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REVIEW

IgE glycosylation and impact on structure and function: A systematic review

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Abstract

The impact of human IgE glycosylation on structure, function and disease mechanisms is not fully elucidated, and heterogeneity in different studies renders drawing conclusions challenging. Previous reviews discussed IgE glycosylation focusing on specific topics such as health versus disease, FcεR binding or impact on function. We present the first systematic review of human IgE glycosylation conducted utilizing the PRISMA guidelines. We sought to define the current consensus concerning the roles of glycosylation on structure, biology and disease. Despite diverse analytical methodologies, source, expression systems and the sparsity of data on IgE antibodies from non-allergic individuals, collectively evidence suggests differential glycosylation profiles, particularly in allergic diseases compared with healthy states, and indicates functional impact, and contributions to IgE-mediated hypersensitivities and atopic diseases. Beyond allergic diseases, dysregulated terminal glycan structures, including sialic acid, may regulate IgE metabolism. Glycan sites such as N394 may contribute to stabilizing IgE structure, with alterations in these glycans likely influencing both structure and IgE-FcεR interactions. This systematic review therefore highlights critical IgE glycosylation attributes in health and disease that may be exploitable for therapeutic intervention, and the need for novel analytics to explore pertinent research avenues.

KEYWORDS
allergy, cancer, glycosylation, IgE, systematic review

1 | INTRODUCTION

IgE is the least prevalent immunoglobulin in human serum, with concentrations below 150 ng/mL in non-atopic individuals.[1] This antibody class is thought to have originally evolved as a first line immune defence against parasites and animal venoms in mammals,[2–5] exerting its immune-stimulating functions via engagement with canonical Fc receptors on a range of effector cells (Figure 1). The significant prevalence of allergic diseases in developed countries has defined IgE for pathogenic roles in allergy.[6–8] A study by the National Centre for Health Statistics placed estimations of allergy prevalence in US children at 27.2%,[9] with previous work indicating a rising prevalence towards allergy in the industrialized world that could see as much as 40% of the population affected.[10] Despite this, understanding of the mechanisms that underly the onset of IgE-mediated allergies and atopic diseases remains limited.

IgE is architecturally similar to other human immunoglobulin classes, comprising two identical heavy-chains and two identical light chains, but carries an additional ε-chain domain where the flexible hinge region of the IgG γ-chain is located (Figure 1).[11,12] The IgE network includes two canonical Fc receptors—the high-affinity FcεRI and the low-affinity CD23/FcεRII; as well as IgE-binding proteins such as Galectin-3, thought to regulate IgE-FcεRI signalling.[12,13] IgE-mediated cross-linking of FcεRI by multivalent allergens mediates mast cell degranulation and synthesis of inflammatory mediators, Th2 cytokines and chemokines involved in allergic responses.[1,12]

Human IgE (hIgE) is heavily glycosylated, with seven potential N-glycosylation sites documented within the ε heavy chain conserved region (Figure 1),[11] yet occupancy, composition and functions of these glycans remain insufficiently understood. In IgG, glycosylation is known to influence inflammatory properties (sialylation),[14,15] ADCC efficacy (fucose)[16,17] and serum clearance rates (mannose)[18]; these attributes are linked to modified FcγR affinities, differential effector functions and cytokine signalling. It is of interest therefore from the perspective of understanding allergic mechanisms and therapy design to clarify the role of glycosylation in the biological and pharmacological effects of IgE.

Allergy has been suggested as a disease of aberrant glycosylation.[19] More recent research reported allergic-derived IgE to carry differential sialylation that may contribute to allergic pathogenicity.[20] Furthermore, the extent of IgE glycosylation may impact the regulation of IgE via endogenous anti-IgE IgG antibodies.[21] Defining if changes in IgE glycosylation could drive allergic mechanisms may aid in the development of novel treatments and approaches to understanding allergy. However, the diffuse nature of literature surrounding hIgE glycosylation renders drawing conclusions on potential associations of IgE glycosylation with disease, allergy, and structural and functional attributes challenging.

Previous reviews on IgE glycosylation have broadly examined literature surrounding health and disease[22]; Fc receptor binding[23]; or have broadly looked at the impact of glycosylation on allergy or antibody function without specific focus on IgE.[24,25] Neither have reviews sought to clarify IgE glycosylation in the context of IgE-based therapeutics, and to date, there has been no systematic review adhering to PRISMA guidelines focused on the hIgE glycan literature. To this end, we sought to address this by systematically reviewing the literature, focusing on studies that investigated human IgEs in the context of glycan composition, structure and function.

In this systematic review, we thus sought to provide a thorough search and evaluation of two facets of hIgE glycan biology: the presence of differential glycosylation associated with disease and potential implications for disease mechanisms and biomarkers; and

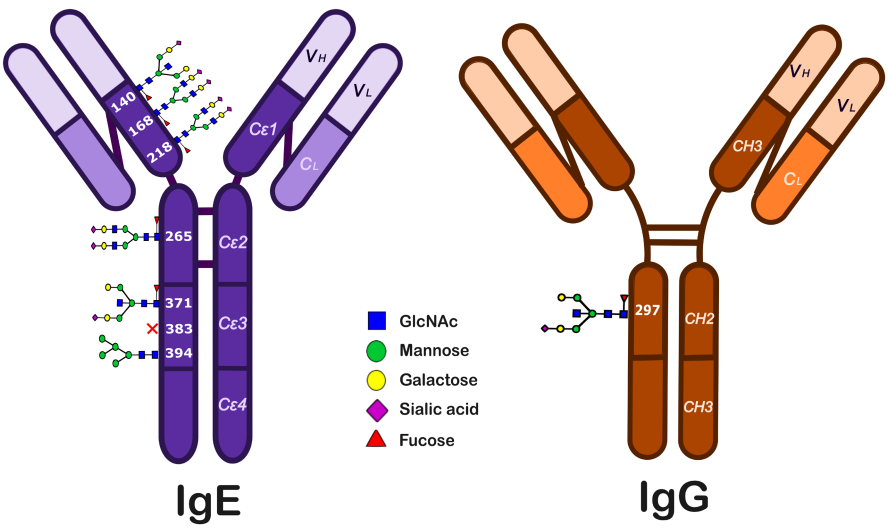

FIGURE 1 Structures and glycosylation sites of IgE and IgG class antibodies. IgE carries an additional CH domain (Cε2) in place of the hinge region found in IgG. Whereas IgG has only one documented conserved N-glycan site, N297; IgE has a total of 7 conserved N-glycosylation sites. One site, N383, is consistently unoccupied. Site N394 is considered evolutionary homologous to IgG's N297 but carries an oligomannose glycan in place of a complex glycan. N394 is the only documented oligomannose glycan on IgE.

the roles of hIgE glycosylation in antibody structure and function, including how this may provide insight into disease mechanisms and novel targets. We review the source of IgE and analytical methodologies. We draw overall conclusions on hIgE glycosylation in relation to structure, biological function and disease mechanisms. Finally, we consider how the current literature may inform future studies in both allergy and development of IgE-based therapeutics.

2 | MATERIALS AND METHODS

2.1 | Search criteria

This systematic review was performed following the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRIMSA) guidelines.[26] Search strategies and inclusion/exclusion criteria were finalized prior to conducting the review. This review protocol has not previously been registered or published. Further details including the PRISMA checklist, search strategy and corresponding results from each database, and a list of excluded studies can be found in the File S1 (see Search Strategies).

2.2 | Study selection

The aim was to identify all relevant published studies providing either compositional analysis of human IgE or insight into the roles of human IgE glycosylation in structure and function. The following inclusion criteria were used:

2.2.1 | Structure and source of immunoglobulin E

Only studies using a form of human-derived IgE, or engineered IgEs or IgE fragments at a minimum containing whole or part of human Fc regions (human, humanized, chimeric) (referred to herewith as IgE), were included. Sources included IgEs derived from human serum and plasma and recombinant human, humanized and chimeric IgE antibodies derived from human, non-human and non-mammalian system cell-based expression systems.

2.2.2 | Investigation

Studies including a form of compositional analysis of IgE glycosylation or investigating structural integrity and/or functional capabilities in relation to glycosylation were included.

2.3 | Data sources

Our initial search included all records listed in the following databases since their inception: Scopus, Web of Science, and Google Scholar and Pubmed/Medline (up to 30 April 2024). No pre-set search filters or language restrictions were used. Grey literature in the form of conference abstracts was to be included, so long as above criteria was met. Theses and pre-prints were ultimately excluded from the analysis.

Detailed search strategies for each individual database are provided in the File S1. We searched title, abstract and keyword fields in Scopus and free terms in Web of Science using the terms "immunoglobulin e" or "IgE" combined with "glycan" or "glycosylation" and "human." For Google Scholar, the same terms but restricted to title only were used to limit number of non-relevant hits. For Pubmed/Medline, we searched titles and abstracts with the terms "immunoglobulin e" or "IgE" in combination with "glycan" or "glycosylation" and "human." Abstracts of retrieved records were manually screened, followed by screening of full texts for studies deemed potentially eligible within the criteria.

2.4 | Data extraction

Titles and abstracts of all identified records from each database search were imported to the commercial reference management software Endnote (Clarivate Analytics). Duplicate records were identified and removed following manual screening. Two investigators (A. McCraw and L. Palhares) finalized the final list of studies for inclusion. For studies including compositional analysis, A. McCraw and J. Hendel performed data extraction. For studies investigating structure and function, A. McCraw and L. Palhares performed data extraction.

3 | RESULTS

3.1 | Overall data trends

Following PRISMA, 41 articles were included for analysis (Figures 2 and 3, Table 1): 19 looked exclusively at glycan composition[11,27–44]; 19 examined structure and function in relation to glycosylation[30,45–62]; and 5 investigated both composition and structure/function.[19,20,63–65] A total of six studies performed site-specific analysis.[11,20,34,39,43,65]

3.1.1 | IgE origin

An overview of expression systems and their corresponding glycosylation patterns is provided in Table S1. Twenty-four studies utilized IgE derived from human sera or plasma (Figure 3); of these, 13 used IgE derived from myeloma.[11,19,28–30,34,36,40,45,53,54,58,60] Six studies used IgE derived from hyperimmune conditions[11,27,41,43,54,58] and IgE from allergic and/or atopic conditions accounted for nine studies.[20,37,41,43,44,52,54,57,65] One study examined serum IgE derived from a patient with a parasitic infection.[58] Four studies used IgE derived from 'healthy' individuals.[11,20,38,39] However, the definitions of

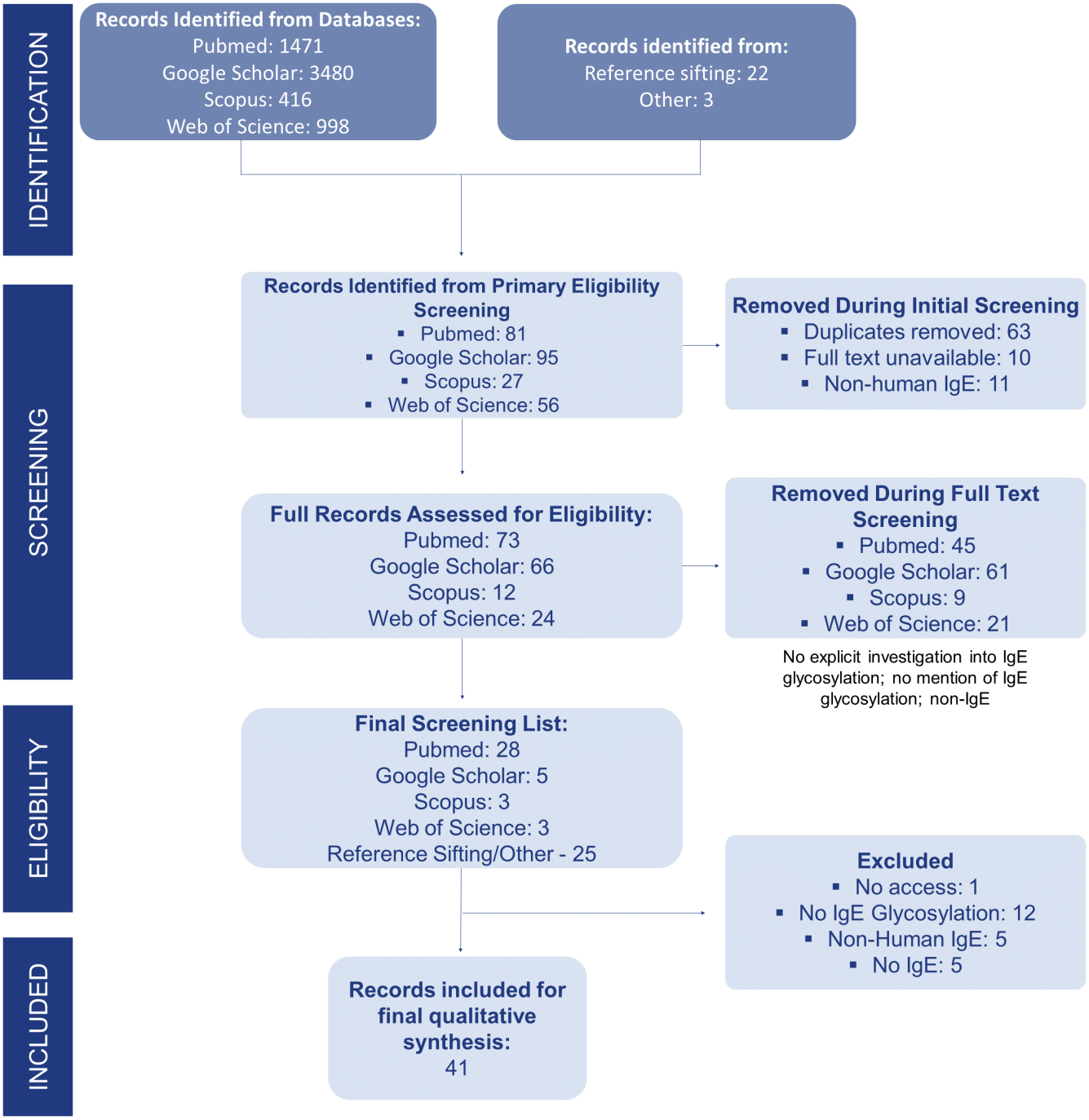

FIGURE 2 PRISMA flow diagram for qualitative synthesis, showing the breakdown of paper inclusion/exclusion at each stage of the screening process. A breakdown of the screening and selection process is provided in Section 2.

'healthy' varied; two defined healthy as 'non-allergic' and the other two specified 'healthy' pooled serum.

Six studies used IgE derived from at least two separate disease states[11,20,41,43,54,58]; of these, four compared IgE glycan composition between these states[11,20,41,43]; and only two included IgE from individuals defined as 'healthy'.[11,20] Seventeen papers utilized recombinant IgE, either full-length or Fc fragments, from mammalian cells,[30–33,35,39,42,47,50–52,55,56,62–65] with murine cell lines being the most represented[30,31,33,35,47,50,51,55,56,62] (Figure 3). Six studies utilized hIgE derived from bacterial or insect expression systems.[46,48–50,59,63]

3.1.2 | IgE type

Thirty-three studies investigated full-length IgE derived from either mammalian cells or human serum.[11,19,20,27–32,34,36–45,51–58,60,62–65] Seven papers used some variation of Fc construct[33,35,46–50] and one

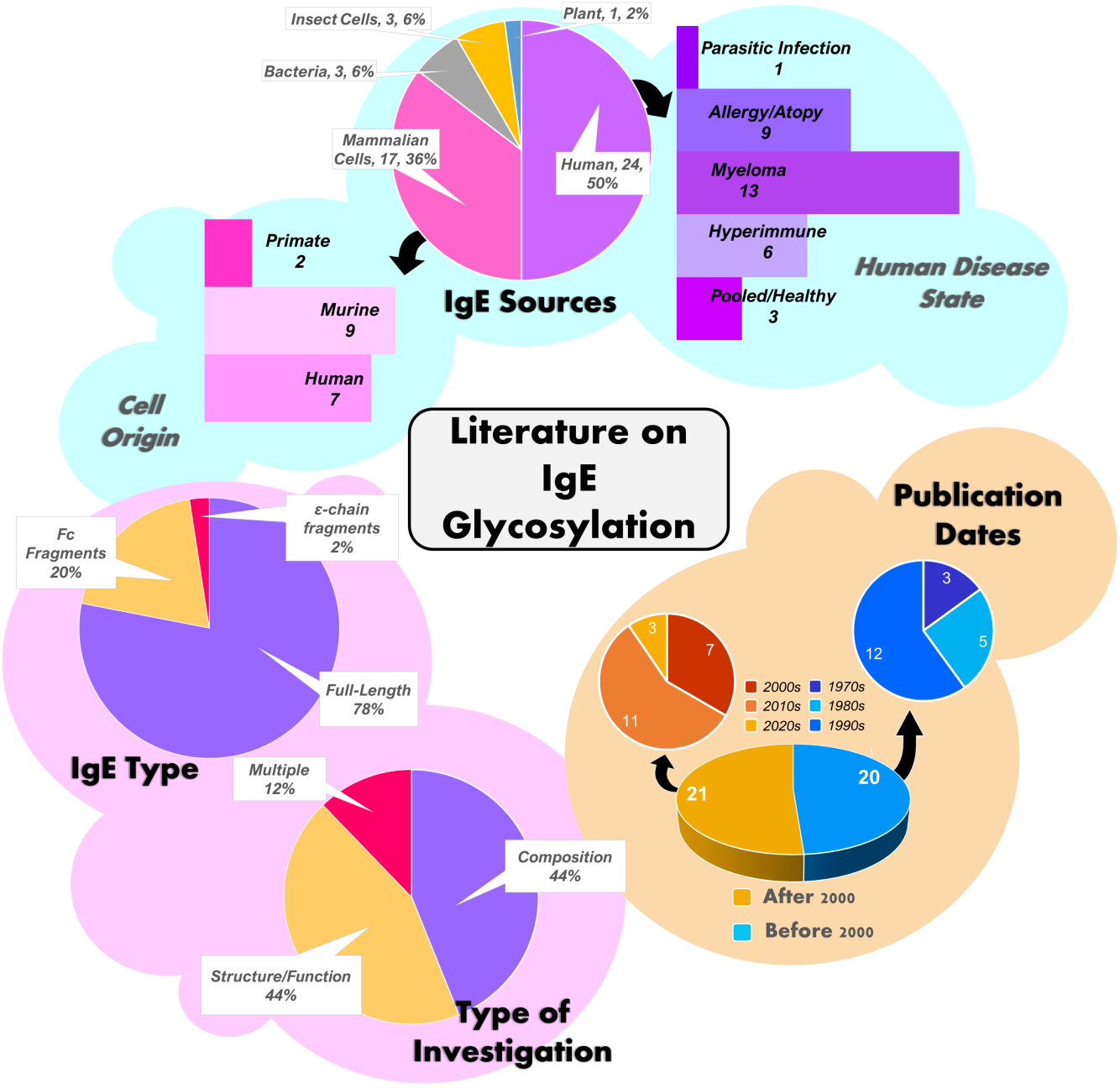

FIGURE 3 Origins and investigations of data on IgE glycosylation generated in the published literature. Data extracted from Table 1. IgE type predominantly consisted of full-length IgEs, followed by Fc fragments and ε-chain fragments. Most IgEs were derived from human sera or from non-human mammalian cells. Of human serum-derived IgE, the majority was sourced from IgE+ myeloma.

used a different protein construct.[59] One paper used a combination of full-length IgE and Fc fragments.[30]

3.1.3 | Form of glycoengineering and analysis

A variety of glycosidases, proteases and glycosyltransferase inhibitors were used for study, either for the purpose of deglycosylation for compositional analysis or for structural/functional investigation (Table 2).

Fourteen studies used endoglycosidases such as Endo-F1 or PN Gase-F,[11,20,27,30–32,40,41,45,46,50,52,64,65] whilst nine used exoglycosidases including neuraminidase and mannosidase[35,40,45,52–54,58,60,64] to glycoengineer IgE. Thirteen used various proteases to digest IgE for the purpose of study.[11,20,28,29,33,34,36,39–41,43,63,65] Finally, six studies used a form of site-specific mutation to glycoengineer

TABLE 1 General overview of papers investigating IgE glycosylation summarizing primary information on IgE source, type and origin.

Paper	Year published	Features investigated	IgE type	Source of IgE
Arnold et al. PMID: 15557177[27]	2004	Composition	Full-length	Human serum
Baenziger et al. Pt. 1 PMID: 4361830[28]	1974	Composition	Full-length	Human plasma
Baenziger et al. Pt. 2 PMID: 4361831[29]	1974	Composition	Full-length	Human plasma
Bantleon et al. PMID: 26943931[63]	2016	Multiple	Full-length	Insect cells; mammalian cells
Batista et al. PMID: 8622947[31]	1996	Composition	Full-length	Mammalian cells
Crescioli et al. PMID: 29360527[32]	2018	Composition	Full-length	Mammalian cells
Fridrikkson et al. PMID: 10727230[33]	2000	Composition	Fc fragments	Mammalian cells
Hinneburg et al. PMID: 26729457[34]	2016	Composition	Full-length	Human plasma
Ikeyama et al. PMID: 3702874[42]	1986	Composition	Full-length	Mammalian cells
Ikeyama et al. PMID: 3683401[35]	1987	Composition	Fc fragments	Mammalian cells
Kochwa et al. PMID: 5290027[36]	1971	Composition	Full-length	Human plasma
Koers et al. PMID: 30850477[37]	2019	Composition	Full-length	Human B cells from PBMC
Koning et al. PMID: 31333671[38]	2019	Composition	Full-length	Human B cells from PBMC, analysis of BCR
McCraw et al. PMID: 36362241[64]	2022	Multiple	Full-length	Mammalian cells
Montero-Morales et al. PMID: 28400175[39]	2017	Composition	Full-length	Mammalian cells; tobacco plant cells; human sera
Plomp et al. PMID: 24308486[11]	2014	Composition	Full-length	Human serum
Rearick et al. PMID: 6830248[40]	1983	Composition	Full-length	Human plasma
Sassi et al. PMID: 24698316[41]	2014	Composition	Full-length	Human serum
Shade et al. PMID: 25824821[65]	2015	Multiple	Full-length	Mammalian cells; human serum
Shade et al. PMID: 32499653[20]	2020	Multiple	Full-length	Human serum
Wu et al. PMID: 26687240[43]	2016	Composition	Full-length	Human serum/plasma
Zavázal et al. PMID: 2408980[44]	1985	Composition	Full-length	Human serum
Basu et al. PMID: 7685756[30]	1993	Structure/Function	Full-length; Fc fragments	Mammalian cells; human plasma
Björklund et al. PMID: 10403487[45]	1999	Structure/Function	Full-length	Human serum
Björklund et al. PMID: 10865116[46]	2000	Structure/Function	Fc fragments	Insect cells
Doré et al. PMID: 28844738[47]	2017	Structure/Function	Fc fragments	Mammalian cells
Garman et al. PMID: 10917520[48]	2000	Structure/Function	Fc fragments	Insect cells
Henry et al. PMID: 10858288[49]	2000	Structure/Function	Fc fragments	Bacteria
Hunt et al. PMID: 15743766[50]	2005	Structure/Function	Fc fragments	Mammalian cells; bacteria
Nettleton et al. PMID: 7613162[51]	1995	Structure/Function	Full-length	Mammalian cells

Disease origin	Expression system/cell type	Additional details	Notes
Hyperimmune	*N/A*		Single donor
Myeloma	*N/A*	Human myeloma IgE P.S.[a]	
Myeloma	*N/A*	Human myeloma IgE P.S.	
N/A	Baculovirus-infected Sf9 cells; HEK293	Expression vector providing human IgG C1, kappa Cl domains and tailored gp67 signal sequences where the IgG domain was replaced by the human IgE heavy chain constant regions CH1–4 containing a C-terminal 5xhis tag	Used an additional recombinant IgE (termed 425 rIgE) derived from HEK293 cells for glycan comparison
N/A	J558L, WEH1-231	Chimeric mouse/human	
N/A	HEK293	Chimeric mouse/human	
N/A	NS0	Human IgE-Fc construct	*Modified to contain only site N394*
Myeloma	*N/A*	Myeloma plasma, Lambda	
N/A	U266	Human myeloma IgE P.S.	
N/A	L-IS11IgE-9		
Myeloma	*N/A*	Human myeloma IgE P.S.	
Allergy	*N/A*		Non-processed sequence reads from peripheral blood and bone marrow mononuclear cells
Healthy	*N/A*		PBMCs and BCR repertoire screening
N/A	HEK293	Chimeric mouse/human	
Pooled serum from healthy volunteers	HEK293b, tobacco plant cells (Nicotiana benthamiana)	TBD Human/mouse chimeric and humanised IgEs	
Hyperimmune; myeloma; pooled healthy	*N/A*		
Myeloma	*N/A*	Human myeloma IgE P.S.	
Hyperimmune; atopy	*N/A*		
Allergy	HEK293		*Also investigated murine IgE*
Non-atopic; allergy	*N/A*		
Hyperimmune; atopy	*N/A*		
Atopy	*N/A*		
Myeloma	CHO, COS	Human myeloma IgE P.S.	
Myeloma	*N/A*	Human myeloma IgE D.E.S, IgE P.S, IgE N.D., IgE U.D, IgE W.T	
N/A	Baculovirus-infected Sf9 cells	Fc fragments containing the constant region of the human ε-chain	
N/A	NS0, HEK293	Human Fcε3-4 and Fc fragments	
N/A	Sf9	Cε3-Cε4 fragments	
N/A	*E. coli*	Construct from human myeloma ND ε chain	
N/A	NSO; *E. coli*	Human Fcε3-4 fragments	
N/A	COS	Human IgE 315–547 Fc fragments	

(Continues)

TABLE 1 (Continued)

Paper	Year published	Features investigated	IgE type	Source of IgE
Plattner et al. PMID: 37073887	2023	Structure/Function	Full-length	Mammalian cells; human
Robertson et al. PMID: 2261464[53]	1990	Structure/Function	Full-length	Human serum
Robertson et al. PMID: 1919004[54]	1991	Structure/Function	Full-length	Human serum
Sayers et al. PMID: 9819207[55]	1998	Structure/Function	Full-length	Mammalian cells
Sayers et al. PMID: 15199058[56]	2004	Structure/Function	Full-length	Mammalian cells
Shibasaki et al. PMID: 1378039[57]	1992	Structure/Function	Full-length	Human serum
Truong et al. PMID: 8418206[58]	1993	Structure/Function	Full-length	Human serum
Vercelli et al. PMID: 2468089[59]	1989	Structure/Function	ε-chain fragments	Bacteria
Wollenberg et al. PMID: 8350053[60]	1993	Structure/Function	Full-length	Human serum[b]
Young et al. PMID: 7543206[62]	1995	Structure/Function	Full-length	Mammalian cells
Zavázal et al. PMID: 8645991[19]	1996	Multiple	Full-length	Human serum

[a], IgE D.E.S.,[105] IgE N.D.[106] IgE P.S.,[107] IgE U.D.,[108] IgE V.L.[109] and IgE W.T.[108]: IgE derived from the sera of specific patients with myeloma.
[b]Exact source is unknown but presumed human serum.

IgE.[51,52,55,56,62,65] An overview of compositional analysis methodologies is shown in Tables S2–S4.

3.2 | Site occupancy

Site occupancy was investigated by six studies.[11,20,34,39,43,65] For site occupancy, most studies were conducted on IgE derived from human sera (Table 3). Early IgE glycosylation studies suggested that IgE carried 3–4 complex glycans and one oligomannose glycan.[28,36] It has since been established that IgE carries up to five complex glycans and one oligomannose glycan considered equivalent to the sole glycan of IgG (Figure 1).[11] Complex glycan sites consist of N140, 168, 218, 265 and 371, whilst N394 carries the oligomannose glycan. An additional consistently unoccupied N-glycosylation site is present at N383 (Figure 4A).

Variance was found in site occupancy levels between studies. Plomp et al. reported partial occupation at N218, N371 and N394, supported by Montero-Morales et al.,[11,39] who additionally reported lower occupancy at N218 and N371 for plant-derived IgE.[39] Shade et al.[20] were mostly in agreement for N218 but found substantially lower occupation rates for N371 (20% compared with an average of 93% for other sites) and full occupation of N394. Sites N140, 168 and 265 were consistently reported to be fully occupied.[11,20]

3.3 | Glycan composition

Examination of N394 shows that it carries between 5 and 9 hexoses,[11,27,33,39,40,64] with five mannose residues the most prevalent glycoform.[11,27] One study optimizing collision energies for Q-TOF (Table S2) detected what appears to be an N394 glycoform carrying a fucose residue.[34] Whilst this is the only study that has reported the presence of fucose on the oligomannose N394 glycan, a site consistently found to be occupied by a high-mannose glycan, core fucosylation of high-mannose glycans has previously been reported in several studies[66–69] and aberrant glycosylation, including increased fucosylation, is a common feature of cancer.[70,71] Since the study reporting a fucose residue on N394 reports a myeloma-derived IgE, it is conceivable that this could relate to the expression of IgE by malignant cells (see Table 1).

Complex-type glycans typically carry sialic acid, galactose, mannose and N-acetylglucosamine (GlcNAc) (Figure 4). The number of terminal sialic acids can vary, with 1–2 residues being the most prevalent,[11,29,34,36,39,43] although glycans can lack sialic acid entirely.[20,27,34] Recombinant IgE produced in mammalian expression systems differed, with up to 4 terminal sialic acids detected on complex tetra-antennary structures.[32,39,64] Most complex glycans carry a core fucose,[11,29,36,39,41,43,64] although non-fucosylated structures were also observed (Table 3).[11,34]

IgE derived from human sera and plasma predominantly presents with mono-antennary or bi-antennary structures.[11,20,39] Tri-antennary and tetra-antennary structures were detected at low rates in disease states.[11,20,41] Recombinant IgE derived from mammalian expression systems showed increased levels of tri-antennary and tetra-antennary structures.[39,64] Bisecting GlcNAc was detected both in recombinant IgE[39,64] and at low levels in serum IgE.[11,39,41,43] From those studies performing site-specific analysis, bisection appeared most prevalent at site N371,[11,39] but can persist at low levels across all complex sites (Figure 4B).

3.4 | Differential glycosylation patterns

Of the 23 studies utilizing serum-derived IgE, only seven investigated potential differential glycosylation between disease states.

Disease origin	Expression system/cell type	Additional details	Notes
Allergy	HEK293; human B-cell hybridomas		Recombinant IgE Clone SUS-11-NBS01
Myeloma	*N/A*	Human IgE P.S.	
Hyperimmune; atopy; myeloma	*N/A*	Human IgE, IgE D.E.S., IgE D.Z.A., IgE P.S.	
N/A	J558L		
N/A	J558L	Human ε gene variants	
Allergy	*N/A*		
Hyperimmune; myeloma; parasitic Infection	*N/A*	Human myeloma IgE P.S.	
N/A	*E.coli*	ε-chain fragments	
Myeloma	*N/A*	Unknown human myeloma IgE	
N/A	CHO, NS0	Human myeloma IgE N.D. Fc region	
Myeloma	*N/A*	Human myeloma IgE kappa protein V.L.	

Of these, three compared IgE derived from different disease states whilst others investigated glycosylation differences between recombinant and serum-derived IgE, or in different IgE preparations.

Plomp et al.[11] compared human IgE derived from patients with hyperimmune syndrome, IgE myeloma and pooled sera from healthy volunteers. Differences arose in levels of specific glycan residues, or in the number of glycan antennae. All structures were fucosylated in myeloma-derived IgE, whilst low levels of non-fucosylated structures were present in both hyperimmune and healthy IgE. Myeloma IgE carried elevated levels of tri-antennary and tetra-antennary structures compared with hyperimmune and healthy state-derived IgEs, where 96% of complex glycans were bi-antennary.[11] Sassi et al.[41] found decreased levels of tri-antennary and tetra-antennary *N*-glycans in hyperimmune patients compared with an atopic dermatitis patient control. Levels of bisected glycoforms were substantially lower in the myeloma IgE dataset; Shade et al. similarly found bisection was increased on non-atopic compared with allergic IgE.[11,20] Interestingly, IgE light chains were found in both glycosylated and non-glycosylated forms in healthy and hyperimmune IgEs, but not in myeloma IgE.[11] Both non-atopic and allergic IgEs carried similar levels of mannose and fucose, whilst sialic acid content was greatly increased in allergic individuals.[20] Of note, although no compositional analysis was given, work by Robertson et al. found evidence suggesting that sialic acid levels on IgE varied within the general population.[53,54] Alongside evidence of differential glycosylation amongst IgEs from allergic, compared with non-allergic individuals,[19] lectin blot analyses may suggest heterogenous glycosylation within the allergic populations as well.[44]

Batista et al. examined IgE secreted from J558L plasmacytoma and WEHI 231 B-cell lymphoma cells, identifying a second IgE isoform secreted by both cell types, and differences in glycosylation not only between cell types but also between ε chain isoforms secreted from B cells. Differences were determined to be partly due to differing sialic acid content.[31] Differential glycosylation within secreted IgE-N.D. produced in U266 B-cell myeloma cells were reported.[39,42] Levels of mannose, sialic acid and galactose in these preparations were comparatively lower to previous data available on the same IgE-N.D.[42] Finally, core differences between sera-derived and recombinant human embryonic kidney (HEK293b) cell-derived IgEs were reported, whilst plant-derived IgE showed similar glycosylation patterns between batches.[39]

3.5 | Structural and functional involvement of IgE glycans

Publications investigating IgE glycosylation in relation to structure and/or function are shown in Table 4.

3.5.1 | Glycosylation in IgE production and secretion

Glycosylation has been considered essential for correct folding and secretion of IgE; however, this has been reported using rat IgE-secreting rat IR162 plasmacytoma cells treated with tunicamycin, a glycosylation inhibitor preventing formation of N glycans,[72] and later replicated by Yamazaki et al. who used mouse IgE generated with HEK293 cells.[61] This contrasts with detectable IgG from cell supernatants in the presence of tunicamycin.[61,73]

A study of peripheral blood and bone marrow B cells from healthy individuals, patients with myasthenia gravis and systemic lupus erythematosus reported a 2-fold higher propensity for IgE and IgG4 to acquire N-linked glycans in the variable regions during somatic

TABLE 2 A summary of all reagents used for the purpose of glycoanalysis, either compositional or structural/functional, including their targets and means of action.

References	Glycosidase	Type	Target	Method of action
Rearick et al. PMID: 6830248[40]	α-Mannosidase	Exoglycosidase	Mannose	Removes terminal mannose residues from oligosaccharides. Linkage specificity can vary
Fridrikkson et al. PMID: 10727230[33]	Asp-N	Endoproteinase	Aspartic and glutamic acids	Hydrolyses peptide bonds on the N-terminal side of aspartic and glutamic acids to produce protein digests. Less active on glutamic acids
Rearick et al. PMID: 6830248[40]	B-*N*-Acetylglucosaminidase	Exoglycosidase	*N*-glycans	Hydrolyses terminal non-reducing β-GlcNAc residues from oligosaccharides
Bantleon et al. PMID: 26943931[63]; Montero-Morales et al. PMID: 28400175[39]; Plomp et al. PMID: 24308486[11]; Shade et al. PMID: 25824821[65]; Shade et al. PMID: 32499653[20]; Wu et al. PMID: 26687240[43]	Chymotrypsin	Serine protease	Tyrosine, phenylalanine, tryptophan, leucine (selective)	Hydrolyses peptide bonds on the C-terminal side of target residues. Secondary hydrolysis will also occur on the C-terminal side of methionine, isoleucine, serine, threonine, valine, histidine, glycine and alanine
Rearick et al. PMID: 6830248[40]	Endo-β-*N*-acetylglucosaminidase (ENGase)	Endoglycosidase	*N*-glycans	Generates free oligosaccharides from glycoproteins by specifically cleaving between the two GlcNAc residues within the diacetylchitobiose core
Basu et al. PMID: 7685756[30,a]; Plattner et al. PMID: 37073887[52]; Shade et al. PMID: 32499653[20]	Endo-F1	Endoglycosidase	Oligomannose and hybrid glycans	Specifically cleaves N-linked or free oligomannose and hybrid glycans but not complex glycans. Cleaves between the two GlcNAc residues in the diacetylchitobiose core to generate a truncated glycan with one GlcNAc remaining on the asparagine residue
Basu et al. PMID: 7685756[30]	Endo F2	Endoglycosidase	Oligomannose and bi-antennary complex glycans	Cleaves between the two GlcNAc residues in the diacetylchitobiose core to generate a truncated glycan with one GlcNAc remaining on the asparagine residue
Basu et al. PMID: 7685756[30]	Endo F3	Endoglycosidase	N-linked or free bi-antennary and tri-antennary complex and Man3GlcNAc glycans	Cleaves between the two GlcNAc residues in the diacetylchitobiose core to generate a truncated glycan with one GlcNAc remaining on the asparagine residue. Core fucosylation increases Endo F3 activity
Basu et al. PMID: 7685756[30]; Björklund et al. PMID: 10865116[46]	Endo-H	Endoglycosidase	High mannose and some hybrid oligosaccharides	Cleaves the chitobiose core of target glycans
Fridrikkson et al. PMID: 10727230[33]	Lys-C	Endoproteinase	Lysine	Specifically hydrolyses at the carboxyl terminal of lysine residues to produce peptide digests
Björklund et al. PMID: 10403487[45]; Ikeyama et al. PMID: 3683401[42]; McCraw et al. PMID: 36362241[64]; Plattner et al. PMID: 37073887[52]; Rearick et al. PMID: 6830248[40]; Robertson et al. PMID: 2261464[53]; Robertson et al. PMID: 1919004[54]; Truong et al. PMID: 8418206[58]; Wollenberg et al. PMID: 8350053[60]	Neuraminidase/Sialidase	Exoglycosidase	Terminal sialic acid residues	Cleaves non-reducing terminal sialic acid residues, both branched and linear. Linkage specificity may vary

TABLE 2 (Continued)

References	Glycosidase	Type	Target	Method of action
Fridrikkson et al. PMID:v10727230[33]	Pepsin	Endoproteinase	Aspartic acid	Unspecific hydrolysis of proteins in acidic media
Arnold et al. PMID: 15557177[27]; Basu et al. PMID: 7685756[30]; Batista et al. PMID: 8622947[31]; Björklund et al. PMID: 10865116[46]; Björklund et al. PMID: 10403487[45]; Crescioli et al. PMID: 29360527[32]; Hunt et al. PMID: 15743766[50]; McCraw et al. PMID: 36362241[64]; Plattner et al. PMID: 37073887[52]; Plomp et al. PMID: 24308486[11]; Sassi et al. PMID: 24698316[41]; Shade et al. PMID: 25824821[65]	PNGase F/*N*-Glycanase	Endoglycosidase	Almost all N-linked glycans	Amidase cleaving between innermost GlcNAc and asparagine residues of most complex, oligomannose and hybrid N glycans
Baenziger et al. PMID: 4361830[28]; Baenziger et al. PMID: 4361831[29]; Kochwa et al. PMID: 5290027[36]; Rearick et al. PMID: 6830248[40]	Pronase	Mixture of non-specific endo- and exo-proteases	Broad specificity for almost all proteins	Digests proteins down to single amino acids
Montero-Morales et al. PMID: 28400175[39]; Plomp et al. PMID: 24308486[11]; Shade et al. PMID: 25824821[65]	Proteinase K	Serine protease/ endoproteinase		Hydrolyses peptide bonds next to carboxyl terminals of aromatic and aliphatic amino acids
Bantleon et al. PMID: 26943931[63]; Hinneburg et al. PMID: 26729457[34]; Montero-Morales et al. PMID: 28400175[39]; Plomp et al. PMID: 24308486[11]; Sassi et al. PMID: 24698316[41]; Shade et al. PMID: 25824821[65]; Shade et al. PMID: 32499653[20]; Wu et al. PMID: 26687240[43]	Trypsin	Serine protease	Lysine and arginine	Cleaves peptides on the C-terminal side of lysine and arginine residues. Reaction is slowed is an acidic residue is on either side of the cleavage site and hydrolysis stops if a proline residue is present on the carboxyl side of the cleavage site

[a]Basu et al. list their enzyme simply as Endo-F.

TABLE 3 A complete summary of studies investigating human IgE glycan composition, including site occupancy, and their findings.

Study	Year published	IgE source	Method of glycan removal	Method of glycan labelling	Analytical method—technique
Arnold et al. 2004 PMID: 15557177[27]	2004	Human; hyperimmunity	PNGase-F in-gel digestion (N-glycans) Chemical release via anhydrous hydrazine (O-glycans)	Flurophore 2AB	NP-HPLC; WAX-HPLC (labelled glycans, separation into neutral, mono-, di- and tri-sialylated structures) MALDI-MS (non-labelled glycans) Exoglycosidase digestion (orthogonal method) LC-ESI-MS/MS (O-linked glycans)
Baenziger et al. Pt. 1 PMID: 4361830[28]	1974	Human; myeloma	Pronase digestion and fractionation; ion exchange chromatography	N/A	Gas–liquid chromatography (monosaccharide composition) Sephadex G-50 gel filtration (MW estimates) Sequential enzymatic and periodate degradation followed by acid hydrolysis and gas–liquid chromatography (structural sequences of oligosaccharide chains) Methylation, hydrolysis into monosaccharides and separation of glycopeptides followed by gas–liquid chromatography and MS (orthogonal method, structural analysis)
Baenziger et al. Pt. 2 PMID: 4361831[29]	1974	Human; myeloma	Pronase digestion and fractionation; ion exchange chromatography	N/A	Gas–liquid chromatography (monosaccharide composition) Sephadex G-50 gel filtration (MW estimates) Sequential enzymatic and periodate degradation followed by acid hydrolysis and gas–liquid chromatography (structural sequences of oligosaccharide chains) Methylation, hydrolysis into monosaccharides and separation of glycopeptides followed by gas–liquid chromatography and MS (orthogonal method, structural analysis)
Bantleon et al. PMID: 26943931[63]	2016	Insect; Sf9	1-D protein in-gel enzymatic digestion (trypsin, chymotrypsin)	N/A	Lectin blot; MALDI-TOF/TOF

Comparative glycosylation	Site-specific analysis	Evidence for O-linked glycosylation	Glycoforms detected within sites	Differential glycosylation between sources	Summary of findings	Notes
No	No	No	Yes	N/A	Oligomannose structures Man4-Man8 comprised 14.2% of the glycan pool, with Man5 the most abundant (8.3%). Remaining 85.8% glycan structures terminated in galactose or sialic acid. Sialic acid linkages shown to be 2,6 linked to galactose. No light chain glycans detected. No evidence for O-linked glycosylation	
No	No	N/A	No	N/A	IgE myeloma protein has 4 oligosaccharide units per heavy chain—1 oligomannose glycan and 3 complex-type glycans. Complex glycans carried sialic acid, fucose, galactose, mannose and N-acetylglucosamine in a ratio of 1 or 2:1:2:3:4 The ratio of mannose to GlcNAc in the oligomannose glycan was 6:2, with around 3 Man residues suggested to be terminal. No evidence of microheterogeneity found within the oligomannose glycan	Did note the possible presence of a 4th complex glycan. Propose a glycan with a core that has alternating mannose and GlcNAc residues instead of a chitobiose core as has since been proven
No	No	N/A	Yes	N/A	Complex glycans contained 1–2 sialic acids along with fucose, galactose, mannose and GlcNAc in molar proportions of 1:2:3:4 respectively; and were compositionally similar to the complex glycans of IgG, IgM and IgA. Primary differences were in the number of terminal sialic acid residues. Single sialic acid glycans had structural consistency with the sialic acid located on the branch originating from position 3 of the core mannose residue. Core structure determined to consist of 1 β-linked mannose, 1 terminal α-linked fucose, and 2 GlcNAc residues, with fucose linked to the Asn-linked GlcNAc. The branch point was suggested to be a disubstituted β-linked mannose. Man–Man bonds found to always be α-linked whilst Man-GlcNAc bonds were uniformly β-linked	
Yes	No	N/A	N/A	Yes	According to lectin blot, rIgE from insect cells presented with higher fucose content (as detected by AAL) and α1,3 mannose (as detected by GNA) compared with a mammalian cell-derived IgE. Glycoproteomic analysis showed predominantly oligo- and paucimannose structures as expected from insect cells	

(Continues)

TABLE 3 (Continued)

Study	Year published	IgE source	Method of glycan removal	Method of glycan labelling	Analytical method—technique
Batista et al. PMID: 8622947[31]	1996	Cells; J558L, WEH1-231	Enzymatic digestion (PNGase F)	N/A	SDS-PAGE
Crescioli et al. PMID: 29360527[32]	2018	Cells; HEK293	Enzymatic digestion (PNGase F)	Procainamide labelling	Lectin Blot; HILIC-LC-ESI MS with MS/MS
Fridrikkson et al. PMID: 10727230[33]	2000	Cells; NS-0	Enzymatic digestion (Lys-C or Asp-N then pepsin)	N/A	FTMS using Nano-ESI and SWIFT-CAD
Hinneburg et al. PMID: 26729457[34]	2016	Human; myeloma	SDS-PAGE and in-gel trypsin digestion	N/A	Nano RP-LC-ESI-QTOF tandem MS
Ikeyama et al. PMID: 3683401[35]	1987	Cells; L-IS11IgE-9		N/A	Phenol-sulfuric acid method (total neutral sugar, mannose used as standard) Periodic acid-resorcinol method (sialic acid, N-acetylneuraminic acid used as standard)

Comparative glycosylation	Site-specific analysis	Evidence for O-linked glycosylation	Glycoforms detected within sites	Differential glycosylation between sources	Summary of findings	Notes
No	No	N/A	N/A	Differential glycosylation between secreted IgE	Work suggested IgE ε chains secreted by different cell types carried differential glycosylation (plasma cells, B lymphocytes). Cells could express ε chain isoforms, with B lymphocytes glycosylating these isoforms to different extents. PNGase-F treatment completely eliminated MW differences between ε chain isoforms as well as between cell types. Neuraminidase treatment allegedly indicated these glycosylation differences to be attributable in part to differing sialic acid content	Neuraminidase results were not shown
Yes	No	N/A	N/A	Yes?	Developed a stable platform for the expression of recombinant IgE. Lectin blot and liquid chromatography–mass spectrometry (LC–MS) glycosylation analyses revealed no significant differences amongst IgEs produced under different conditions (purified from supernatants of Freestyle293F-CSPG4 IgE (pVITRO) or Expi-CSPG4 IgE cultured at 0.5×10^6 for 4 days, (0.5 M/mL 4D), 5×10^6 for 2 days (5 M/mL 2D), 5×10^6 for 1 day (5 M/mL 1D) or 11×10^6 cells/mL for 1 day (11 M/mL 1D)) LC–MS glycosylation analyses showed a reduction in MAN5 oligomannose structure in new preparations compared with pVITRO IgE reduced MAN5 in the new IgE is insufficient to affect IgE binding or functionality	
No	No	N/A	Yes	N/A	Glycoforms ranging from 5 to 9 hexoses were detected for site N394. Site N394 was fully glycosylated	Fc constructs
No	Yes	N/A	Yes	N/A	Unable to detect sites N140, N168 or N265—possibly too hydrophobic and under applied conditions, were likely retained irreversibly on the stationary phase. Site N218 had 3 different detectable glycoforms: H5N5S2F1; H5N5S1F1; H5N5F1. Site N371 had only one composition: H5N5. Site N394 had 8 detected glycoforms ranging from H5-H9N2 plus H5N4F1, H5N4 and H5N3	
Yes	No	No	N/A	Yes	Neutral sugar calculated as mannose was 23.6 moles/dimer, described as half of that from human IgE produced in U266 cells. N-acetylneuraminic acid was 4.0 moles/dimer. Evidence for glycan heterogeneity within Fc fragment preparations	

TABLE 3 (Continued)

Study	Year published	IgE source	Method of glycan removal	Method of glycan labelling	Analytical method—technique
Ikeyama et al. PMID: 3702874[42]	1986	Cells; U266	Enzymatic digestion (Neuraminidase)	N/A	Phenol-sulfuric acid method (total neutral sugar, mannose used as standard) GLC (quantitation of neutral and amino sugars) Periodic acid-resorcinol method (sialic acid, N-acetylneuraminic acid used as standard) Neuraminidase treatment
Kochwa et al. PMID: 5290027[36]	1971	Human; myeloma	Pronase digestion and fractionation	N/A	Anthrone reaction (hexoses, corrected for fucose content) Cysteine-sulfuric acid reagent (fucose) Thiobarbituric Assay of Warren (sialic acid) Boas modification of the Elson-Morgan reaction (hexosamines)
Koers et al. PMID: 30850477[37]	2019	Human; B cells; healthy, myasthenia gravis, systemic lupus erythematosus	N/A	N/A	Analysis of Ab repertoire data sets from peripheral blood and bone marrow B cells by next-generation sequencing
Koning et al. PMID: 31333671[38]	2019	Human; non-allergic	N/A	N/A	Analysis of peripheral blood B-cell transcripts

Comparative glycosylation	Site-specific analysis	Evidence for O-linked glycosylation	Glycoforms detected within sites	Differential glycosylation between sources	Summary of findings	Notes
No	No	N/A	N/A	Differential glycosylation between secreted IgE	Total carbohydrate content found to be 10.3%. Compared with human myeloma IgE-ND from previous work, mannose, sialic acid and galactose levels were all comparatively lower, whilst fucose and GlcNAc levels were similar. No evidence for O-linked glycosylation. Evidence of differential glycosylation within secreted IgE preparations	Did not identify source of differential glycosylation but ruled out differential sialylation
No	No	N/A	No	N/A	IgE carbohydrate content determined to comprise 12.1% of total MW and contained a mixture of hexose, fucose, sialic acid and hexosamine. Glucosamine was the only detectable amino sugar. Postulated a total of 4 carbohydrate chains with the following compositions and MWs: Glycan 1—H10N5S1F2, MW 3227 Glycan 2—H12N?S2F1, MW 2826 Glycan 3—H5N8S0.5, MW 2589 Glycan 4—H5N8S0.5, MW 2589	Previous work by Bennich and Johansson[108] had found 5 glycan chains, so noted the possibility that one glycopeptide peak may have contained 2 identical glycopeptides.
No	No	N/A	N/A	N/A	IgE has a 2-fold propensity for Fab glycan acquisition compared with IgG1 and IgA, which appears to be a hallmark of Th2-like responses (IgG4 similar). The frequency of non-germline N-glycosylation sites in the V region was significantly higher in bone marrow IgE. These differences could not be explained by elevated levels of SHM or obvious differences in VH allele usage. This suggests altered positive selection pressure for enhanced levels of Fab glycosylation in Th2-like Ab responses	Found no differences in the acquisition of N-glycosylation sites in autoantibody-mediated immune disease patients but didn't look at IgE. Postulated involvement of endogenous sugar-binding lectins that may drive selection pressure for Fab glycosylation in Th2-skewed responses
No	No	N/A	N/A	N/A	IgE VDJ has a moderate SHM burden but acquires more N-linked glycosylation sites through SHM than IgG or IgA. The overall prevalence of N-linked glycosylation motifs (germline- and SHM-derived) is significantly higher in IgE than IgM, IgA and IgG. This is predominantly due to the preservation of germline-encoded N-linked glycosylation motifs in IgE. N-glycosylation may hold roles in the expansion and maintenance of IgE-expression B cells in non-allergic individuals. Other proposed roles may be a disease specific role for N-linked glycosylation motif acquisition for selection of IgE-expressing B cells; or non-specific obstruction of antigen recognition and subsequent inhibition of BCR affinity maturation	

TABLE 3 (Continued)

Study	Year published	IgE source	Method of glycan removal	Method of glycan labelling	Analytical method—technique
McCraw et al. PMID: 36362241[64]	2022	Cells; HEK293	Enzymatic digestion (PNGaseF)	Procainamide labelling	HILIC-LC-ESI MS
Montero-Morales et al. PMID: 28400175[39]	2017	Cells; HEK293 Human; sera, unknown, plant-derived wild-type-HER2-IgE and ΔXF-HER2-IgE constructs grown in Nicotiana benthamiana	Proteolytic digestion (trypsin, proteinase K or a combination of trypsin and chymotrypsin)	N/A	LC-ESI-QTOF-MS/MS

Comparative glycosylation	Site-specific analysis	Evidence for O-linked glycosylation	Glycoforms detected within sites	Differential glycosylation between sources	Summary of findings	Notes
No	No	N/A	Yes	N/A	Oligomannose structures carried between 5 and 9 mannose residues. Complex glycans carried a mixture of bi-, tri- and tetra-antennary structures with up to 4 terminal sialic acid residues and a core fucose. Some glycans carried bisecting GlcNAc and there was evidence for presence of GalNAc	
Yes	Yes	N/A	Yes	Yes	Similar glycan occupancy rates for serum IgE to those found by Plomp et al; as well as between recombinant cell- and serum-derived IgE. All IgE variants showed complex glycans at sites N140, 168, 218, 265 and 371. Site N283 was completely unoccupied. Site 394 carried an oligomannose glycan. Complex glycan sites in serum IgE carried a mixture of mono- and bi-sialylated bi-antennary glycans with core fucose, and some bisected structures; with a total of 4 detected glycan species. Levels of bisection were increased at site 371. IgE produced in HEK293 cells presents with significant glycan diversity, with at least 20 different glycoforms identified. These consisted predominantly of galactosylated tri- and tetra-antennary N-glycans. As for serum IgE, 371 presented with predominantly bisected bi-antennary structures. For N394, serum and HEK IgE both carried oligomannose structures ranging from 5 to 9 mannoses. Structural modelling suggests presence of glycosylation at N371 may interfere with glycosylation of N383	Could not differentiate between bisected bi-antennary structures and tri-antennary structures, so presence of bisected structures was assumed based on previous serum IgE analyses

TABLE 3 (Continued)

Study	Year published	IgE source	Method of glycan removal	Method of glycan labelling	Analytical method—technique
Plomp et al. PMID: 24308486[11]	2014	Human; hyperimmunity, myeloma, pooled healthy	SDS-PAGE and in-gel enzymatic digestion (trypsin, chymotrypsin, proteinase K) In-gel or in-solution PNGase-F deglycosylation	2-AA reductive labelling	Nano-RP-LC-ESI ion trap-MS(/MS) (glycopeptides) LC–MS/MS and UHPLC (labelled glycans)
Rearick et al. PMID: 6830248[40]	1983	Human; myeloma	Pronase and endoglycosidase enzymatic digestion (ENGase, neuraminidase, galactosidase, mannosidase)	N/A	Methylation and acetolysis, borohydride reductions with radioactive tagging. Sequential enzyme digestions with neuraminidase, 3-galactosidase, β-N-acetylglucosaminidase, and a-mannosidase SDS-PAGE Paper chromatography and electrophoresis
Sassi et al. PMID: 24698316[41]	2014	Human; hyperimmunity, atopy	Trypsin and PNGase-F digestion	N/A	Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometric analysis.
Shade et al. PMID: 25824821[65]	2015	Cells; HEK293 Human; allergy	SDS-PAGE and in-gel enzymatic digestion (as for Plomp et al); enzymatic digestion (PNGase F, EndoF1).	N/A	Nano-LC–MS/MS (site-specific) HCD-MS/MS (identification of differences between chromatographically resolved isomers)

Comparative glycosylation	Site-specific analysis	Evidence for O-linked glycosylation	Glycoforms detected within sites	Differential glycosylation between sources	Summary of findings	Notes
Yes	Yes	N/A	Yes	Yes	Light chain shown to be present in both a glycosylated and non-glycosylated form in both healthy and hyperimmune IgEs but not in myeloma IgE. Sites N140, 168, 218, 265, 371 were all found to be solely occupied by complex-type glycans whilst N394 was occupied by a oligomannose glycan. The number of mannose residues on N394 ranged from 2 to 9, with the 5 mannose form being the most prevalent. Galactose residues were present on all complex glycan antennae with most structures all carrying 1–2 sialic acid residues. All complex glycans carried a core fucose in myeloma-IgE whereas low levels of non-fucosylated structures were found in hyperimmune and healthy IgE. 96% of complex glycans were bi-antennary in hyperimmune and healthy IgE compared with elevated levels of tri- and tetra-antennary structures in myeloma IgE. Bisected glycoforms were abundant at N371 but otherwise uncommon; and substantially lower in myeloma IgE. N383 was unoccupied. Sites N218, N371, N394 were partially occupied	Sialylation levels were suspected to be higher than detected in this study. Myeloma IgE glycosylation was markedly different from healthy and hyperimmune IgE
No	No	N/A	Yes	N/A	Demonstrated the oligomannose structure on human IgE was similar to that from rat IgE, carrying 5–9 mannose residues of which all were α-linked, with the exception of the innermost mannose residue which was β-linked to GlcNAc	Challenged the oligomannose glycan structure proposed by Baenziger et al. Suggested that as the oligomannose glycan structure was not unique to IgE, it was less likely it had specific involvement in IgE receptor binding
Yes	No	N/A	N/A	Yes	Decreased levels of tri- and tetra-antennary N-glycans in patients compared with controls. Otherwise no significant differences	No site-specific analysis
No	Yes	N/A	Yes	N/A	N394 carried an oligomannose glycan whilst all other sites carried complex-type glycans. All but one site in OVA-human IgE carried predominantly complex antennary structures	Site-specific analysis performed for recombinant IgE only

TABLE 3 (Continued)

Study	Year published	IgE source	Method of glycan removal	Method of glycan labelling	Analytical method—technique
Shade et al. PMID: 32499653[20]	2020	Human; non-atopic, allergy	Proteolysis (trypsin, chymotrypsin)	N/A	Nano LC–MS/MS
Wu et al. PMID: 26687240[43]	2016	Human; hyperimmunity, atopy	SDS-PAGE and in-gel enzymatic digestion (trypsin, chymotrypsin)	N/A	Nano-HPLC, LC–MS/MS
Zavázal et al. PMID: 2408980[44]	1985	Human; atopy	N/A	N/A	Lectin binding

Comparative glycosylation	Site-specific analysis	Evidence for O-linked glycosylation	Glycoforms detected within sites	Differential glycosylation between sources	Summary of findings	Notes
Yes	Yes	N/A	Yes	Yes	Sites N140, 168, 265 and 394 were fully occupied. Sites N218 and 371 were partially occupied. Site N383 was unoccupied Similar levels of mannose (oligomannose glycan) and fucose residues were identified on both non-atopic and atopic IgE. Levels of bisecting GlcNAc and terminal galactose were significantly increased on non-atopic IgE. Increased sialic acid levels on atopic IgE weren't sex- or age-dependent. Sialic acid content on total IgE was the key distinguishing factor between atopic and non-atopic IgE	
Yes	Yes	N/A	Yes	No	Site N383 was unoccupied, whilst N394 carried an oligomannose glycan. All other detected sites carried complex-type glycans. N265 was unmapped due to digestion process. Site N140 carried a mixture of mono- and bi-sialylated structures. All glycoforms were fucosylated and bi-antennary. Site N168 carried a core fucosylated desialylated bi-antennary glycan. Site N218 was structural diverse, with major structures being bi-antennary with fucose and sialic acid. Small amounts of non-fucosylated and non-sialylated glycans were detected; with some possibility of both mono- and tri-antennary structures. Site N371 consisted of predominantly mono-sialylated bi-antennary glycans, but tri- and mono-antennary as well as bi-sialylated glycoforms were also detected. Site N394 carried a mixture of high mannose and paucimannose structures	PGM3 mutation patients and an atopic dermatitis patient show similar glycan profiles. Did not rule out presence of other diseases and defined atopic dermatitis as a healthy control
No	No	N/A	N/A	N/A	Variable histamine release via lectin binding across different individuals may suggest heterogeneity in IgE glycosylation leading to differential lectin recognition. Concanavalin A showed differential binding across IgE from different atopic sera	Speculative as glycosylation status of IgE not confirmed

TABLE 4 Studies investigating IgE glycosylation in relation to structure and/or function.

Study	Year published	IgE source	IgE characteristics	Form of glycoengineering	Glycan/s investigated	Confirmation of glycosylation status
Bantleon et al. PMID: 26943931[63]	2016	Baculovirus-infected Sf9 cells (insect); HEK293 cells	Full-length	N/A	All	Lectin blot; mass spectrometry
Basu et al. PMID: 7685756[30]	1993	Human myeloma; CHO and COS cells	Full-length (myeloma); Cε2-Cε4 Fc fragments	Enzymatic deglycosylation (PNGase-F, Endo-F, Endo-H, ENGase)	N265, 371, 383, 394	SDS-PAGE
Björklund et al. PMID: 10403487[45]	1999	Human myeloma; human sera	Full-length	Enzymatic deglycosylation (PNGase-F, sialidase)	All	SDS-PAGE; lectin binding
Björklund et al. PMID: 10865116[46]	2000	Baculovirus-infected Sf9 cells (insect)	rCε2-3 & rCε2-4 Fc fragments	Enzymatic deglycosylation (PNGase-F, Endo-H)	N265, 371, 383, 394	Immunoblotting
Doré et al. PMID: 28844738[47]	2017	NS0 and HEK293 cell lines	Fcε3-4 fragments	N/A	N371, 383, 394	Mass spectrometry
Garman et al. PMID: 10917520[48]	2000	Baculovirus expression system (insect)	Fc constructs	N/A	N394	
Henry et al. PMID: 10858288[49]	2000	*E.coli*	Recombinant Cε3 fragments	N/A	N/A	N/A
Hunt et al. PMID: 15743766[50]	2005	NS0 and *E.coli*	Fcε3-4 fragments	Enzymatic deglycosylation (PNGase-F)	N371, 383, 394	SDS-PAGE, Schiff's staining, mass spectrometry
McCraw et al. PMID: 36362241[64]	2022	HEK293 cells	Full-length	Enzymatic deglycosylation (Neuraminidase)	All sialic acid residues	Mass spectrometry
Nettleton et al. PMID: 7613162[51]	1995	COS cells	Cε3-4 Fc fragments	Site Mutation	N371, 383, 394	Western Blot

Structural/functional attributes investigated	Conclusions	Structural involvement indicated	Functional involvement indicated	Additional notes
FcεRI binding and functional activities	Regardless of variant glycosylation provided by insect expression system, Sf9-derived IgE retains comparable FcεRI binding and αIgE-mediated degranulation of RBL-SX38 cells to a HEK293-derived IgE	N/A	Maybe	Supports conclusions that N394/oligomannose glycans are the obligate glycan structure of IgE
Structural stability; FcεRIα binding	Glycans hold no roles in mediating function but are important for structural stabilization	Yes	No	No evidence for O-linked glycosylation. Deglycosylated Ab typically aggregated but non-aggregated deglycosylated Ab showed comparable binding to glycosylated Ab
Recognition by domain-specific anti-IgE Abs; rFcεRIα	Extent of deglycosylation impacted binding profile of around a 1/3 of α-Cε2 mAbs but not other domain-specific mAbs. rFcεRIα binding was substantially reduced following PNGase-F treatment but not sialidase treatment alone. Glycan sites in Cε3, particularly N394, may have structural involvement	Yes	Yes	
Structural stability; FcεRIα binding	N-glycosylation, particularly N394, in the Cε2-4 domains likely have roles in stabilizing structures involved in FcεRI binding	Yes	Yes	Found no evidence of aggregation following deglycosylation. At least 2 out of 4 potential glycan sites confirmed to be occupied
Structural stability	N394 does not contribute to thermal stability of IgE	No	N/A	Results contrast with IgG results
FcεRI binding	No involvement of N394 in FcεRI binding	N/A	No	
FcεRI binding	Found no requirement for glycosylation in FcεRI binding	N/A	No	
FcεRI binding	Deglycosylation of fragments produced in NS0 cells did not impact folding. Deglycosylated material showed faster dissociation from FcεRI but similar on-rates to glycosylated material	No	Maybe	
FcεRI and CD23 binding	Desialylation of IgE did not appear to impact FcεRI binding but may impact CD23 binding. Desialylation does not appear to structurally impact IgE	Maybe	Yes	
FcεRI binding	Mutations in N371 and N383 retained FcεRI binding. Fragments with N394 mutations were incapable of binding FcεRI but was indeterminate if this was through structural stability or functional requirement	Maybe	Yes	Impact on binding could be due to structural impact

(Continues)

TABLE 4 (Continued)

Study	Year published	IgE source	IgE characteristics	Form of glycoengineering	Glycan/s investigated	Confirmation of glycosylation status
Plattner et al. PMID: 37073887[52]	2023	Human sera (atopic); HEK293 cells and human B-cell hybridomas	Full-Length	Enzymatic deglycosylation (PNGase-F; Endo-F1; Neuraminidase); Site Mutation	All (Enzymatic deglycosylation); N394 (site mutation)	Unconfirmed
Robertson et al. PMID: 2261464[53]	1990	Human myeloma	Full-length	Enzymatic deglycosylation (Neuraminidase)	All sialic acid residues	Unconfirmed
Robertson et al. PMID: 1919004[54]	1991	Human sera (Hyper-IgE, Atopic Dermatitis, myeloma)	Full-length	Enzymatic deglycosylation (Neuraminidase)	All sialic acid residues	SDS-PAGE
Sayers et al. PMID: 9819207[55]	1998	J558L cells	Full-length	Site Mutation	N371, N394	Unconfirmed
Sayers et al. PMID: 15199058[56]	2004	J558L cells	Full-length	Site Mutation	N371	Unconfirmed
Shade et al. PMID: 32499653[20]	2020	Human sera (allergic, non-atopic); Expi293 cells	Full-length	Enzymatic deglycosylation (Neuraminidase)	All sialic acid residues	Mass spectrometry; lectin blot
Shade et al. PMID: 25824821[65]	2015	HEK293 cells	Full-length	Enzymatic deglycosylation (PNGase-F, Endo-F1); site-specific mutation	All; N371, N383, N394	Mass spectrometry
Shibasaki et al. PMID: 1378039[57]	1992	Human sera (allergy/ atopy)	Full-length	N/A	All	Lectin recognition
Truong et al. PMID: 8418206[58]	1993	Human myeloma; human sera (Hyper-IgE syndrome, parasitic infection)	Full-length	Enzymatic deglycosylation (Neuraminidase)	All sialic acid residues	Unconfirmed
Vercelli et al. PMID: 2468089[59]	1989	*E.coli*	Recombinant ε chain fragments	N/A	N/A	N/A

Structural/functional attributes investigated	Conclusions	Structural involvement indicated	Functional involvement indicated	Additional notes
Omalizumab binding and activity	Removal of N394 reduced omalizumab's ability to bind IgE and block IgE:CD23 interactions. There was no impact of sialylation on omalizumab:IgE interactions. Glycan arrays indicated conformational changes in the absence of specific glycosylation may trigger changes in omalizumab binding activity.	Maybe	Yes	Impact on binding could be due to structural impact, but was not fully defined
Galectin-3 binding	Treating myeloma IgE with neuraminidase resulted in increased recognition by hGal3	N/A	Yes	
Galectin-3 binding	Recognition by Gal3 varied between patient samples. Non-reactive samples showed increased Gal3 recognition following neuraminidase treatment. Appears to be differential sialylation within patient IgE populations as well as between patients	N/A	Yes	
FcεRI binding	N371 mutation had a moderate effect on FcεRI binding kinetics; whilst N394 mutants showed no detectable binding activity. N394 is essential for correct IgE folding in mammalian cells. N371 mutants showed increased binding to CD23 by more than 10-fold	Yes	Yes	CD23 data not shown and appears to remain unpublished
CD23 binding	No involvement of N371 in IgE-CD23 interactions	N/A	No	Previous results may have been due to mutation of nearby Lys352
FcεRI binding and functional activities	Increased sialic acid content found on allergic-derived IgE compared with non-allergic which appeared to correlate with increased mast cell degranulation for allergic-derived IgE. Results could be replicated by neuraminidase treatment of rIgE. Reduced phosphorylation downstream of FcεRI found with low sialic acid IgE. No difference in FcεRI or allergen binding	N/A	Yes	
FcεRI binding and functional activities	PNGase-F treatment attenuated FcεRI binding and degranulation. Glycan requirement mapped to Cε3 domain—N371 and 383 mutations had no impact whilst N394 mutations abrogated FcεRI binding	N/A	Yes	No confirmation of PNGase F activity. Did not determine if samples aggregated following deglycosylation as previously reported
Basophil degranulation	Variation in IgE glycosylation may impact histamine release via lectin cross-linking	N/A	Maybe	
Galectin-3 binding	Observed differential Gal3 binding to IgE proposed to be due to differential glycosylation. Gal3 binding was significantly increased following neuraminidase treatment. Sialylation may modulate Gal3 recognition of IgE	N/A	Yes	
CD23 binding	Found no requirement for glycosylation in CD23 binding. Enzymatic deglycosylation of an intact myeloma IgE led to increased CD23 binding (unshown). Results implied decreased glycosylation may increase CD23 binding	N/A	Maybe	

(Continues)

TABLE 4 (Continued)

Study	Year published	IgE source	IgE characteristics	Form of glycoengineering	Glycan/s investigated	Confirmation of glycosylation status
Wollenberg et al. PMID: 8350053[60]	1993	Human myeloma	Full-length	Enzymatic deglycosylation (Neuraminidase)	All sialic acid residues	Unconfirmed
Young et al. PMID: 7543206[62]	1995	CHO-L761H and NS0 cells	Recombinant IgE-Fc	Site Mutation	N265, N371	SDS-PAGE
Zavázal et al. PMID: 8645991[19]	1996	Human myeloma/ allergy	Full-length	N/A	All	Mass spectrometry, lectin binding

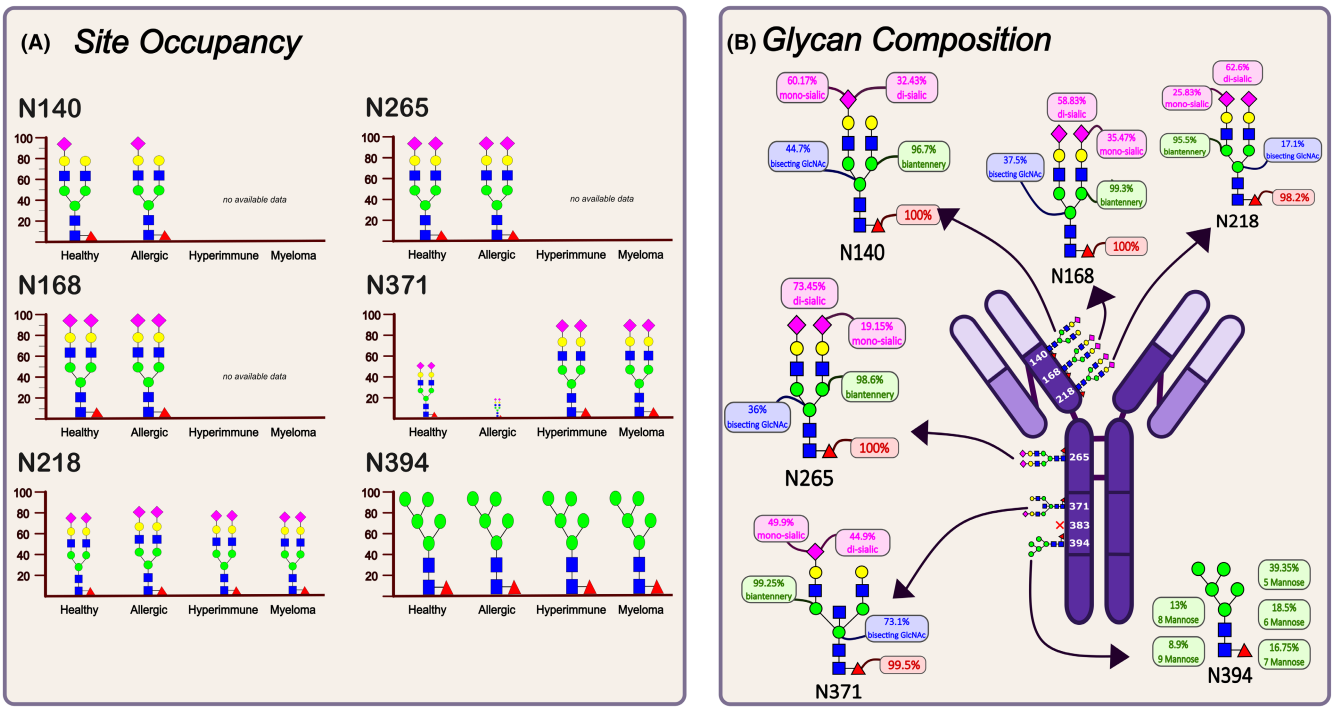

FIGURE 4 Site occupancy and glycan composition for IgE derived from human sera. (A) Average rates of site occupancy for the occupied glycans of IgE. Data given as % rate of occupancy. Healthy pooled IgE, $N=2$[20,39]; hyperimmune IgE, $N=1$[11]; myeloma IgE, $N=1$[11]; allergic IgE, $N=1$.[20] (B) Average rates of glycan features for individual glycan sites. Glycans shown for each site are depicted with the most common glycan features. N140, $N=3$[11,20,39]; N168, $N=3$[11,20,39]; N218, $N=3$[11,20,39]; N265, $N=2$ (sialic acid, fucose), $N=1$ (bisection, antennae)[11,20]; N317, $N=2$[11,39]; N394, $N=2$.[20,39]

hypermutation (SHM), compared with IgG1 or IgA. No differences in N-glycosylation site frequencies or in N-linked glycan acquisition were found between patients and healthy groups. These findings led to the suggestion that a heightened propensity for Fab region glycosylation during B-cell affinity maturation may represent a hallmark for Th2-biased immune responses.[37] A study of B cells from healthy

Structural/functional attributes investigated	Conclusions	Structural involvement indicated	Functional involvement indicated	Additional notes
Galectin-3 binding	Desialylation of IgE increased Gal3 binding. Neu-IgE bound Langerhans cells only in the presence of exogenous Gal3. Function of exogenous Gal3 appeared to depend on sialylation state of IgE, mediating binding of low sialic acid IgE to LC whilst inhibiting high sialic acid IgE-binding. Sialylation may act as a means of modulating IgE activity	N/A	Yes	
FcεRI and CD23 binding	Double N265 N371 glycan mutants had comparable FcεRI binding to unmodified IgE-Fcs; but affinity for CD23 was far higher compared to wild-type IgE-Fc. This affect was traced to N265 due to underglycosylation of N371 in wild-type IgE-Fc. Proposed glycosylation decreases affinity for CD23 which is overcome by the Fab regions, with extent of glycosylation potentially modulating IgE effector functions	N/A	Yes	
Allergic initiation	Differences in IgE glycosylation appear to contribute to pathological mechanisms of allergy	N/A	Yes	

individuals showed that although IgE features a lower SHM burden potentially due to the limited presence of IgE+ B cells in germinal centres, it not only retains germline-encoded but also acquires more N-glycan motifs through SHM compared with IgG and IgA.[38] This may suggest increased pressure for IgE class antibodies to acquire N-glycans, a phenomenon potentially linked to a role of glycans at these sites in masking antigen recognition, thus impairing affinity maturation to regulate IgE levels. Whether these processes are dysregulated in allergic conditions remains to be elucidated.

3.5.2 | IgE glycosylation and structure

Nine studies investigated glycan involvement in IgE structure, predominantly focusing on N394 (Figure 1), with limited investigation into other glycans or specific glycan residues. Findings were inconsistent.

One study reported that mutations in N394 or the nearby residue T396 resulted in human IgE Fc region fragments incapable of binding FcεRIα. These findings could suggest the involvement of this glycan site in structural integrity and Fc receptor-binding affinity.[51] Further work involving Fc fragments suggested roles for glycosylation in maintaining structural stability; however, the extent of glycan involvement differed between studies.[30,46] Basu et al.[30] reported aggregation following deglycosylation, assigning a role for Fc glycans in preserving structure/conformation integrity. Björklund et al.[46] found no aggregation, although reported a specific role for N394 in preserving FcεRI-binding site integrity. Methodology varied, with different enzyme combinations of Endo-F/Endo-H and PNGase-F/Endo-H (Table 2) used by Basu et al. and Björklund et al., respectively. Deglycosylation of recombinant Fc fragments produced in NS0 cells was reported not to impact Fc folding; however, the presence of interchain disulphide bridges in IgE Fc fragments not present in the full Fc may have far greater impact on structural stability, potentially masking glycan contributions in such experiments.[50]

Regarding full-length IgE, size-exclusion chromatography (SEC) and circular dichroism (CD) showed no aggregation, but CD was thought to reveal a small shift following EndoF1 treatment, interpreted as a change in IgE secondary structure.[65] N394 does not appear to modulate thermal stability of IgE Fcε3-4 fragments.[47] No significant structural changes or aggregation were detected following desialylation of full-length IgE, but complete lack of impact on structure could not conclusively be drawn.[64]

Glycosylation in full-length IgE may act to mask potential binding epitopes on specific domains: PNGase-F treatment (Table 2) was found to improve reactivity of several anti-Cε2 mAbs and substantially reduced rFcεRIα binding.[45] Aggregation was ruled out as a potential cause by fractionating IgE preparations to isolate non-aggregated IgE. In comparison, whilst sialidase treatment (Table 2) could similarly improve anti-Cε2 mAb reactivity, it had no impact on rFcεRIα binding.[45]

3.5.3 | IgE glycosylation and its impact on IgE interactions with FcεRI

Most studies focused on N394, with few investigating other Fc glycan sites or sialic acid residues. Studies investigating N394 in FcεRI interactions tended to use IgE fragments as opposed to full-length IgE.

Mutation of asparagine residues to alternative residues for sites N394 and N371 revealed that loss of N371 had only minor impact on binding kinetics, whilst N394 mutants showed no detectable binding activity to FcεRIα and inability to drive FcεRI-mediated degranulation.[55] Structural impact was not determined.

N394 was declared essential for FcεR binding: whether this is through structural changes or direct effects on FcεR binding was undetermined.[51] Later work declared N394 as essential for overall IgE functional activity.[65] However, no significant contributions of N394 to the FcεRI-binding interface were observed when using insect cell-derived Cε3-4 Fc fragments.[48] A second study using full-length IgE from an insect expression system found comparable FcεRI binding and degranulation to a HEK293-derived IgE; this may support oligomannose glycans as an obligate requirement for functional activity.[63]

Escherichia coli-produced IgE-Fc fragments and isolated Cε3 domains, completely lacking glycosylation, nonetheless showed near-full FcεRI affinity,[49] whilst deglycosylated Fc-Cε3-4 fragments from NS0 cells retained FcεRI binding, albeit with approximately 4-fold lower affinity, attributed to faster receptor off-rates. On-rates were comparable to those in glycosylated preparations.[50] Previous studies have also observed differing binding kinetics following deglycosylation.[74–76] Unlike those produced in mammalian cells, *E. coli*-derived proteins typically are required to undergo a refolding process in order for a functionally active protein to be generated. This carries a risk that this refolding step may yield a differentially folded structure to that of the natively expressed molecule. Thus, any differences between mammalian system-produced and *E. coli*-derived IgE-Fc domains may be due to non-physiologically folded structure rather than the presence or absence of glycans.

Obligate glycan requirement was mapped to the Cε3 domain.[65] N371 site mutations retained similar binding constants to wild-type (WT) IgE and ability to drive IgE-mediated degranulation, although one study demonstrated slightly altered degranulation of N371 mutants compared with WT.[51,55,62,65] Mutation of N383 or N265 similarly did not impact FcεRI interactions.[51,62] Mutations of N394 through changing Asn to an alternative residue, however, rendered Fc fragments incapable of binding FcεRI or driving FcεRI-mediated degranulation.[51,55,65] Mast cell degranulation was slightly decreased when cells were sensitized via a Cε1 domain glycan mutant (Figure 1, N140, N168, N218): this was speculated to indicate a minor requirement of these glycans in IgE function, possibly through modulation of Fab arm flexibility.[65]

PNGase-F treatment of different IgE-Fc fragments was shown to attenuate FcεRI binding.[45,46] However, it has not been possible to uncouple glycan modification from structural alteration. Similar but less pronounced effects were observed when using Endo-H (Endoglycosidase, Table 2).[46] More work would be required to confirm these findings and determine the underlying causes of these observations.

IgEs derived from allergic individuals may differ in their ability to drive FcεRI-mediated functions compared with IgEs derived from non-allergic states. Alongside evidence of differential glycosylation amongst IgEs from allergic individuals compared with non-allergics,[19] IgE antibodies from allergic individuals are reported to have higher sialic acid content, correlating with increased degranulation and allergic activity.[20] These may potentially be attributed to differences in FcεR engagement and immune cell signalling. Sialidase treatment (Table 2) alone could not replicate the effects of PNGase-F treatment on FcεRI in early studies,[45] although more recent work reported that desialylation of IgE reduced mast cell degranulation and FcεRI signalling.[20] However, another study using desialylated IgE reported no difference in FcεRI recognition or binding compared with WT-IgE.[64]

3.5.4 | IgE glycosylation and CD23/FcεRII Interactions

Few studies investigated IgE glycosylation with regard to CD23 interactions. Whilst the CD23-IgE interaction is known to be enhanced by calcium, no requirement of IgE glycosylation for binding CD23 is reported.[59,77] However, increased binding to CD23 was observed when IgE was deglycosylated via enzymes[64] or site mutation.[62]

IgE-Fc fragments mutated to lack both N265 and N371 glycan sites had 10-fold higher affinity for CD23 compared with WT-IgE-Fc,[62] an effect traced to N265. As double-mutant Fc fragments had comparable CD23 binding to full-length IgE, the authors suggested that the Fab region may increase CD23 affinity sufficiently to overcome glycan hindrance.[62] Desialylation alone appears sufficient to increase CD23 recognition and binding compared with WT-IgE.[64] Site N371 was confirmed to have no involvement in IgE-CD23 interactions.[56]

3.5.5 | IgE glycosylation and non-Fc receptor IgE-binding proteins

Limited investigation has been carried out on other IgE-binding proteins such as Galectin 3 (Gal3). Gal3 interactions with IgE appear predominantly modulated by IgE sialic acid levels, suggesting that Gal3 preferentially binds restricted IgE glycoforms.[53,54,58,60] Evidence supports heterogenous sialylation within the general population[53] and between hyperimmune patients[54] as determined by variable Gal3 recognition of serum IgE from different patient cohorts.

In agreement with these reports, Gal3 binding of WT-IgE appears limited, but interactions can be substantially increased via sialidase treatment[53,58]; an observation also reported using hyperimmune syndrome-derived IgE.[54] Gal3 may act to modulate IgE interactions with cells: Gal3 expressed on neutrophils differentially bound IgE according to levels of IgE sialylation, with decreased sialylation leading to increased binding.[58] Similarly, IgE binding to Langerhans cells was modulated by exogenous Gal3 produced by keratinocytes, an interaction enhanced by decreased IgE sialylation.[60] Gal3 could also inhibit binding of sialylated WT-IgE to FcεRI, speculated to be mediated by Gal3-FcεRI interactions sterically hindering IgE binding to FcεRI.[60]

Recent work has shed light on the mechanisms of the anti-IgE mAb omalizumab, which is approved for the treatment of IgE-mediated diseases.[78,79] Plattner et al. traced the binding requirement of omalizumab to the presence of oligomannose, specifically N384, on IgE; with both Endo-F1-treated IgE (Table 2) and site mutation of N394 glycan site able to abrogate omalizumab binding to IgE. These findings highlight a potential requirement of oligomannose for omalizumab function.[52]

4 | DISCUSSION

4.1 | Differential glycosylation of IgE from different sources and different disease states

Differential IgG glycosylation is shown across healthy states and different diseases including inflammatory autoimmune conditions such as rheumatoid arthritis,[80,81] HIV[82] and in some settings serve as a prognostic biomarker of disease severity or progression.[83,84] However, few observations have been made regarding IgE glycosylation in disease. IgE carries no known conserved O-glycosylation motifs, and no evidence for O-linked glycosylation is reported within myeloma-derived IgE.[27,42] However, composition of its N-glycans, particularly those carrying complex-type glycans (Figure 1), may vary between sources and disease states. In the literature, site-specific analysis of myeloma-derived IgEs reported elevated levels of tri-antennary and tetra-antennary structures and decreased bisected glycoform levels compared with both hyperimmune IgE syndrome- and control-derived IgEs,[11] which would align with reports of aberrant glycosylation associated with cancer.[70,71,85] Allergy-derived IgEs similarly display altered glycosylation, with increased sialylation and lower levels of bisection compared with non-allergic IgE reported,[20] and sialylation levels may vary between IgE secretory sources.[31] These may align with suggestions of allergy as a disease of altered glycosylation.[19]

Whilst in IgG, changes in glycosylation patterns have been linked to loss of tolerance and onset of autoimmune disease,[80,86] limited work has been performed with regard to IgE glycosylation and autoimmunity, particularly IgE-mediated autoimmune conditions.[87] It would be of interest to define whether, as for IgG, predictive autoimmune-associated glycosylation patterns exist for IgE. Literature already suggests the presence of differential IgE glycosylation patterns in humans: although unconfirmed, observations surrounding the requirements of glycosylation for recognition of IgE by omalizumab have prompted speculation that glycosylation differences could explain non-response to omalizumab treatment,[52] and findings from earlier works suggest patterns of differential sialylation within the general population.[54] Additional investigation, with a focus on site-specific analysis, would be of great interest to clarify findings within the literature.

Recombinant mammalian cell-derived IgEs show similarities in glycan structures to myeloma-derived IgEs, as well as presenting with significant glycan diversity even between preparations from the same cell line.[39,42] As such, recombinant IgEs may not accurately reflect 'healthy' IgE. Although non-human systems may provide more consistent glycosylation patterns,[39,63] the presence of non-human glycan structures including xylose (in plant expression systems) and α-Gal (in non-human mammalian cell lines) may further complicate results. Additionally, the use of bacterial expression systems, whilst removing the complexity of glycosylation, is unlikely to accurately represent the attributes of human IgE.

Very low serum IgE levels in healthy states present challenges for obtaining adequate antibody yields: consequently, many studies lack true 'healthy' controls to compare IgE in health versus disease. Studies largely rely on IgE from disease states such as myeloma or hyperimmune conditions, a significant limitation. Recent advancements in IgE-based technologies such as IgE-specific purification matrixes[64] and novel serum-based purification protocols[88] may increase the availability of healthy serum IgE for study and comparative evaluations.

4.2 | Glycans as an IgE regulatory mechanism

Alongside the presence of seven conserved N-glycan sites, higher selection pressure for IgE to acquire N-glycans in its variable regions during SHM has been reported. This has been suggested to denote putative roles of glycans in masking antigen recognition and promoting IgE regulation. Furthermore, similar observations for IgG4 to acquire N-glycan motifs are thought to indicate a propensity for increased glycosylation of Th2 antibodies.[37,38] Both IgE and IgG4 are implicated in the Th2 response,[89] allergy and atopy. It is possible therefore that glycosylation could act as a regulatory mechanism for controlling antibody levels in Th2 immune responses, a process that may be dysregulated in allergic or autoimmune conditions.

Observations surrounding differential IgE sialylation may support this, noted to vary both within the general population, and more specifically in allergic patient-derived IgEs.[20,54] Sialylation levels could represent a form of IgE regulation, particularly via Galectin 3 where sialylation levels can modulate Gal3-IgE interactions, impairing IgE engagement with canonical FcRs on immune effector cell populations.[53,54,58,60] As increased sialylation can prolong glycoprotein half-lives through masking galactose residues from recognition by the asialoglycoprotein receptor.[90] This may account for heightened levels of IgE observed in allergic/atopic individuals and dysregulated IgE metabolism,[91] and it is consistent with observations of altered sialylation in allergic individuals.[20] Hypersensitivity has been proposed as a disease of glycosylation, based on differences in glycosylation of serum-derived IgE from allergic versus non-allergic individuals. These findings suggest that allergy may be associated with altered IgE glycosylation.[19]

Sialylation may therefore represent a regulatory function that becomes dysregulated in IgE-mediated diseases. Low levels of sialylation that are subsequently increased during hypersensitivity potentially contribute to dysregulated IgE metabolism and could help explain the higher levels of serum IgE detected within patient blood.

4.3 | Glycans and IgE structure

With regard to the role of glycans on IgE production and secretion, the clearest evidence to-date have been collected with rodent antibodies. Rat and mouse IgE secretion is inhibited in the presence of broad-spectrum glycosylation inhibitors such as tunicamycin.[61,72] At least for rodent IgE, this may suggest a fundamental contribution of glycans to structural maintenance or control of antibody assembly and production. Evidence for structural contributions of glycans in human IgEs are unclear. Reports of aggregation following PNGase-F-mediated deglycosylation vary,[30,45,46] and investigations of the human Cε3 domain following PNGase-F treatment suggested that subtle changes may contribute to reduced binding to FcεRI.[45] Whilst IgE Fc fragments can successfully be produced without glycans in bacterial systems such as *E.coli*, it is possible that the requirement for refolding may yield a non-physiologically folded structure. Robust studies interrogating antibody structural characteristics following glycoengineering of IgE in future studies may provide important insight.

The most convincing argument for a structural contribution arises from observations made regarding CD23. Whilst desialylation of IgE does not appear to affect FcεRI binding,[20,45,64] it may contribute to binding to CD23.[64] This is supported by observations that glycosylation may decrease CD23 affinity via a shielding effect, partially mitigated by the Fab region in full-length IgE.[59,62] Whether this is due to steric hindrance or changes in molecular conformation remains unclear; however, IgE desialylation was reported to improve reactivity of certain anti-Cε2 mAbs, something that may suggest effects on IgE conformation.[45] Similarly, deglycosylation of IgE substantially reduced omalizumab binding, indicating the possibility that glycosylation that may be linked to conformational changes that impact binding to omalizumab.[52] Of interest, recent work using murine IgEs and IgGs found roles for auto-antibodies recognizing IgE, in IgE regulation, with observations that these auto-antibodies could be specific for IgE glycans and thus preferentially bind glycosylated IgE.[21,92] Whilst CD23 was found to be required for clearance of IgG:IgE complexes from the circulation, the functionality of these anti-IgE auto-antibodies appeared dependent on IgE glycosylation, with auto-antibodies raised in response to deglycosylated IgE showing decreased activity compared with those raised in response to glycosylated IgE.[21] These findings suggest an involvement of IgE glycosylation in mediating regulatory responses through IgG; however, whether this interaction is attributable to a direct involvement of IgE glycans or a passive structural conformation imparted by the presence of glycosylation and whether these phenomena apply to human IgE remain to be defined. Together, however, these data suggest that potential conformational changes in the IgE structure resulting from changes to glycosylation may substantially impact the ability of anti-IgE antibodies to recognize and engage with IgE.

Interpretation of existing data suggests glycan-mediated stabilization of IgE conformation may occur upon binding to the FcεRI,[50] and, similarly, that removal of glycans may trigger conformational

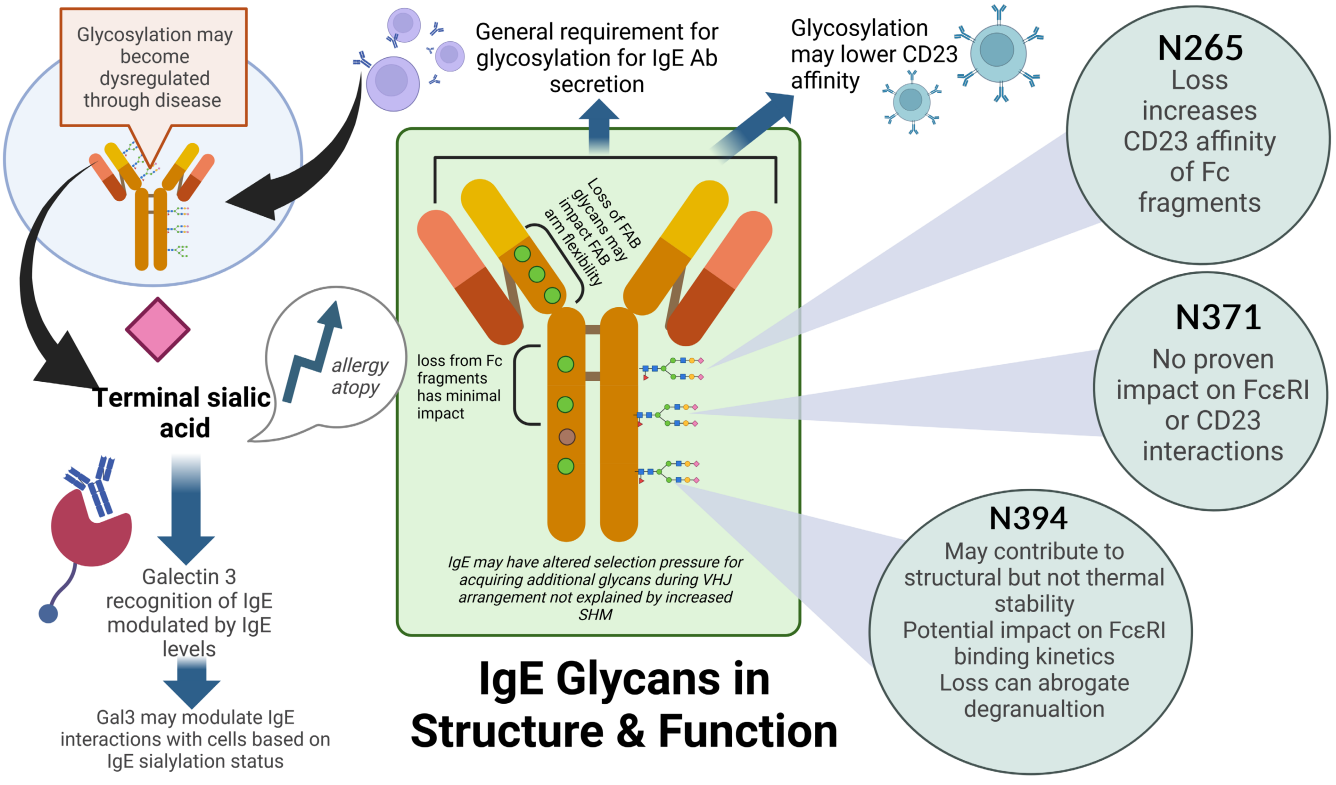

FIGURE 5 Graphical summary of the impact of glycans on IgE structure and function, showing the main conclusions of this literature review. Evidence from the literature suggests that terminal residues such as sialic acid may have an impact on IgE interactions with receptors such as Gal3 and may be involved in regulation of IgE homeostasis. Created with BioRender.com.

changes leading to an opening of IgE's bent conformation.[45,93] Conformational changes in the IgE structure upon FcεR binding are known to contribute to the slow dissociation rates from FcεRI[94]; observations that glycoengineered IgE may have modified FcεR-binding kinetics suggest a conformation impact that should be further investigated.[50,64] It is likely that changes in glycosylation may trigger modest changes in IgE structure, promoting a more open conformation that may subsequently influence interactions with IgE receptors or binding proteins.

4.4 | Glycans and IgE function

Separating structural from functional impact is challenging, especially considering the disparate analytical and mechanistic methodologies (Table S4) conducted for the glycovariants across studies. *E.coli*-derived IgE Fc fragments, which lack glycan structures, still bind FcεRI[49,95] and IgEs expressed in cellular systems, both human and otherwise, retain FcεRI binding despite the presence of heterogenous glycoforms. These argue against an innate requirement of IgE glycosylation for function.

N394 is the only residue with consistent evidence for functional contribution, since IgEs mutated to lack N394 are incapable of binding FcεRI or mediating degranulation.[51,55,65] Production of fully functional IgEs from insect-based systems, where glycosylation is primarily oligo- or paucimannose in nature, lends further credence to the proposal of N394, or, more broadly, oligomannose glycans as the obligate IgE glycan.[46,48,63]

In comparison, mutation of sites N371,[51,55,62,65] N383[51] or N265[62] have little impact on FcεR binding or functionality,[65] although there is some suggestion that glycans in the Cε1 domain (Figure 1, N140, N168, N218) may impact functionality: loss exerted moderate decreases in mast cell degranulation.[65] This again suggests a requirement for N394 for basic function, or perhaps maintenance of the base IgE structure required for function. Contrastingly, other glycans may provide a regulatory mechanism, as suggested for sialic acid; or help finetune interactions with Fc receptors via minor structural changes, such as in the case of CD23. It is conceivable that aberrant IgE glycosylation such as higher levels of sialic acid in pathological states resulting in reduced CD23 affinity may impair IgE regulation via engagement and contribute to disease pathology.

5 | CONCLUSIONS

Overall, certain glycan features may impact IgE structure and stability, and terminal glycans such as sialic acid residues may contribute to the regulation of IgE clearance rates via interactions with non-canonical receptors, potentially acting as a regulatory mechanism and often associated with allergic diseases (Figure 5). However, several methodological limitations exist that may curtail conclusions (Box 1), and thus, further work is needed to determine structural and functional roles of IgE glycans (Box 2). For instance, limited availability of IgE from healthy populations for study and comparison with IgEs derived from different pathological conditions presents a significant challenge. This is further hampered by lack of understanding of how 'healthy' state may differ between genders and ethnicities. Different expression systems may not accurately reflect glycan composition in specific pathological states, leading to inconsistencies in structural and functional evaluations; for instance, most studies demonstrating some level of contribution of N394 in IgE functions utilized mammalian expression systems[30,45,51,55,65] to generate IgE, whilst reports that typically found no contributions employed non-mammalian expression systems.[48–50] To that end, further consideration of the choice of expression system and its impact on IgE functional attributes is warranted. In addition to reported roles in immune regulation, allergy and autoimmunity, there is growing interest in the use of IgE monoclonal antibodies for cancer treatment, in the field of AllergoOncology.[96] Evidence of anti-tumour functions was reported in a Phase 1 clinical trial of the first-in-class Folate Receptor alpha-specific MOv18-IgE.[97] Additional IgEs and other IgE formats such as bispecifics[98] are now being explored for therapeutic application in different settings such as melanoma,[99] multiple myeloma,[100] pancreatic cancer[101] and prion disease.[102] Thus far, limited attention has been given to the impact of IgE glycosylation on therapeutic efficacy. Glycoengineering of therapeutic IgGs has resulted in development of afucosylated antibodies capable of driving enhanced ADCC.[103] With suggestions that heightened sialylation may enhance IgE effector functions and potentially mediate clearance rates,[20,104] understanding glycan composition of recombinant IgEs and glycoengineering may contribute to the therapeutic application of this class.

BOX 1 Common Limitations Affecting Studies of IgE Glycosylation.

- **Disparate methodologies**, including the use of different cell expression systems for antibody production and glycoengineering, and glycoanalytical tools.
- **Limited investigation into the impact of altering glycan site or glycan composition on structure**, often no simultaneous structural evaluations and functional analysis are conducted to ascertain a direct involvement of glycosylation.
- **Limited technologies for obtaining IgE,** either from human blood or tissues or from cell culture supernatants, in sufficient quantities for study.
- **Low levels of IgE from healthy states**, where serum IgE concentration is often low present challenges in obtaining sufficient quantities of antibody for evaluation.
- **Lack of consensus on the definition of 'healthy'** in comparative studies with different 'disease' states means that any differential glycosylation between health and disease, age groups and biological sex may be under-reported.

BOX 2 Suggested Areas for Improvement in Studies of IgE Glycosylation in Health, Structure and Function and Different Pathological Conditions.

- **Development of guidelines for the definition and inclusion of healthy volunteers, for example** 'healthy' state definition could be free from allergy, atopic disease, autoimmune or other inflammatory immune conditions or malignancy at the time of sample harvesting; age and sex matching of healthy volunteer samples with patient samples.
- **Incorporation of new technologies specific for the purification of IgE** may aid in the harvesting of IgE from serum, particularly from non-allergic individuals. Additional work into techniques for the purification of IgE from human serum may benefit the field, particularly for improving accessibility to IgEs from samples with low antibody titres.
- **Structural analyses aligned with functional interrogation of IgE**, to understand the contributions of glycans in antibody structural characteristics and biological functions.
- **Investigation of the impact of IgE glycosylation on interactions with CD23, IgE-binding proteins and anti-IgE antibodies**, investigation into the functional consequences of these interactions and their involvements in IgE activity could shed further light on allergic mechanisms and other conditions where IgE is dysregulated.

New investigations utilizing modern and higher throughput analytical technologies and studies in larger cohorts are required to help understand the impact of glycans on IgE-mediated diseases and to inform treatment design. Overall, the broad and varied nature of existing literature highlights multiple avenues for future research surrounding IgE glycosylation to both explore novel avenues and clarify existing findings.

AUTHOR CONTRIBUTIONS

Conceptualization: A.J.M., S.C., S.N.K. and A.C. Methodology: A.J.M., S.N.K., A.C. and L.C.P. Investigation: A.J.M., L.C.P. and J.H. Resources: A.J.M., A.C., L.C.P. and A.S. Data curation: A.J.M., J.H., R.A.G., A.C., M.V.H. and A.S. Writing—original draft preparation: A.J.M, S.N.K., L.C.P. and A.C. Writing—review and editing: A.J.M., G.K.W., S.N.K., R.A.G., L.C.P., D.I.R.S., J.M., A.C. and S.C. Supervision: G.K.W. and S.N.K. Project administration: A.C. and S.C. Funding acquisition: D.I.R.S., S.N.K. and G.K.W. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

S. N. Karagiannis is a founder and shareholder of Epsilogen Ltd. S. N. Karagiannis declares patents on antibody technologies. L. C. Palhares is funded by a grant by Epsilogen Ltd. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. D.I.R. Spencer & R.A. Gardner are employed, and J. Hendel was employed by Ludger Ltd. a company that commercializes glycan analytics. All other authors have declared that no conflict of interest exists.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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