites—two in the Fab region (Asn166, Asn211) and three within the Fc (Asn263, Asn337, and Asn459) (Fig. 3). As discussed above, both IgG and IgE have conserved N-glycans between the Cγ2 or equivalent Cε3 domains of their Fc regions that are critical for function; the corresponding residue in IgA1 and IgA2 is instead a Cys (Cys299) that mediates a disulfide bond across the two heavy chains (Fig. 3). The Cα2 N-glycans (Asn263 in IgA1 or Asn263 and Asn337

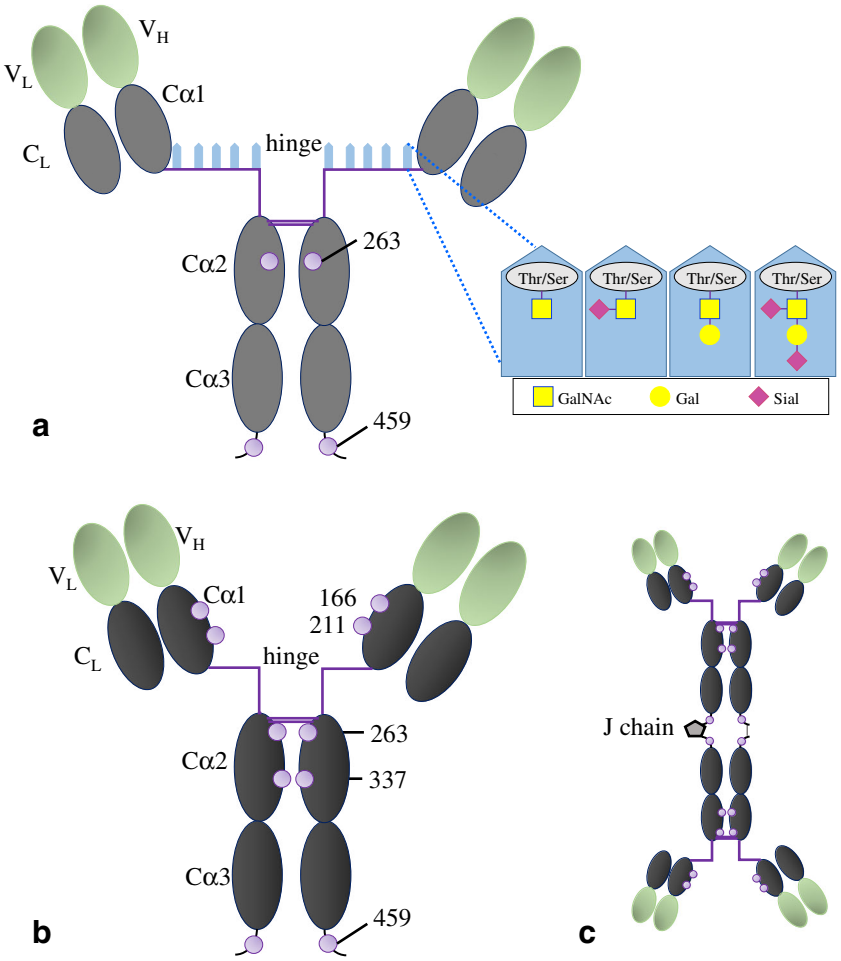

Fig. 3 IgA domain structure and glycosylation. IgA can be divided into two subclasses, IgA1 and IgA2. They differ in their hinge region, the sensitivity to proteases, and their distribution in serum and mucosa. **a** The domain organization of IgA1, which features an elongated, O-glycosylated hinge region. In addition to five potential O-glycosylation sites in the hinge, IgA1 has N-glycosylation sites at Asn263 in the Cα2 domain and Asn459 in the tailpiece. *Inset* shows several potential O-glycan structures found on the IgA1 hinge. The left two structures are under-galactosylated; such glycans are found on IgA1 in patients with IgA nephropathy. Other structures not illustrated are galactosylated O-glycans with a single sialic acid attached to either the GalNAc or Gal residue. **b** The domain architecture of IgA2, which has a deletion in the hinge region that removes the potential O-glycosylation sites and shortens the hinge compared to IgA1. IgA2 has three additional biantennary complex N-glycosylation sites per heavy chain (Asn166, Asn211, Asn337) compared to IgA1. **c** Schematic of dimeric IgA2, formed via disulfide bonds between cysteines in the IgA tailpiece and the J (joining) chain. Disulfides also form directly between IgA tailpieces in the opposite heavy chains. Polymeric IgA (and IgM) containing J chain can be transcytosed across mucosal epithelial cells by the polymeric Ig receptor (pIgR) and released as secretory IgA or IgM, each of which is a covalent complex of a polymeric IgA or IgM with the pIgR ectodomain

in IgA2) are instead completely solvent-exposed, as seen in crystal structures of IgA1-Fc bound to either FcαRI or SSL7 [93, 94]. The diversity of N-glycan structures found on the IgA1 Fc region is much higher than observed for IgG, with primarily biantennary and some triantennary structures at the Asn263 site and larger triantennary or even tetraantennary structures at the tailpiece site; furthermore, IgA1 N-glycans are much more frequently sialylated than IgG N-glycans [95, 96]. In the case of secretory IgA, there are N-glycans present on both the secretory component (SC; this is the cleaved pIgR ectodomain) and the J chain. The SC contains seven N-glycosylation sites with a wide range of glycotopes that are predominantly sialylated, fully galactosylated biantennary glycans with unusual fucosylation linked to Gal rather than the core GlcNAc. J chain has a single N-glycosylation site with mostly sialylated biantennary structures, with core fucosylation about half of the time. Interestingly, the unusual SC N-glycans contain epitopes recognized by bacterial adhesins, allowing SIgA to function both in innate and adaptive immunity [97]. Furthermore, SC is anchored to the mucus layer at the epithelial surface via its glycans, improving its efficiency at blocking bacterial invasion by "immune exclusion" [98].

In addition to N-glycosylation, IgA1 (as well as IgD) antibodies are heavily O-glycosylated in the hinge region, with five potential O-glycosylation sites in the IgA1 hinge region

(Fig. 3). These O-glycans are composed of *N*-acetylgalactosamine (GalNAc), O-linked to serine or threonine residues, followed by a terminal Gal residue; either or both of the GalNAc or Gal groups can be sialylated. Undergalactosylation of IgA1 O-glycans, which leaves a terminal GalNAc residue, is linked to the autoimmune kidney disease IgA nephropathy [99, 100].

IgA N- and O-glycans play important biological roles, mediating interactions with a variety of immune receptors [96, 101]. Dimeric and polymeric IgA activate the lectin pathway of the complement cascade by binding to the carbohydrate recognition domain (CRD) of MBL [102]. Likewise, galectins 1, 3, and 8 all bind to IgA1 to varying degrees via its O-glycans [103, 104]. The asioaloglycoprotein receptor, involved in clearance primarily of IgA2 by the liver, binds IgA N-glycans [105, 106]. Transferrin receptor, in addition to its well-known role in iron homeostasis, binds IgA1 in a glycan-dependent manner and plays a role in mesangial cell activation by IgA1-containing immune complexes in IgA nephropathy [107, 108]. Finally, an IgA1/IgD receptor on T cells binds each isotype via hinge O-glycans [109]. Early reports suggested that variation in IgA glycosylation could impact its interaction with FcαRI, the primary IgA-specific activating receptor, although a detailed biophysical analysis indicated that IgA1 N-glycans did not significantly alter the affinity of IgA1 for FcαRI [110], even though the IgA1 N-glycans do contact FcαRI in the crystal structure [93].

In patients suffering from autoimmune IgA nephropathy as well as Sjögren's Syndrome, IgA levels are found to be elevated. In IgA nephropathy, changes in expression and activity of particular glycosyltransferases in B cells can lead to undergalactosylation of the O-glycans. The resulting terminal GalNAc residue (with or without a sialic acid) acts as a neo-epitope and can be recognized by anti-glycan IgG, forming circulating immune complexes that form deposits in the mesangial region of the glomerulus [99, 100]. Patients with Henoch-Schönlein purpura also have IgA1 with undergalactosylated O-glycans [111, 112]. In contrast, patients with Sjögren's syndrome have increased IgA levels, which are highly sialylated. This hypersialylation is proposed to prevent clearance of pathogenic IgA, while high O-galactosylated IgA was shown to activate complement more efficiently [100, 113–116]. A study of Ab responses in hyperimmunoglobulinemia D, where dysregulation of IgD production leads to high levels of IgD and, in most patients, increased IgA1, demonstrated that O-sialylation changes the affinity to IgD receptors on T cells to which IgD and IgA are competing for, skewing the balance and modulating T cell activation upon IgD receptor binding [117].

IgA is an unusual Ab isotype in that it can trigger either activating or inhibitory responses, primarily depending on its multimeric state. Monomeric IgA induces inhibitory ITAMi signaling via FcαRI, whereas multivalent antigen-bound IgA activates robust inflammatory responses through the same receptor via the classic ITAM pathway [17, 118]. Importantly, ITAMi inhibitory signaling by FcαRI and other ITAM receptors including FcγRIIA and FcγRIIIA can dampen signaling by activating IgE or IgG receptors [118–120]. In parallel, secretory IgA can engage SIGN-R1 (murine homolog of DC-SIGN) via N-glycan mannose residues to induce tolerogenic dendritic cells and the expansion of Tregs [121]. Given the ability of IgA to induce inhibitory and anti-inflammatory responses, it is perhaps not surprising that epidemiological studies have shown an inverse association of IgA levels and allergic airway disease [122]. Cholera toxin, a common mucosal adjuvant, was shown to suppress allergic inflammation in a mouse model of asthma via induction of antigen-specific secretory IgA [123].

IgM Glycosylation

IgM is often described as a natural Ab, produced in the early phase of the immune response with no requirement for class switch. The often low affinity and polyreactive IgM antibodies build up an effective first line of defense by forming pentamers and hexamers. In turn, this increases the avidity and allows for efficient activation of the complement system [12, 124]. IgM pentamers can be transcytosed to mucosal sites by pIgR since they contain the J chain, which is not present in hexamers [7]. IgM contains five N-glycosylation sites, three of which feature biantennary complex N-glycans (Asn171, Asn332, Asn395) and two adopt oligomannose structures (Asn402, Asn563) (Fig. 4) [7, 38]. Terminal GlcNAc of IgM was shown to bind MBL, while GlcNAc was not accessible when antigen is bound to IgM pentamers. That suggests that complement activation by antigen-IgM complexes is not possible. However, MBL might have an impact on removal of aggregated IgM [125]. The level of IgM N-sialylation was reported to influence internalization by T cells, leading to suppression of activation and downregulation of gene expression, e.g., pro-inflammatory cytokine expression (IFN-γ, IL-17, IL-6) [126]. More than that, follicular lymphoma (FL) B cell receptors (BCR) often sustain the IgM isotype with conserved *N*-oligomannose. Those FL IgM+ mannose-rich B cells were characterized to interact with tumor-associated monocyte-derived M2 macrophages in FL niches. Here, mannose structures engage DC-SIGN to further B cell activation [127].

IgD Glycosylation

IgD has three glycosylation sites in the Fc domain (Asn354, Asn445, Asn496), of which the Asn354 glycan is oligomannose and the other two are complex N-glycans (Fig. 5) [128–130]. The Asn354-oligomannose was characterized to be required for proper protein folding [38]. Like IgA1, IgD also has up to five

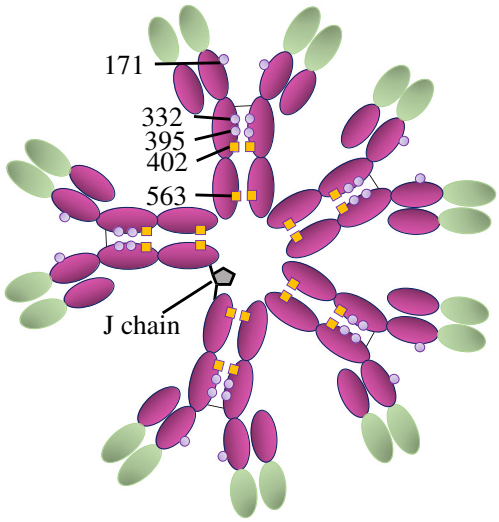

Fig. 4 Polymeric IgM domain structure and glycosylation. IgM, like IgA, has a C-terminal tailpiece extension on each heavy chain. The tailpiece can form disulfide bonds with other IgM heavy chains and with the J chain to form a pentameric polymeric IgM species. IgM can also form hexamers without J chain. The IgM constant domains contain five N-linked glycosylation sites, of which three are of the biantennary complex form (Asn171, Asn332, Asn395, *light violet circles*) and two of the oligomannose type (at Asn402, Asn563, *yellow squares*).

O-glycosylation sites on the hinge region [130]. Studies of IgD binding to Fc delta receptor (FcδR) showed that both GlcNAc and galactose of IgD Cδ1 and Cδ3 are implicated in binding to FcδR [131]. In autoimmune nephropathy and hyperimmunoglobulinemia D, IgD was observed to be more galactosylated and less sialylated when compared to healthy controls. This may contribute to enhanced FcδR engagement on T cells and to T cell activation, driving the pathogenesis of autoimmunity [117, 132].

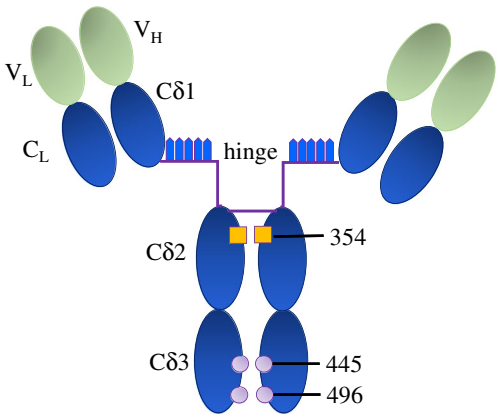

Fig. 5 IgD domain structure and glycosylation. IgD, like IgA1, features an O-glycosylated hinge region as well as N-glycans in the Fc region. The constant domains contain three N-linked glycosylation sites, of which two are of the biantennary complex form (Asn445 and Asn496, *light violet circles*) and one is a branching oligomannose at Asn354 (*yellow squares*). The hinge region contains five potential O-glycosylation sites (*blue prisms*)

Conclusions

It is well established that immunoglobulins contain conserved glycosylation sites in the Fc and Fab fragments which can be of the complex biantennary form or the oligomannose structures, dependent on the isotype of the immunoglobulin. Variations in the specific glycosylation form, such as sialylation, or even complete de-glycosylation, at each site may affect the interaction of the immunoglobulin with its receptors. Significantly more is known about the effects of (de)glycosylation of IgG isotypes on various diseases than the other non-IgG Ig isotypes, where modification of the Fc glycosylation pattern of IgG changes the affinity to type I FcγRs and type II FcRs and consequently modulates the pro- or anti-inflammatory properties of immunoglobulins. Therefore, the effects of changes in glycosylation are much better understood in the areas of autoimmunity and cancer therapy, where IgG is the dominant immunoglobulin, than in the field of allergy, where the more predominant immunoglobulin is IgE.

The understanding of the effects of glycosylation on allergic disease is not as well understood nor apparently as straight forward. The publication of seemingly contradictory findings about the role of glycosylation of IgE and its binding to its high affinity receptor has been frustrating. The confusion about the role of glycosylation of IgE is further made by the unexpected lack of a role for glycosylation and the interaction of IgE with the lectin receptor or low-affinity IgE receptor also known as CD23. The effect of glycosylation and the interaction of IgE with other lectin receptors such as Galectin-9 still needs to be assessed as well [133, 134].

The recent detailed report by Shade et al. [84•] appears to have moved us toward to a more definitive role for specific glycosylation of IgE at the asparagine-394 location of Cε3 as necessary for IgE interaction with the high affinity IgE receptor. This report identifies the one specific glycosylation site as the only crucial one for alteration in the binding of IgE to FcεRI. Confirmation by another laboratory and further elucidation of this effect in allergic development and mediation will be imperative in the next few years. In addition, an understanding of possible further effects on immune complex formation and aggregation outside of the FcεRI will be essential to define in order to move forward in using this information for the development of preventative and therapeutic treatments.

Lastly, the role of IgA and IgG as possible inhibitors or modulators of an allergic response adds another layer to the role of glycosylation of Ig to allergy and asthma. IgA levels have been directly correlated with the clinical success of allergy immunotherapy [122, 135, 136] though the mechanism and meaning of this association are not clear and thus the role of glycosylation of IgA in this area is not completely understood. However, it is known that monomeric IgA can induce

inhibitory ITAMi signals via FcαRI that downregulate inflammatory responses of IgE and IgG receptors [17]. Likewise, SIgA in the mucosa has been shown to suppress allergic asthma symptoms [123], and SIgA binding to DC-SIGN via N-glycan mannose residues was able to induce tolerogenic dendritic cell responses [121]. Further work is needed to better understand the potential of serum and secretory IgA for suppression of allergic reactions as well as asthma.

IgG has been proposed as a direct inhibitor of IgE-mediated anaphylaxis [30–32, 35], and animal studies have directly supported this inhibitory role for IgG [33, 137–139]. Animal studies have also strongly suggested that IgG can mediate anaphylaxis [140–144], though this too has not been directly proven in humans [140]. The effects of various glycosylations on the enhancing or diminishing nature of the severity of allergic reactions by these so-called blocking antibodies, as well as the possible mediation of anaphylaxis by IgG, are other areas of important investigation that should take place in the near future.

Compliance with Ethical Standards

Conflict of Interest Dr. Herr is a consultant for Mapp Biopharmaceutical, Inc. Dr. Strait, Ms. Epp, and Ms. Sullivan declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- Of importance

1. Wright JD, Chu H-M, Huang C-H, Ma C, Chang TW, Lim C. Structural and physical basis for anti-IgE therapy. Sci Rep. 2015;5:11581.
2. Furness AJS, Vargas FA, Peggs KS, Quezada SA. Impact of tumour microenvironment and Fc receptors on the activity of immunomodulatory antibodies. Trends Immunol. 2014;35(7):290–8.
3. Hodi F, O'Day S, McDermott D, Weber R, Sosman J, Haanen J, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
4. Yamane-Ohnuki N, Satoh M. Production of therapeutic antibodies with controlled fucosylation. MAbs. 2009;1(3):230–6.
5. Irani V, Guy AJ, Andrew D, Beeson JG, Ramsland PA, Richards JS. Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases. Mol Immunol. 2015;67(2):171–82.
6. Fanning GWLJ, Connor AM. Development of the immunoglobulin repertoire. Clin Immunol Immunopathol. 1996;79(1):1–14.
7. Schroeder HWJ, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010;125:S41–52.
8. Tonegawa S. Somatic generation of antibody diversity. Nature. 1983;302(14):575–81.
9. Mandel B. Neutralization of poliovirus: a hypothesis to explain the mechanism and the one-hit character of the neutralization reaction. Virology. 1976;69(2):500–10.
10. Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch JV. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. Cell. 2014;158(6):1243–53.
11. DiLillo DJ, Palese P, Wilson PC, Ravetch JV. Broadly neutralizing anti-influenza antibodies require Fc receptor engagement for in vivo protection. J Clin Invest. 2016;126(2):1–6.
12. Ricklin D, Hajishengallis G, Yang K, Lambris J. Complement—a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785–97.
13. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Daëron M, et al. Polymorphic variants for human IgG subclasses Specificity and affinity of human Fcg receptors and their polymorphic variants for human IgG subclasses. Blood. 2009;113(16):3716–25.
14. Vidarsson G, Dekkers G, and Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol*., 2014;5: no. OCT 1–17.
15. Kubota R, Niwa M, Satoh S, Akinaga K, Shitara, and Hanai N. Engineered therapeutic antibodies with improved effector functions. Cancer Sci. 2009;100:1566–1572
16. Chan AC, and Carter PJ. Therapeutic antibodies for autoimmunity and inflammation. Nat. Rev. Immunol. 2010;10:301–316
17. Blank U, Launay P, Benhamou M, Monteiro RC. Inhibitory ITAMs as novel regulators of immunity. Immunol Rev. 2009;232(1):59–71.
18. • Pincetic A, Bournazos S, DiLillo DJ, Maamary J, Wang TT, Dahan R, et al. Type I and type II Fc receptors regulate innate and adaptive immunity. Nat Immunol. 2014;15(8):707–16. **This article delineates how the IgG Fc domain modulates the affinity to Fc receptors (type I and II). It gives insights about the downstream pro-inflammatory or immunomodulatory pathways by engaging type I or type II FcRs**.
19. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol. 2008;8(1):34–47.
20. Boesch AW, Brown EP, Cheng HD, Ofori MO, Normandin E, Nigrovic PA, et al. Highly parallel characterization of IgG Fc binding interactions. MAbs. 2014;6(4):915–27.
21. Wang TT, Maamary J, Tan GS, Bournazos S, Davis CW, Krammer F, et al. Anti-HA glycoforms drive B cell affinity selection and determine influenza vaccine efficacy. Cell. 2015;162(1):160–9.
22. • Massoud AH, Yona M, Xue D, Chouiali F, Alturaihi H, Ablona A, et al. Dendritic cell immunoreceptor: a novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells. J Allergy Clin Immunol. 2014;133(3):853–863.e5. **This article documents that the sialylated fraction of IVIG induces regulatory T cells. Binding of sialylated IgG of IVIG to the C type lectin receptor DCIR ameliorates the airway hyperresponsiveness (AHR)**.
23. Isakov N. Immunoreceptor tyrosine-based activation motif (ITAM), a unique module linking antigen and Fc receptors to their signaling cascades. *J. Leukoc. Biol*. 1997;61:6–16.
24. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science. 2005;310(5753):1510–2.
25. Diebolder CA, Beurskens FJ, De Jong RN, Koning RI, Strumane K, Lindorfer MA, et al. Complement is activated by IgG hexamers assembled at the cell surface. Science. 2014;343(6176):1260–3.
26. Davies AM, Jefferis R, Sutton BJ. Crystal structure of deglycosylated human IgG4-Fc. Mol Immunol. 2014;62(1):46–53.
27. Larché M, Akdis C, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol. 2006;6:761–71.
28. Möbs C, Slotosch C, Loffler H, Jakob T, Hertl M, Pfutzner W. Birch pollen immunotherapy leads to differential induction of

regulatory T cells and delayed helper T cell immune deviation. J Immunol. 2010;184(4):2194–203.
29. Möbs C, Ipsen H, Mayer L, Slotosch C, Petersen A, Würtzen PA, et al. Birch pollen immunotherapy results in long-term loss of Bet v 1-specific TH2 responses, transient TR1 activation, and synthesis of IgE-blocking antibodies. *J. Allergy Clin. Immunol.* 2012;130(5):1108–1116.
30. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol. 2014;133(3):621–31.
31. Aalberse RC, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. J Immunol. 1983;130(2):722–6.
32. Mothes N, Heinzkill M, Drachenberg KJ, Sperr WR, Krauth MT, Majlesi Y, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: Reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. Clin Exp Allergy. 2003;33(9):1198–208.
33. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and FcγRIIb cross-linking. J Clin Invest. 2006;116(3):833–41.
34. Burton OT, Logsdon SL, Zhou JS, Medina-Tamayo J, Abdel-Gadir A, Noval Rivas M, et al. Oral immunotherapy induces IgG antibodies that act through FcγRIIb to suppress IgE-mediated hypersensitivity. J Allergy Clin Immunol. 2014;134(6):1310–1317.e6.
35. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. J Allergy Clin Immunol. 2015;135(5):1249–56.
36. Jefferis R, Lund J. Interaction sites on human IgG-Fc for FcγR: current models. Immunol Lett. 2002;82(1–2):57–65.
37. Parekh RB, Dwek RA, Sutton BJ, Fernandes DL, Leung A, Stanworth D, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature. 1985;316(6027):452–7.
38. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol. 2007;25:21–50.
39.• Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science (80-). 2006;313(5787):670–3. **This article confirms that Fc sialylation modulates the affinity to Fc gamma receptors and demonstrate that Fc sialylation switches IgG to anti-inflammatory properties**.
40. Sazinsky SL, Ott RG, Silver NW, Tidor B, Ravetch JV, Wittrup KD. Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. Proc Natl Acad Sci U S A. 2008;105(51):20167–72.
41.• Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and dectin-1. Nat Med. 2012;18(9):1401–6. **This article demonstrates that high galactosylation of IgG is crucial for the inhibitory property of IgG1 immune complexes, through promotion of an association between FcγRIIB and dectin-1 and ultimately results in a blockade of C5a effector functions in vitro and C5a-dependent inflammatory responses in vivo**.
42. Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Gloria Meng Y, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. J Biol Chem. 2002;277(30):26733–40.
43. Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, et al. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem. 2003;278(5):3466–73.
44. Natsume A, Wakitani M, Yamane-Ohnuki N, Shoji-Hosaka E, Niwa R, Uchida K, et al. Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded bispecific antibody comprising of two single-chain antibodies linked to the antibody constant region. J Biochem. 2006;140(3):359–68.
45. Kanda Y, Yamada T, Mori K, Okazaki A, Inoue M, Kitajima-Miyama K, et al. Comparison of biological activity among nonfucosylated therapeutic IgG1 antibodies with three different N-linked Fc oligosaccharides: the high-mannose, hybrid, and complex types. Glycobiology. 2007;17(1):104–18.
46. Mori K, Iida S, Yamane-Ohnuki N, Kanda Y, Kuni-Kamochi R, Nakano R, et al. Non-fucosylated therapeutic antibodies: the next generation of therapeutic antibodies. Cytotechnology. 2007;55(2–3):109–14.
47. DiLillo D, Ravetch JV. Differential Fc-receptor engagement drives an anti-tumor vaccinal effect. Cell. 2014;161(5):1035–45.
48. Anthony RM, Wermeling F, Karlsson MCI, Ravetch JV. Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci U S A. 2008;105(50):19571–8.
49. Anthony R, Kobayashi T, Wermeling F, Ravetch J. Intravenous gammaglobulin suppresses inflammation through a novel TH 2 pathway. Nature. 2011;475(7354):110–3.
50. Anthony RM, Nimmerjahn F. The role of differential IgG glycosylation in the interaction of antibodies with FcγRs in vivo. Curr Opin Organ Transplant. 2011;16(1):7–14.
51. Schwab I, Mihai S, Seeling M, Kasperkiewicz M, Ludwig RJ, Nimmerjahn F. Broad requirement for terminal sialic acid residues and FcγRIIB for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. Eur J Immunol. 2014;44(5):1444–53.
52. Schwab I, Biburger M, Krönke G, Schett G, Nimmerjahn F. IVIg-mediated amelioration of ITP in mice is dependent on sialic acid and SIGNR1. Eur J Immunol. 2012;42(4):826–30.
53. Fiebiger BM, Maamary J, Pincetic A, and Ravetch JV. Protection in antibody- and T cell-mediated autoimmune diseases by antiinflammatory IgG Fcs requires type II FcRs. *Proc. Natl. Acad. Sci*. 2015;112(18):E2385–94.
54. Rombouts Y, Ewing E, van de Stadt LA, Selman MHJ, Trouw LA, Deelder AM, et al. Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann. Rheum. Dis*. 2013;1–10.
55.• Ohmi Y, Ise W, Harazono A, Takakura D, Fukuyama H, Baba Y, et al. Sialylation converts arthritogenic IgG into inhibitors of collagen-induced arthritis. Nat Commun. 2016;7:1–12. **This article describes that low IgG Fc sialylation correlates with severity of rheumatoid arthritis in humans and mice. The article also demonstrates that IgG Fc sialylation suppresses the development of collagen-induced arthritis (CIA)**.
56. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med. 1995;1(3):237–43.
57. Bondt A, Selman MHJ, Deelder AM, Hazes JMW, Willemsen SP, Wuhrer M, et al. Association between galactosylation of immunoglobulin G and improvement of rheumatoid arthritis during pregnancy is independent of sialylation. J Proteome Res. 2013;12(10):4522–31.
58. Trbojević Akmačić I, Ventham NT, Theodoratou E, Vučković F, Kennedy NA, Krištić J, et al. Inflammatory bowel disease associates with proinflammatory potential of the immunoglobulin G glycome. Inflamm Bowel Dis. 2015;21(6):1237–47.

59. Dong X, Storkus WJ, Salter RD. Binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells. J Immunol. 1999;163:5427–34.
60. Arnold JN, Dwek RA, Rudd PM, Sim RB. Mannan binding lectin and its interaction with immunoglobulins in health and in disease. Immunol Lett. 2006;106(2):103–10.
61.• Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, et al. J Clin Invest. 2015;125(11):4160–70. **This report demonstrates that sialylation of IgG Fc reduces the binding affinity of IgG to C1q thus impairing its complement-dependent cytotoxicity and provides an FcγR-independent mechanism for sialylation to limit the inflammatory properties of IgG**.
62. Harre U, Lang SC, Pfeifle R, Rombouts Y, Frühbeißer S, Amara K, et al. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. Nat Commun. 2015;6:6651.
63. Collins ES, Galligan MC, Saldova R, Adamczyk B, Abrahams JL, Campbell MP, et al. Glycosylation status of serum in inflammatory arthritis in response to anti-TNF treatment. Rheumatology. 2013;52(9):1572–82.
64. Yamada K, Ito K, Furukawa JI, Nakata J, Alvarez M, Verbeek JS, et al. Galactosylation of IgG1 modulates FcγRIIB-mediated inhibition of murine autoimmune hemolytic anemia. J Autoimmun. 2013;47:104–10.
65. Ito K, Furukawa J, Yamada K, Tran NL, Shinohara Y, Izui S. Lack of galactosylation enhances the pathogenic activity of IgG1 but Not IgG2a anti-erythrocyte autoantibodies. J Immunol. 2014;192(2):581–8.
66. Oefner CM, Winkler A, Hess C, Lorenz AK, Holecska V, Huxdorf M, et al. Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs. J Allergy Clin Immunol. 2012;129(6):1647–55.
67. Hess C, Winkler A, Lorenz AK, Holecska V, Blanchard V, Eiglmeier S, et al. T cell-independent B cell activation induces immunosuppressive sialylated IgG antibodies. J Clin Invest. 2013;123(9):3788–96.
68. Mahan AE, Jennewein MF, Suscovich T, Dionne K, Tedesco J, Chung AW, et al. Antigen-specific antibody glycosylation is regulated via vaccination. PLoS Pathog. 2016;12(3):e1005456.
69. Ackerman ME, Crispin M, Yu X, Baruah K, Boesch AW, Harvey DJ, et al. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. J Clin Invest. 2013;123(5):2183–92.
70. Gould HJ, Sutton BJ. IgE in allergy and asthma today. Nat Rev Immunol. 2008;8(3):205–17.
71. Sutton BJ, Davies AM. Structure and dynamics of IgE-receptor interactions: FcERI and CD23/FcERII. Immunol Rev. 2015;268(1):222–35.
72. Dorrington KJ, Bennich HH. Structure-function relationships in human immunoglobulin E. Immunol Rev. 1978;41:3–25.
73. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, et al. The biology of IgE and the basis of allergic disease. Annu Rev Immunol. 2003;21(1):579–628.
74. Galli S, Tsai M. IgE and mast cells in allergic disease. Nat Med. 2013;18(5):693–704.
75. Berin MC. *Pathogenesis of IgE-mediated food allergy*. Clin Exp Allergy. 2015;45(10):1483–96. doi:10.1111/cea.12598.
76. Marichal T, Starkl P, Reber LL, Kalesnikoff J, Oettgen HC, Tsai M, et al. A beneficial role for immunoglobulin E in host defense against honeybee venom. Immunity. 2013;39(5):963–75.
77. Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R. Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity. 2013;39(5):976–85.
78. Starkl P, Marichal T, Gaudenzio N, Reber LL, Sibilano R, Tsai M, et al. IgE antibodies, FcεRIα, and IgE-mediated local anaphylaxis can limit snake venom toxicity. J Allergy Clin Immunol. 2016;137(1):246–257.e11.
79. Mukai K, Tsai M, Starkl P, Marichal T, and Galli SJ. IgE and mast cells in host defense against parasites and venoms. Semin Immunopathol. 2016;38(5):581–603. doi:10.1007/s00281-016-0565-1.
80. Young RJ, Owens RJ, Mackay GA, Chan CM, Shi J, Hide M, et al. Secretion of recombinant human IgE-Fc by mammalian cells and biological activity of glycosylation site mutants. Protein Eng. 1995;8(2):193–9.
81. Vercelli D, Helm B, Marsh P, Padlan E, Geha RS, Gould H. The B-cell binding site on human immunoglobulin E. Nature. 1989;338(6217):649–51.
82. Nettleton M, Kochan J. Role of Glycosylation Sites in the IgE Fc Molecule. Int Arch Allergy Immunol. 1995;107:328–9.
83. Helm B, Marsh P, Vercelli D, Padlan E, Gould H, Geha R. The mast cell binding site on human immunoglobulin E. Nature. 1988;331:180–3.
84.• Shade K-TC, Platzer B, Washburn N, Mani V, Bartsch YC, Conroy M, et al. A single glycan on IgE is indispensable for initiation of anaphylaxis. J Exp Med. 2015;212(4):457–67. **This article demonstrates that the oligomannose structure at Asn394 of IgE is substantial for IgE-binding to Fc epsilon RI, mast cell activation and development of anaphylaxis**.
85. Johansen F-E, Braathen R, Brandtzaeg P. Role of J chain in secretory immunoglobulin formation. Scand J Immunol. 2000;52(3):240–8.
86. Bakema JE, Van Egmond M. Immunoglobulin A: a next generation of therapeutic antibodies? MAbs. 2011;3(4):352–61.
87. Novak J, Julian BA, Mestecky J, Renfrow MB. Glycosylation of IgA1 and pathogenesis of IgA nephropathy. Semin Immunopathol. 2012;34(3):365–82.
88. Novak J, Julian BA, Tomana M, Mestecky J. IgA glycosylation and IgA immune complexes in the pathogenesis of IgA nephropathy. Semin Nephrol. 2008;28(1):78–87.
89. Mestecky J, Raska M, Julian BA, Gharavi AG, Renfrow MB, Moldoveanu Z, et al. IgA nephropathy: molecular mechanisms of the disease. Annu Rev Pathol Mech Dis. 2013;8(1):217–40.
90. Mostov KE. Transepithelial transport of immunoglobulins. Cardiovasc Res. 1994;12:63–84.
91. Hamburger AE, Bjorkman PJ, Herr AB. Structural insights into antibody-mediated mucosal immunity. Curr Top Microbiol Immunol. 2006;308:173–204.
92. Norderhaug IN, Johansen FE, Schjerven H, Brandtzaeg P. Regulation of the formation and external transport of secretory immunoglobulins. Crit Rev Immunol. 1999;19(5–6):481–508.
93. Herr AB, Ballister ER and Bjorkman PJ. Insights into IgA-mediated immune responses from the crystal structures of human FcαRI and its complex with IgA1-Fc. *Nature*. 2003;423:614–620.
94. Ramsland PA, Willoughby N, Trist HM, Farrugia W, Hogarth PM, Fraser JD, Wines BD. Structural basis for evasion of IgA immunity by Staphylococcus aureus revealed in the complex of SSL7 with Fc of human IgA1. Proc Nat Acad Sci USA. 2007;104(38):15015–56. http://www.pnas.org/content/104/38/15051
95. Mattu TS, Pleass RJ, Willis AC, Kilian M, Wormald MR, Lellouch AC, et al. The glycosylation and structure of human serum IgA1, Fab, and Fc regions and the role of N-glycosylation on Fca receptor interactions. J Biol Chem. 1998;273(4):2260–72.
96. Gomes MM, Herr AB. IgA and IgA-specific receptors in human disease: structural and functional insights into pathogenesis and therapeutic potential. Springer Semin Immunopathol. 2006;28(4):383–95.
97. Royle L, Roos A, Harvey DJ, Wormald MR, Van Gijlswijk-Janssen D, Redwan ERM, et al. Secretory IgA N- and O-

glycans provide a link between the innate and adaptive immune systems. J Biol Chem. 2003;278(22):20140–53.
98. Phalipon A, Cardona A, Kraehenbuhl JP, Edelman L, Sansonetti PJ, Corthésy B. Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. Immunity. 2002;17(1):107–15.
99. Suzuki H, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, Renfrow MB, et al. The pathophysiology of IgA nephropathy. J Am Soc Nephrol. 2011;22:1795–803.
100. Suzuki H, Moldoveanu Z, Hall S, Brown R, Vu H, Novak L, et al. IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. J Clin Invest. 2008;118(2):629–39.
101. Ben Mkaddem S, Christou I, Rossato E, Berthelot L, Lehuen A, Monteiro RC. IgA, IgA receptors, and their anti-inflammatory properties. In: Daeron M, Nimmerjahn F, editors. Fc receptors. Cham: Springer International Publishing; 2014. p. 221–35.
102. Roos A, Bouwman LH, van Gijlswijk-Janssen DJ, Faber-Krol MC, Stahl GL, Daha MR. Human IgA activates the complement system via the mannan-binding lectin pathway. J Immunol. 2001;167(5):2861–8.
103. Sangeetha SR, Appukuttan PS. IgA1 is the premier serum glycoprotein recognized by human galectin-1 since T antigen (Galß1 to 3GalNAc-) is far superior to non-repeating N-acetyl lactosamine as ligand. Int J Biol Macromol. 2005;35(5):269–76.
104. Cederfur C, Salomonsson E, Nilsson J, Halim A, Öberg CT, Larson G, et al. Different affinity of galectins for human serum glycoproteins: galectin-3 binds many protease inhibitors and acute phase proteins. Glycobiology. 2008;18(5):384–94.
105. Stockert RJ, Kressner MS, Collins JC, Sternlieb I, Morell AG. IgA interaction with the asialoglycoprotein receptor. Proc Natl Acad Sci U S A. 1982;79(20):6229–31.
106. Rifai A, Fadden K, Morrison SL, Chintalacharuvu KR. The N-glycans determine the differential blood clearance and hepatic uptake of human immunoglobulin (Ig)A1 and IgA2 isotypes. J Exp Med. 2000;191(12):2171–82.
107. Moura IC, Centelles MN, Arcos-Fajardo M, Malheiros DM, Collawn JF, Cooper MD, et al. Identification of the transferrin receptor as a novel immunoglobulin (Ig)a1 receptor and its enhanced expression on mesangial cells in Iga nephropathy. J Exp Med. 2001;194(4):417–26.
108. Moura IC. Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy. J Am Soc Nephrol. 2004;15(3):622–34.
109. Rudd PM, Fortune F, Patel T, Parekh RB, Dwek RA, Lehner T. A human T-cell receptor recognizes 'O'-linked sugars from the hinge region of human IgA1 and IgD. Immunology. 1994;83(1):99–106.
110. Gomes MM, Wall SB, Takahashi K, Novak J, Renfrow MB, Herr AB. Analysis of IgA1 N-glycosylation and its contribution to FcαRI binding. Biochemistry. 2008;47(43):11285–99.
111. Saulsbury FT. Alterations in the O-linked glycosylation of IgA1 in children with Henoch-Schonlein purpura. J Rheumatol. 1997;24(11):2246–9.
112. Lau KK, Wyatt RJ, Moldoveanu Z, Tomana M, Julian BA, Hogg RJ, et al. Serum levels of galactose-deficient IgA in children with IgA nephropathy and Henoch-Schönlein purpura. Pediatr Nephrol. 2007;22(12):2067–72.
113. Kokubo T, Yoshiyuki H, Iwase H, Horii A, Tanaka A, Nishikido J, et al. Evidence for Involvement of IgA 1 hinge glycopeptide in the IgA1-IgA1 interaction in IgA nephropathy. J Am Soc Nephrol. 1997;8:915–9.
114. Yanagihara T, Brown R, Hall S, Moldoveanu Z, Goepfert A, Tomana M, et al. In vitro-generated immune complexes containing galactose-deficient IgA1 stimulate proliferation of mesangial cells. Results Immunol. 2012;2:166–72.
115. Horynova M, Raska M, Clausen H, Novak J. Aberrant O-glycosylation and anti-glycan antibodies in an autoimmune disease IgA nephropathy and breast adenocarcinoma. Cell Mol Life Sci. 2013;70(5):829–39.
116. Barratt J, Smith AC, Feehally J. The pathogenic role of IgA1 O-linked glycosylation in the pathogenesis of IgA nephropathy. Nephrology. 2007;12(3):275–84.
117. De Wolff JF, Dickinson SJ, Smith AC, Molyneux K, Feehally J, Simon A, et al. Abnormal IgD and IgA1 O-glycosylation in hyperimmunoglobulinaemia D and periodic fever syndrome. Clin Exp Med. 2009;9(4):291–6.
118. Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffié C, et al. Identification of FcαRI as an inhibitory receptor that controls inflammation: dual role of FcRγ ITAM. Immunity. 2005;22(1):31–42.
119. Aloulou M, Mkaddem SB, Biarnes-Pelicot M, Boussetta T, Souchet H, Rossato E, et al. IgG1 and IVIg induce inhibitory ITAM signaling through FcγRIII controlling inflammatory responses. Blood. 2012;119(13):3084–96.
120. Mkaddem SB, Hayem G, Jönsson F. Shifting FcγRIIA-ITAM from activation to inhibitory configuration ameliorates arthritis. J Clin Invest. 2014;124(9):3945–59.
121. Diana J, Moura IC, Vaugier C, Gestin A, Tissandie E, Beaudoin L, et al. Secretory IgA induces tolerogenic dendritic cells through SIGNR1 dampening autoimmunity in mice. J Immunol. 2013;191(5):2335–43.
122. Gloudemans AK, Lambrecht BN, and Smits HH. Potential of immunoglobulin A to prevent allergic asthma. Clin Dev Immunol. 2013;2013:542091.
123. Smits HH, Gloudemans AK, van Nimwegen M, Willart MA, Soullie T, Muskens F, et al. Cholera toxin B suppresses allergic inflammation through induction of secretory IgA. Mucosal Immunol. 2009;2(4):331–9.
124. Kishore U, Gupta SK, Perdikoulis MV, Kojouharova MS, Urban BC, Reid KBM. Modular organization of the carboxyl-terminal, globular head region of human C1q A, B, and C chains. J Immunol. 2003;171(2):812–20.
125. Arnold JN, Wormald MR, Suter DM, Radcliffe CM, Harvey DJ, Dwek RA, et al. Human serum IgM glycosylation: Identification of glycoforms that can bind to Mannan-binding lectin. J Biol Chem. 2005;280(32):29080–7.
126. Colucci M, Stöckmann H, Butera A, Masotti A, Baldassarre A, Giorda E, et al. Sialylation of N-linked glycans influences the immunomodulatory effects of IgM on T cells. J Immunol. 2015;194(1):151–7.
127. Amin R, Mourcin F, Uhel F, Pangault C, Ruminy P, Dupré L, et al. DC-SIGN-expressing macrophages trigger activation of mannosylated IgM B-cell receptor in follicular lymphoma. Blood. 2015;126(16):1911–20.
128. Melliss SJ, Baenzigere U. Structures of the oligosaccharides present at the three asparagine-linked glycosylation sites of human IgD. J Biol Chem. 1983;258(19):11546–56.
129. Mellis SJ, Baenziger JU. Structures of the O-glycosidically linked oligosaccharides of human IgD. J Biol Chem. 1983;258(19):11557–63.
130. Arnold JN, Radcliffe CM, Wormald MR, Royle L, Harvey DJ, Crispin M, et al. The glycosylation of human serum IgD and IgE and the accessibility of identified oligomannose structures for interaction with mannan-binding lectin. J Immunol. 2004;173:6831–40.
131. Amin AR, Tamma SM, Oppenheim JD, Finkelman FD, Kieda C, Coico RF, et al. Specificity of the murine IgD receptor on T cells is for N-linked glycans on IgD molecules. Proc Natl Acad Sci. 1991;88(20):9238–42.
132. Smith AC. O-glycosylation of serum IgD in IgA nephropathy. J Am Soc Nephrol. 2006;17(4):1192–9.

133. Niki T, Tsutsui S, Hirose S, Aradono S, Sugimoto Y, Takeshita K, et al. Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE-antigen complex formation. J Biol Chem. 2009;284(47):32344–52.
134. Nakakita SI, Itoh A, Nakakita Y, Nonaka Y, Ogawa T, Nakamura T, et al. Cooperative interactions of oligosaccharide and peptide moieties of a glycopeptide derived from IgE with galectin-9. J Biol Chem. 2016;291(2):968–79.
135. Wright BL, Kulis M, Orgel KA, Burks AW, Dawson P, Henning AK, et al.. Component-resolved analysis of IgA, IgE, and IgG4 during egg OIT identifies markers associated with sustained unresponsiveness. Allergy 2016. doi:10.1111/all.12895.
136. Jay DC, Nadeau KC. Immune mechanisms of sublingual immunotherapy. Curr Allergy Asthma Rep. 2014;14(11):1–7.
137. Burton OT, Noval-Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. Immunity. 2014;41(1):141–51.
138. Allen LC, Kepley CL, Saxon A, Zhang K. Modifications to an Fcgamma-Fcepsilon fusion protein alter its effectiveness in the inhibition of FcepsilonRI-mediated functions. J Allergy Clin Immunol. 2007;120(2):462–8.
139. Tam SW, Demissie S, Thomas D, Daëron M. A bispecific antibody against human IgE and human FcgammaRII that inhibits antigen-induced histamine release by human mast cells and basophils. Allergy. 2004;59(7):772–80.
140. Finkelman FD, Khodoun MV, and Strait R. Human IgE-independent systemic anaphylaxis. J Allergy Clin Immunol. 2016;137(6):1674–80. doi:10.1016/j.jaci.2016.02.015.
141. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. J Allergy Clin Immunol. 2002;109(4):658–68.
142. Jönsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, et al. Mouse and human neutrophils induce anaphlaxis. J Clin Invest. 2011;121(4):1484–96. doi:10.1172/JCI45232.
143. Beutier H, Gillis CM, Iannascoli B, Godon O, England P, Sibilano R, et al. IgG subclasses determine pathways of anaphylaxis in mice. J Allergy Clin Immunol. 2016;pii:S0091-6749(16)30158–0. doi:10.1016/j.jaci.2016.03.028.
144. Khodoun MV, Strait R, Armstrong L, Yanase N, Finkelman FD. Identification of markers that distinguish IgE- from IgG-mediated anaphylaxis. Proc Natl Acad Sci U S A. 2011;108(30):12413–8.