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IgE Glycosylation in Health and Disease

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Abstract

IgE are absolutely required for initiation of allergy reactions, which affect over 20% of the world's population. IgE are the least prevalent immunoglobulins in circulation with 12-h and 2-day half-lives in mouse and human serum, respectively, but an extended tissue half-life of 3-weeks bound to the surface of mast cells by the high affinity IgE receptor, FcεRI (Gould and Sutton 2008). Although the importance of glycosylation to IgG biology is well established, less is known regarding the contribution of IgE glycosylation to allergic inflammation. IgE has seven and nine N-linked glycosylation sites distributed across human and murine constant chains, respectively. Here we discuss studies that have analyzed IgE glycosylation and its function, and how IgE glycosylation contributions to health and disease.

1 IgE Biology in Health and Disease

IgE antibody induced inflammation is nearly synonymous with allergic disease, which affects one in five individuals worldwide. IgE are central to the mechanisms of immediate type hypersensitivity reactions. These antibodies are also somewhat enigmatic given that a comprehensive understanding of IgE biology has yet to be established. IgE antibodies are the subclass found least in the serum compared to IgM, IgG and IgA, with a concentration of 50–100 ng/ml, markedly lower than IgG (5–10 mg/ml). Compared to its long tissue half life of weeks, IgE serum half life is only 2–3 days (Vieira and Rajewsky 1988). Structurally, it differs from IgG in that it contains four domains in the constant region (Cε1–4, Fig. 1a) compared to three in IgG (Gould et al. 2003). In the constant domains, receptor binding occurs, yielding IgE effector functions. The IgE receptor, FcεRI, has strikingly high affinity for the antibody, $\sim 10^{11} \text{ M}^{-1}$, and is the source of the long tissue phase of IgE (Chang 2000). FcεRI coats the surface of cells including mast cells, basophils, as well as dendritic cells, Langerhans cells and eosinophils in humans. Notably, there are structural variations in FcεRI present in these cell types which result in differential effects upon binding. Mast cells and basophils express FcεRI as a tetramer, $\alpha\beta\gamma_2$, with the α unit responsible for IgE binding at the Cε3 domain. The β chain of the receptor is mainly responsible for signal amplification and is notably absent in the trimeric form of FcεRI, $\alpha\gamma_2$ present on dendritic cells, Langerhan cells, and eosinophils. The γ subunit is critical as it is the main structure responsible for orchestrating intra-cellular signaling pathways (Kraft and Kinet 2007). Allergen binding to IgE and subsequent cross linking of FcεRI receptors activates ITAMs

and subsequent downstream signaling. Specifically, FcεRI is responsible for the canonical symptoms of immediate type hypersensitivity reactions detailed below.

There is a second IgE receptor, known as both CD23 and FcεRII. This is often referred to as the “low affinity” receptor given that the affinity coefficient, 10^6 – 10^7 M⁻¹, is dwarfed by that of FcεRI. This immunoglobulin receptor has a trimeric C-type lectin like globular domains extracellularly, one transmembrane domain and a short cytoplasmic domain. Interestingly, CD23 can be either membrane bound or soluble forms. CD23 is expressed across a variety of cells including T and B lymphocytes, follicular dendritic cells, and epithelial cells (Armitage et al. 1989; Rieber 1993; Yu et al. 2003). Upon activation, membrane bound CD23 causes suppression of IgE production by B cells. Secreted CD23 can exist in monomeric or trimeric structures and can either down or up-regulate IgE synthesis, depending of its oligomerization (McCloskey et al. 2007). Taken together, CD23 is a potent factor in maintaining IgE homeostasis. Additional cells including antigen-presenting and gut epithelial cells utilize CD23 for transcytosis cross epithelial barriers and subsequent allergen presentation to the immune system (Palaniyandi et al. 2011).

As mentioned above, IgE antibodies are present in very low levels in the serum, particularly compared to other immunoglobulin subclasses. This suggests that control of IgE production is very tightly regulated. After initial and successful VDJ recombination yields a functional B cell receptor, production of eventually IgE occurs as a result of IgM+ and IgD+ B cell activation. Tissue Th2 cells as well as Tfh and Th2-like cells in lymphoid organs have been identified as possible sources of cytokines to promote B cell preferential class switch to IgE (Reinhardt et al. 2009). Class switch recombination (CSR) to IgE is triggered upon ligation of CD40, certain toll like receptors or BAFF in the context of IL4 exposure (Geha et al. 2003). Transcription promoters lie just upstream of DNA switch (S) regions and are responsible for “selection” of particular S recombinations and resultant specific antibody subclass production. In the case of IgE, ligation of the IL-4 receptor triggers STAT6 activation which is phosphorylated and translocates to the nucleus, binding the IgE promoter (Hebenstreit et al. 2006). For counter-regulation, Bcl-6 can bind sites in the IgE promoter which overlap with STAT6 binding loci, leading to inhibition of IgE class switch (Harris 1999). Notably, class switch to IgE can occur in two possible ways- direct switch from IgM to IgE or IgM to IgG and finally IgE. In B cells stimulated by IL4 and LPS, IgG1 was produced initially and IgE several hours later (Mandler 1993). Wesemann et al. then showed similar sequential production in the context of IL4 and anti-CD40 stimulation (Wesemann et al. 2011). Preferential class switch between IgG and IgE is determined by various factors. Intriguingly, the IgG1 switch region contains the most target sequences for AID, the enzyme responsible for class switching, and IgE the fewest. The IgG1 switch region may also block accessibility to the IgE S region (Misaghi et al. 2013) and accessibility to the IgE S region also seems to occur with a delayed rate (Wesemann et al. 2012). Taken together, control of class switching to IgE is an important means through which the antibody level can be tightly controlled. In addition, and critically, sequential class switch to IgE results in high-affinity antibody, perhaps owing to the longer timeline and persistent exposure to AID, also responsible for affinity maturation. Direct class switch from IgM directly to IgE results in lower affinity antibodies (He et al. 2015). This has significant functional repercussions.

When considering where and when IgE class switch recombination, production and memory occur there are some puzzling curiosities. First and foremost, there seems to be an overall lack of IgE memory B cells found in immunologic tissues (Yang, Sullivan, and Allen 2012). Typically, in lymphoid germinal centers (GCs), B cell proliferation occurs with concurrent affinity maturation and positive selection via interactions with follicular dendritic cells and Tfh cells. Therefore, GC interactions are critical for the eventual development of memory B cells and plasma cells resulting in long lived antibody memory (Janeway et al. 2001). Murine studies demonstrated while IgE⁺ B cells were not found in spleen or lymph nodes, plasma cells represented the preponderance of IgE producing cells in these animals. These cells were noted to have undergone sequential switching, with an IgG intermediate, followed by rapid differentiation to IgE expressing plasma cells (Erazo et al. 2007). He et al. were able to show that GC IgE⁺ cells do exist but that they expressed significantly lower expression of surface antibody, weak BCR signaling and did not populate GC light zone. This suggested that IgE⁺ cells in germinal centers were dying prematurely in the maturation process (He et al. 2013). As noted previously, sequential class switching from IgG to IgE seems to provide the most significant source of high affinity antibody. Thus, IgG⁺ B cells have been hypothesized as the source of IgE memory. B220⁺ CD138⁻ IgG1⁺ B cells have been identified as the cell population responsible for significant IgE memory production (He et al. 2013). Further, the same group identified a murine IgG1 memory B cell subset which was able to generate IgE⁺ plasma cells and produce high affinity, functional antibody, able to trigger anaphylaxis in a murine model (He et al. 2017). Therefore, IgG⁺ B cells serve as a main reservoir for IgE memory along with IgE⁺ plasma cells. It is notable that direct class switch from IgM to IgE results in low affinity antibody production and short lived GC IgE⁺ cells. Together, these cells and mechanisms are the source of both high and low affinity IgE antibodies and maintain the functional pool of IgE (He et al. 2015).

IgE antibodies have gained significant attention as the incidence of allergic diseases has increased substantially. The World Allergy Organization estimates that a staggering 30–40% of the world's population suffers from one or more allergic conditions (Pawankar et al. 2011). This contributes to substantial burden of illness and the economic implications are massive, thought to cost billions of dollars in medical visits and lost productivity (2010). Atopic diseases are considered to be one of the principle manifestations of IgE driven pathology. As discussed above, allergen specific IgE antibodies are bound to the surface of mast cells and basophils via Fc binding to the FcεRI receptor. Allergen cross linking of cell bound IgE results in cellular degranulation and canonical symptoms of allergic inflammation. Immediate release of mediators causes rapid onset of vascular dilation and permeability, bronchoconstriction, leukocyte extravasation and smooth muscle contraction. Cellular activation also results in synthetic processes which generate mediators of “late phase” reactions (Holgate 1982). These processes result in clinical symptoms including potentially fatal anaphylaxis. Allergen specific IgE can be utilized for diagnostics in allergy and is a target of therapies in asthma, environmental/food/venom allergies and atopic dermatitis.

Yet, significant IgE biology extends beyond allergic inflammation and atopic diseases. It has been implicated in protection against parasitic infection and venom exposures. Additionally, several non-allergic diseases also have IgE implicated in their pathogenesis. As previously

described, IgE antibodies coat the surface of mast cells via the FcεRI receptor. Mast cells localize to vascularized tissues and are especially concentrated at mucosal and skin surfaces (Gurish and Austen 2012). In the context of parasitic infections, both parasite specific and non-specific IgE antibodies markedly increase (Anthony et al. 2007). There is speculation that these increased IgE levels may trigger mast cell activation at mucosal surfaces leading to various biologic functions which ultimately lead to worm expulsion. In general, specific mechanisms responsible for these actions are not well elucidated (Fitzsimmons et al. 2014). In fact, in mice infected with *Schistosoma mansoni*, significant manipulations of IgE levels did not specifically modulate host pathology from infection, though may have contributed to liver pathology (Jankovic 1997). The specific biologic relevance of IgE with regard to parasitic immunity remains to be deciphered.

As they are in parasitic diseases, mast cells are also felt to confer innate protection against toxic proteins such as those from insects and snakes. In murine models, venom proteins administered in vivo trigger mast cells to release pre-formed heparin that contribute to toxin neutralization (Mukai et al. 2016). Mast cell proteases have also been shown to directly reduce toxicity of venoms (Metz et al. 2006). Specific IgE antibodies against insect venoms are used as diagnostic tools to identify persons allergic to these proteins who may be at risk of anaphylaxis with subsequent exposures. It has therefore been presumed that anti-venom IgE antibodies are pathogenic. However, with consideration of mast cell protective functions in the context of toxin exposures, several groups have questioned whether venom specific IgE may play a role in facilitating mast cell protective responses. Mice immunized with whole bee venom generated both IgG1 and IgE venom specific antibodies. Immunized mice were more resistant to subsequent exposure to lethal dosing of the same venom. IgE deficient and FcεRF^{-/-} mice did not demonstrate this same resistance. Passive immunization with bee venom antibody containing serum also provided protection upon bee venom administration in IgE deficient mice (Marichal et al. 2013). In mice who were administered repeated immunization with phospholipase A2, known to be a potent allergenic component of honeybee venom, protection was established against subsequent lethal honeybee venom. This protection did not occur in mice who were B cell deficient or in those with dysfunctional FcεRI (Palm et al. 2013). Given that most humans with venom specific IgE have never had anaphylaxis in the context of exposure, Mukai et al. speculate that improved protection against venomous exposures may be an “ancient and fundamental” function of IgE and mast cells (Mukai et al. 2016).

IgE has largely been implicated in allergic diseases. However, their presence in diseases primarily involving immune system dysregulation have raised questions as to a more global immunologic role for IgE antibodies. As an example, decades ago, self-reactive IgE antibodies and IgE containing immune complexes were found in patients with systemic lupus erythematosus (SLE) (Miyawaki S 1973). It has been well established that self reactive immune complexes lead to production of type I interferons by plasmacytoid dendritic cells in SLE (Ronnblom 2001). Recently, Henault et al. demonstrated that the presence of DNA-IgE autoantibodies in human SLE caused self-DNA triggered TLR9 activation in plasmacytoid dendritic cells facilitated by the interaction of IgE/FcεRI. This lead to markedly increased production of IFN-α (Henault et al. 2016). They speculate that self dsDNA-IgE complexes may contribute to increased loss of tolerance and exacerbation

of pathology as a result. In fact, higher levels of self-reactive dsDNA-IgE in the serum correlated with disease severity.

Immune deficiencies provide another clinical example in which IgE is elevated in the context of immune dysregulation. Hyper-IgE (HIES) or Job syndrome is a complex disease in which IgE levels can reach levels of more than 20,000 IU/L, orders of magnitude higher than normal (Sowerwine et al. 2012). The disease presents with a mysterious combination of symptoms including *Staph aureus* abscesses, eczematous rashes, recurrent sinopulmonary infections, chronic candida infections, eosinophilia, fungal infections, and EBV and VZV infections (Siegel et al. 2011). Notably, patients with HIES also present with osseous abnormalities including craniofacial dysmorphism, delayed tooth eruption and high arched palate (Nieminen et al. 2011). Malignancy affects significant numbers of these people as well, involving both blood and solid organs. These clinical manifestations are the result of STAT3 mutations on chromosome 17. This leads to abnormalities in the IL-6 pathway (Holland et al. 2007) and, significantly, marked inhibition of the Th17 immunologic response (Milner et al. 2008). The specific mechanisms responsible for the marked elevation in IgE levels is not fully known. Interestingly, Liu et al. recently showed that STAT3 deletion in keratinocytes of mice with eczema-like inflammation surprisingly lead to a marked elevation in IgE levels (Liu 2017). Cutaneous inflammation in this model was induced by *S. aureus*, which is also responsible for significant disease pathology in patients with HIES. Other immune deficiencies including DOCK8 deficiency, TYK2 deficiency, Omenn's syndrome, Wiscott-Aldrich syndrome, DiGeorge syndrome, Netherton and IPEX syndromes have elevated IgE as a hallmark (Mogensen 2016). Once again, how IgE occurs in and affects these conditions is poorly understood and studies are ongoing to improve understanding.

2 IgE Structure

IgE and IgG are perhaps the most clinically relevant immunoglobulin classes. Further, there are a number of superficial similarities shared between these two molecules. Both are monomeric immunoglobulins comprised of two identical heavy chains and two identical light chains, and exert their canonical effector functions through interactions with Fc receptors expressed by innate cells. IgE heavy chains have four constant domains, and a single variable domain, and light chain has single constant and variable domains. Both IgE and IgG have been shown to adopt open and closed structures, that result in different Fc receptor binding profiles. While a single N-linked glycosylation site is present in all IgG, IgE is the most heavily glycosylated monomeric antibody.

Seven N-linked glycosylation sites are distributed across the constant domains of the human IgE heavy chains, while 9 sites are found on mouse IgE (Fig. 1a). One site, at asparagine (N) 394 on human IgE, is conserved across all mammalian IgE. The glycan that occupies the conserved site has an oligomannose structure (Fig. 1a, b). Indeed, this site is reported to be orthologous to the IgG glycosylation site (Wurzberg et al. 2000). Indeed, crystal structure of IgE and IgG Fcs feature a glycan, which is consist with glycans having a fixed position in the molecule. On human IgE, the remaining sites barring N383, which is unoccupied, contain mono- and di-sialylated complex biantennary glycans. Indeed, the published data on

IgE from allergic patients is currently lacking, as analysis are restricted to IgE from serum of non-atopic (Plomp et al. 2013), hyperimmune (Arnold et al. 2004; Plomp et al. 2013), IgE myeloma (Plomp et al. 2013), and hyper IgE (Wu et al. 2016) patients, and recombinant sources (Shade et al. 2015). The IgE molecule adopts a bent horseshoe structure, where the Fab and Fc domains are folded towards each other. Further, the IgE Fab is rigid, unlike the flexible IgG hinge. It is thought the IgE molecule can rotate around its central axis reciprocally refolding. To date, no studies have detected the presence of O-linked glycosylation in IgE (Arnold et al. 2004; Plomp et al. 2013; Wu et al. 2016).

3 IgE N394 Glycosylation Site and Oligomannose Glycan

Important structural studies have revealed that IgE binds to FcεRI at the Cε3 domains and CD23 at both Cε3 and Cε4 domains of the Fc (Garman et al. 2000; Holdom et al. 2011). The interaction to each receptor is dependent on IgE conformation and is mutually exclusive, with FcεRI binding IgE in an open and CD23 in a closed conformation. The changes in conformation upon binding to one receptor disables binding to the other receptor. Over the past decade, strategies to alleviate IgE-mediated allergic diseases have generally been devised to prevent IgE-FcεRI interaction. This includes an anti-IgE therapeutic antibody Omalizumab that binds to an epitope overlapping FcεRI and directly prevents binding of serum free IgE to FcεRI. Another engineered protein inhibitor, designed ankyrin repeat protein (DARPin E2_79) also binds to the Cε3 and accelerates the dissociation of IgE from FcεRI on preformed complexes through an allosteric mechanism (Kim et al. 2012). Recently, a single-domain antibody derived from llama, sdab 026, have shown to inhibit IgE-FcεRI interaction by locking IgE in a closed conformation similar to CD23 binding and prevents the open conformation that allows the FcεRI interaction (Jabs et al. 2018).

Crystal structures of human IgE Fc also showed the glycans attached to N394 on the Cε3 domains occupy the cavity between two Fcs. This glycosylation site is evolutionary conserved across mammalian species and homologous to the key glycan attached on the IgG Fc (Jabs et al. 2018; Holdom et al. 2011; Wan et al. 2002; Garman et al. 2000; Sondermann et al. 2000). Previous studies have shown that IgG Fc glycans are critical for maintaining antibody structural conformation. Removal of the Fc glycans results in a more closed conformation in the IgG Fcs and forfeits the functional conformation important for Fcγ receptor binding (Krapp et al. 2003; Feige et al. 2009). In comparison, conflicting studies between 1980 and early 2000 that used aglycosylated IgE produced from *Escherichia coli* or deglycosylated IgE or IgE Fc after enzymatic treatments make it hard to determine whether glycosylation modulates IgE functions.

The earliest studies using *E. coli*-derived recombinant IgE Fc or Cε3 domains revealed that aglycosylated IgE binds to FcεRI and can elicit IgE-mediated inflammation (Helm et al. 1998a, b; Henry et al. 2000). However, these proteins require additional manipulation including solubilization and refolding after recovery from bacterial insoluble inclusion bodies, which may compromise the native state of conformation. Later studies using enzymes to deglycosylate IgE Fc produced from mammalian cell lines or IgE derived from myeloma or serum showed incoherent results. While one study showed that deglycosylated IgE Fc retained binding to FcεRI with minimal difference in a radioligand competition

binding assay (Basu et al. 1993), another showed a 4-fold lower binding affinity to FcεRI by SPR (Hunt et al. 2005). These results deviated from another study that showed significant impairment to FcεRI binding upon enzymatic deglycosylation (Bjorklund et al. 1999, 2000). On the other hand, all studies that genetically disrupted the N-linked glycosylation site N394 on IgE or IgE Fc by mutating asparagine-394 to glutamine (N394Q) or threonine (N394T), or alternatively disrupting the site by substituting the third amino acid in the Asn-X-Ser/Thr sequon threonine-396 to alanine (T396A), all abolished FcεRI binding and the subsequent IgE-mediated degranulation (Sayers et al. 1998; Shade et al. 2015; Jabs et al. 2018).

The functional significance of the glycan on IgE Fc was further exemplified by the in vivo work using mouse models (Shade et al. 2015). The equivalent of N394 is N384 in mouse. In mouse, genetic mutation (N384Q or T386A) or enzymatic digestion of IgE by PNG to remove all N-glycans lost the ability to bind FcεRI in vivo in ear mast cells and in vitro in ELISA or cell-based binding assays. As expected, IgE lacking N384 glycans were also unable to initiate immediate anaphylaxis in a passive cutaneous anaphylaxis mouse model. In a reciprocal experiment where all N-linked glycosylation sites were mutated except N384 (N384 only), this IgE was still able to elicit anaphylactic response similar to the WT, demonstrating that the glycan on the conserved site in IgE Fc is absolutely critical for IgE effector functions.

In contrast to the complex-type glycans on IgG Fc, all studies including crystal structure and glycopeptide mass spectrometry, uniformly identified the glycan attached to N394 is of oligomannose structures, with 2–9 mannose residues attached to two GlcNAc and Man₅GlcNAc₂ being the major form. IgE were sourced from monoclonal myeloma-derived, recombinant from different mammalian cell lines, serum of non-diseased donors, and serum of patients with hyperimmune conditions, IgE myeloma, atopic dermatitis or hyper IgE syndrome (Dorrington and Bennich 1978; Wan et al. 2002; Arnold et al. 2004; Holdom et al. 2011; Plomp et al. 2013; Shade et al. 2015; Wu et al. 2016). N-linked glycans of secreted mammalian proteins are typically processed to an intermediate oligomannose structure before further processed to hybrid and complex glycans in the lumen of the ER and the Golgi. As mentioned, all other N-linked sites of IgE are composed of complex glycans. Thus, it is unique for glycans at N394 to retain the oligomannose structures from being converted to complex type. The mouse IgE homologue also harbors oligomannose glycans at the conserved site.

The functional significance of glycans at N394 and its exclusive composition of oligomannose structures provide a unique therapeutic opportunity to target this glycan. Unlike some of the other glycosidases that cleave all types of N-linked glycans, Endoglycosidase F1 selectively hydrolyses the oligomannose and hybrid structures and leaves complex structures unaffected. Mirroring genetically disruption of N394 glycosylation site on IgE, EndoF1-treated recombinant or native allergic human IgE lost its ability to bind FcεRI-expressed cells and elicit degranulation in mast cells. Mouse IgE digested with EndoF1 was also unable to initiate anaphylaxis in vivo (Shade et al. 2015). Being buried in the interstitial space between Fcs, the importance of N394 glycan is likely to maintain IgE protein structure as IgG glycans (Feige et al. 2009). Circular dichroism that measures the secondary structure of proteins revealed a shift in spectrum upon the loss of

oligomannose glycans in IgE after EndoF1 treatment, reflecting changes in IgE three-dimensional conformation (Shade et al. 2015). This change likely explains the loss of functional protein folding necessary to bind FcεRI and initiate allergic inflammation.

In contrast to the requirement of IgE glycans in FcεRI interaction, deglycosylated myeloma IgE was reported to augment binding to CD23 on B cells (Vercelli et al. 1989). This finding reinforces the idea that IgE conformation decides the exclusive interaction with FcεRI or CD23. Perhaps the loss of glycans on IgE leads to a more closed conformation, thus promoting CD23 and preventing FcεRI binding.

4 Other N-Linked Glycosylation Sites on IgE

IgE is one of the most heavily glycosylated antibody class. Besides N394, IgE has six other glycosylation sites, of which five are known to have complex glycans attached (Fig. 1a, b). While oligomannose glycans are composed of only GlcNAc and mannose residues, complex glycans have additional sugars including fucose that attaches to the core structure and galactose or sialic acid to the terminal. To date, studies that described complex glycans on human recombinant, myeloma-derived or native IgE all revealed fucosylated sialylated structures to be the major complex glycoforms (Dorrington and Bennich 1978; Arnold et al. 2004; Plomp et al. 2013; Shade et al. 2015; Wu et al. 2016). Previous study has reported that deglycosylated IgE has a propensity to form aggregate (Basu et al. 1993). Thus, glycans on IgE probably contribute significantly to the water solubility of IgE and removal of these hydrophilic, acidic glycans may increase exposure of hydrophobic regions and results in aggregation. Various studies that mutated glycosylation sites with complex glycans attached in IgE Fc have thus far suggested a minimal role in IgE- FcεRI interaction. IgE/IgE Fc with single or double mutations at N265 and N374 sites was still able to bind FcεRI with the same affinity as the WT and initiate degranulation (Nettleton and Kochan 1995; Young et al. 1995; Sayers et al. 1998; Shade et al. 2015). Interestingly, mutation of all N-linked sites in Cε1 domain of Fab portion showed a slight reduction in IgE-mediate degranulation (Shade et al. 2015), suggesting perhaps complex glycans in Cε1 modulates IgE interaction with the antigen.

Genetic disruption of the glycosylation site removes the entire glycan structure and disregards the roles that are dependent on the heterogeneity of the complex glycans. Studies of the complex glycans on IgG Fc demonstrated that these glycans are heterogeneous and can display more than 30 different glycoforms at a single site in healthy individuals (Kaneko et al. 2006; Wuhler et al. 2007). These glycoforms reflect physiological processes and differ in diseases, serving as potential disease biomarkers. For example, changes in sialylation of IgG autoantibodies have been associated with clinical manifestation of the autoimmune and inflammatory diseases (Matsumoto et al. 2000; van de Geijn et al. 2009; Scherer et al. 2010; Ackerman et al. 2013a, b). Importantly, changes in glycoforms markedly modulate protein functions. When IgG is equipped with sialic acid, IgG shifts its receptor binding preferences and mediates anti-inflammatory activity (Kaneko et al. 2006; Anthony et al. 2008; Sondermann et al. 2013; Pincetic et al. 2014). Further, IgG that lack fucose has enhanced antibody-dependent cellular cytotoxicity as a result of increased affinity to FcγRIIIA by 50-fold (Shields et al. 2002; Ferrara et al. 2011).

In comparison to IgG, the role of complex glycans in IgE is not known. Studies of the β -galactoside-binding lectins, galectins, have perhaps suggested the involvement of complex glycans in IgE-mediated functions. Galectin-3 has been shown to bind mouse IgE and activate IgE-sensitized rat basophilic leukemia cells (Frigeri et al. 1993; Liu et al. 1993; Zuberi et al. 1994). On the other hand, another galectin, galectin-9, has been shown to reduce mast cell degranulation and passive anaphylaxis by binding to IgE and inhibit its interaction with antigen (Niki et al. 2009). It is likely that these galectins bind to the complex glycans on the IgE to exert its regulatory roles. However, it is not clear how these two lectins work together in vivo, for example, whether they bind to the same glycans on IgE and their relevance in human physiology.

5 Conclusions and Future Perspectives

Despite its discovery over 50 years ago, much remains to be discovered relating to IgE. As mentioned above, it is not clear precisely what makes allergen-specific IgE pathogenic in some individuals, and inert in other. However, their role in diseases of intense public health interest mandate ongoing pursuit of a comprehensive rendering of their function and regulation. IgE is the most heavily glycosylated monomeric antibody, and the conserved glycan at N394 has an important role in conformational folding and binding to Fc ϵ RI. This work is in early stages and dedication to continued unraveling of the story is critical. However, even in these early stages of understanding, it is interesting to speculate as to how glycosylation could be a major factor in treatment of IgE-associated diseases in the future. As described previously, galectins may play a significant role in connecting IgE to its downstream effector functions. Recent studies have identified a role for Galectin-3 in the regulation of the IgE receptor, Fc ϵ RI. Utilizing RNAi screening experiments, one group recently demonstrated Gal-3 as a negative regulator of Fc ϵ RI triggered mast cell degranulation. Their data suggests Gal-3 may affect surface Fc ϵ RI internalization, receptor mobility, F-actin polymerization, and Fc ϵ RI ubiquitination, all of which would profoundly impact mast cell signaling (Bambouskova et al. 2016). As altered variability in IgE glycosylation may impact galectin binding, it is certainly notable that activating or blocking interactions could yield substantial impact on the Fc ϵ RI and mast cell function. This would have marked impact on allergic inflammation. As a notable clinical correlate, Galectin-3 has been increasingly identified as a biomarker in various allergic diseases. A recent study identified Gal-3 as a marker increased in eosinophilic asthma but notably decreased in neutrophilic asthma (Gao et al. 2015). This finding was extended by Riccio et al. demonstrating Gal-3 as a predictive marker for positive response to anti-IgE treatment of asthma. Subjects positive for Gal-3 at baseline had more improvement in bronchial wall thickening, eosinophilic inflammation, and smooth muscle involvement (Riccio et al. 2017). Beyond asthma, a specific Gal-3 polymorphism has recently been identified as a significant genetic predictor of allergy to beta-lactam antibiotics (Cornejo-Garcia et al. 2016). These examples provide early clinical evidence for what is an increasing connection between IgE antibodies, glycosylation, and co-receptors with biologic relevance.

With IgE glycosylation likely to be a significant factor in its biology and effector function, identification of relevant patterns associated with clinical disease will be critical. Once the specific glycan patterns associated with pathology are identified and the basic biology

elucidated, novel diagnostic and therapeutic therapies can be extrapolated. Glycoengineering is an exciting future prospect where in vivo generation of antibody specific glycosylation could be utilized for reduction in inflammation. Indeed, modulation of IgG and tumor glycosylation has been reported by a number of groups, highlighting the therapeutic potential of these approaches (Albert et al. 2008; Xiao et al. 2016; Pagan et al. 2018). This may be one strategy of many to target IgE glycosylation for therapeutic benefit in the futures.

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Fig. 1. Human IgE and its glycans.

a Schematics of IgE structure and glycosylation. Predicted N-glycosylation sites in each constant domain (Cε) are represented by closed and open circles for complex and oligomannose glycans, respectively. **b** Complex glycans are composed of fucose (red), GlcNAc (blue), mannose (green), galactose (yellow circles), and sialic acid (pink). Core complex structure is shown in dotted box. Oligomannose glycans are composed of GlcNAc and between 5 and 9 mannoses