CDG and immune response: From bedside to bench and back

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Abstract

Glycosylation is an essential biological process that adds structural and functional diversity to cells and molecules, participating in physiological processes such as immunity. The immune response is driven and modulated by protein-attached glycans that mediate cell-cell interactions, pathogen recognition and cell activation. Therefore, abnormal glycosylation can be associated with deranged immune responses. Within human diseases presenting immunological defects are Congenital Disorders of Glycosylation (CDG), a family of around 130 rare and complex genetic diseases. In this review, we have identified 23 CDG with immunological involvement, characterised by an increased propensity to – often life-threatening – infection. Inflammatory and autoimmune complications were found in 7 CDG types. CDG natural history(ies) and the mechanisms behind the immunological anomalies are still poorly understood. However, in some cases, alterations in pathogen recognition and intracellular signalling (e.g. TGF-² 1, NFAT and NF-º B) have been suggested. Targeted therapies to restore immune defects are only available for PGM3-CDG and SLC35C1-CDG. Fostering research on glycoimmunology may elucidate the involved pathophysiological mechanisms and open new therapeutic avenues, thus improving CDG patients' quality of life.

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Synopsis: This review provides an update on the clinical, biochemical and cellular aspects of immunological involvement in CDG.

Details of the contributions of individual authors

Carlota Pascoal and Rita Francisco participated in the conception and design of the article, analysing and reviewing the literature data. The authors obtained final approval of the version to be published.

Tiago Ferro participated in the design of the article outline, drafted figures and critically revised all important intellectual content.

Vanessa dos Reis Ferreira participated in the conception, design and in critically revising the manuscript for important intellectual content.

Jaak Jaeken participated in the conception, design and analysis of the article; the author was involved in drafting the manuscript and critically revising it for important intellectual content. He also helped to draft the figures of the manuscript.

Paula Videira designed the article outline, participated in its conception and in critically revising it for important intellectual content. She is the guarantor of the article, accepts full responsibility for the work submitted and/or the conduct of the study, had access to the data, and controlled the decision to publish.

All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. All authors gave final approval of the version to be published.

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Keywords: Congenital Disorders of Glycosylation (CDG), Immune system, Inflammation, Infection, Autoimmune disease, Immunodeficiency

List of Abbreviations

APC - Antigen presenting cell

(pre-)BCR – (precursor) B-cell receptor

CDG – Congenital Disorder(s) of Glycosylation

DC – Dendritic cell

DC-SIGN - DC-specific intercellular adhesion molecule-3-grabbing non-integrin

ECM - Extracellular matrix

Ecgp96 - Endothelial cell glycoprotein 96

ER - Endoplasmic reticulum

G-CSF - Granulocyte colony-stimulating factor

GalNAc - N-acetylgalactosamine

GDP - Guanosine diphosphate

GlcNAc – N-acetylglucosamine

IBD - Inflammatory bowel disease

Ig - Immunoglobulin

IgAN - IgA nephropathy

IV - Intravenous

IAV - *Influenza A virus*

LAD II – Leukocyte adhesion deficiency II

(s)Le^x – (Sialyl-)$Lewis^x$

LLOs - Lipid-linked oligosaccharides

LPS – Lipopolysaccharide

MBL – Mannose-binding lectin

MHC - Major histocompatibility complex

NFAT - Modified nuclear factor of activated T-cells

NK - Natural killer

PRR – Pathogen recognition receptor

RTE - Recent thymic emigrant

SCN - Severe congenital neutropenia

Ser – Serine

TCR – T cell receptor

Thr – Threonine

TLR – Toll-like receptor

TREC - TCR excision circle

UDP - Uridine diphosphate

V-ATPase - Vacuolar ATPase

1. Introduction

Glycosylation is the assembly, processing and addition of sugars (glycans) to proteins and lipids. It is one of the most ubiquitous, complex and essential post-translational modifications, with various critical biological and physiological functions. Glycans typically decorate cell surface and secreted proteins as well as some cytosolic proteins. Indeed, at least 50 % of the human proteome is estimated to be glycosylated.[1] Structurally, glycans are relevant since they often comprise a significant portion of glycoproteins.[2] Function-wise, glycans ensure protein maturation and stability, functional modulation (e.g. immunogenicity), or even confer new functions to proteins (e.g. through glycan recognition by lectins), among others.[3–6]

The glycosylation machinery comprises a set of proteins that process, transport and assemble monosaccharides derived from the primary metabolism into linear or branched glycan chains.[7] The resulting glycoconjugates are highly diverse, which stems from the glycosylation type, the nature and combination of the constituting sugars, as well as from the glycosidic bonds involved. The sugar code has been considered the third alphabet of life.[8] The glycome, which refers to all sugars of an organism whether free or present in more complex molecules, constitutes the biological code with the greatest information diversity that populates an organism.[9] Unsurprisingly, most proteins have several glycoforms that can be expressed within the same cell or be cell/tissue/organ-specific. This

diversity has multiple implications in protein function, cellular interaction/communication and, ultimately, in organ homeostasis.[10–13]

The immune system is a paradigm of glycosylation control and fine-tuning. Glycoimmunology is a relatively recent research field that has the ambitious aim of further unravelling and understanding the complexity of the glycan landscape in the immune system/response. The immune response relies upon countless cell-cell contacts, recognition of self- and non-self-antigens, with glycans being present in virtually all the constituents of the immune system. Hence, defects in glycosylation may have a differential impact on the immune status, resulting in a broad spectrum of clinical manifestations and outcomes.[14]

Glycans and glycosylation abnormalities have been linked to several human diseases. These include congenital disorders of glycosylation (CDG), cancer, autoimmune and neurological diseases. Thus, glycans are central to many homeostatic control mechanisms.[11,15–17] However, the underlying molecular pathways responsible for such effects remain to be understood.

CDG are a growing family of rare, heterogeneous metabolic diseases. To date, 133 CDG have been identified. This number is expected to rise in parallel with the knowledge of glycosylation pathways, the improvement in diagnostics and the increased awareness of these conditions among the medical and scientific communities.[15,18] CDG patients frequently show multi-organ involvement and a sizable number exhibit immunological dysfunction.[19]

The main aims of this review are to:

(1) provide a general overview on the role of glycosylation in host-pathogen recognition and immune response;

(2) update the clinical information on immunological involvement in CDG, and;

(3) explore the underlying cellular and molecular mechanisms behind immune dysfunction in CDG.

1.1 General overview of glycan biosynthesis

The main glycosylation types in humans are protein N- and O-glycosylation. N-glycosylation is the most extensively studied pathway and takes place in different cellular compartments. It is initiated in the cytoplasm with the formation of activated sugars (e.g. GDP-mannose and UDP-glucose) and the synthesis of dolichol-linked precursor oligosaccharides in the endoplasmic reticulum (ER) membrane. In the ER lumen, the precursor oligosaccharide assembled onto the lipid carrier, is transferred to an asparagine (Asn) residue of a nascent protein by an oligosaccharyltransferase. The terminal N-glycosylation step concerns the trimming and processing of the glycans and takes place both in the ER and Golgi complex. The inter-organellar transport system plays a major role in this phase generating a wide array of glycans with increasing degrees of branching and complexity.[20]

In contrast with N-glycans, O-glycans are built by monosaccharide transfers catalysed by glycosyltransferases mainly located in the Golgi complex. The initial step of the most prevalent type of O-glycosylation occurs in the rough ER or the *cis* Golgi with the transfer of N-

acetylgalactosamine (GalNAc) to the hydroxyl group of a serine (Ser) or threonine (Thr) residue of the protein. This core structure (Ser/Thr-GalNAc) can then be processed to generate different mucin-type core structures, which may be further elongated and modified. Besides GalNAc-O-glycans, other O-glycans containing either fucose or xylose as the first monosaccharide may also be synthetized.[20]

Glycosaminoglycans (GAGs) are a specific type of O-glycans that occurs in an abundant type of heteropolysaccharides found predominantly at the extracellular matrix (ECM) and cell membranes. GAGs are negatively-charged, long, unbranched molecules containing repetitive disaccharide units. Generally, these disaccharides are composed of alternating uronic acid (either glucuronic acid or iduronic acid) and hexosamine units (glucose, GalNAc or N-acetylglucosamine-GlcNAc). The variation and differential arrangement of these monosaccharides gives rise to different types of GAGs (e.g. heparin and heparan sulfate). Proteoglycans are GAGs associated with proteins, generally through an O-glycosidic bond to a Ser residue via a tetrasaccharide linker composed of one glucuronic acid, two galactose, and one xylose residues.[20]

1.2. The immune system and immune response

The immune response has the ultimate mission to defend us against infections, protecting the integrity and homeostasis of the organism as a whole. Both innate and adaptive immune responses are elicited and rely on pathogen/antigen recognition, by either direct cell-cell contact or cytokine- and chemokine-mediated actions. The innate immune response identifies and responds to pathogen-associated molecular patterns via different cell types and proteins (e.g. complement system and pathogen recognition receptors - PRRs) that mediate phagocytosis, direct cytotoxicity, and eliminate extracellular microbes.

The adaptive response includes B and T cells, whose surface receptors have high specificity for the antigen and are clonally distributed on individual cells. Importantly, since some clonally expanded B and T cells differentiate into memory cells, the adaptive immunity offers long-lasting protection through immunological memory.[21,22] While B cells can directly recognize many different soluble antigens, T cells can only recognize them if they are presented by antigen presenting cells (APCs) via the major histocompatibility complex (MHC) molecules. Figure 1A illustrates the cell types and mechanisms of both immunity processes as well as the timeline of the intervention of each mechanism.

An efficient immune response demands a constant and complex molecular and cellular cross-talk to detect pathogens, to distinguish self from non-self and to enhance the response to forthcoming infections. All interventions of the immune response are indispensable for the prevention and eradication of infections, as well as to to prevent immune dysfunction. These complex events on the one hand, stimulate inflammation and on the other hand, inhibit it. When this delicate balance is disrupted, one of two scenarios takes place: (1) loss of immune tolerance leading to autoimmunity or (2) immunosuppression, resulting in higher susceptibility to infections, and cancer development and progression.

1.3. How human glycans affect the immune response

Glycans, due to their ubiquitous expression in practically all cell surface and secreted proteins, add yet another layer of intricacy and diversity to the immune response. Both glycans and glycan-binding proteins create a platform of contact and communication between immune cells. Here, the pivotal and widespread role of glycosylation is made evident by the array of molecules of the immune system that are glycosylated. These include immunoglobulins, adhesion molecules (e.g. integrins and selectins), PRRs (e.g. Toll like receptors – TLRs - and lectins) and complement molecules, cytokines, chemokines and (many of) their receptors.[23–32]

The role of human glycans in the immune response is depicted in two interrelated scenarios: (1) all pathogens require contact with host glycans for infection; (2) glycans ornamenting molecules of the immune response influence their properties, the subsequent signaling pathways, and, consequently, the triggered immune response.

Pathogens can interact directly with host glycans through glycan-binding proteins (e.g. pili) expressed by the pathogens.[33] Glycans can also indirectly influence pathogen recognition by promoting or hindering ligand accessibility at the host cell surface.[34,35] Table 1 includes examples of direct and indirect glycan recognition by pathogens. Interestingly, the glycan composition of the pathogen itself, can serve to escape immune surveillance, recognition and elimination.[36] In some cases, microorganisms can even appropriate the host-glycan to escape immune system-mediated recognition and destruction.[37] In the context of human defects of glycosylation, the glycan dependence seems not only to render some infectious agents unable to infect patients with specific CDG but also to render some CDG resistant to particular pathogens.[38,39]
These findings can provide strategies to control infections by microorganisms that are unable to adapt to glycan-depleted cellular landscapes.

Table 1 – Host glycan recognition by pathogens to promote infection and evade the immune response

Glycan recognition, mimicry and evasion by pathogens			
Direct recognition			
Pathogen / disease	**Host glycan**	**Mechanism**	**Reference**
Escherichia coli / urinary t infection	galactose-² 1,4-D-galactose	P pili mediated adhesion	[33]
E. coli K1 / meningitis	N-glycans on the Ecgp96 glycoprotein	Outer membrane protein A- mediated recognition	[40]
Pseudomonas aeruginosa / respiratory tract infections	D-galactose L-fucose	Lec A lectin Lec B lectin Recognition and adhesion Rpithelium permeability	[41,42]
*Influenza A virus/*flu	Sialylated cell surface glycans	Hemagglutinin (host internalization) and neuraminidase (release)	[43]
Polyomavirus	Sialylated glycans, non-sialylated GAGs	Major capsid proteins / for internalization	[44]
Indirect recognition			
Hepatitis B virus / hepatitis	Core-fucosylated N-glycans	Envelop proteins / facilitate endocytosis	[35]
Staphylococcus aureus/	O-glycosylated mucins	Protect epithelial cells / hinder	[34,45,46]

detrimental bacterial infection		pathogen access	

Glycosylation of host immune receptors governs the proper initiation of the immune response. Since glycans usually constitute a significant portion of the whole receptor, they may affect how receptors fold and associate among themselves (or with other proteins), as well as their stability and half-life.[47,48] Most PRRs, namely TLRs, calcium-dependent C-type lectins (such as dectin-1 and DC-specific intercellular adhesion molecule-3-grabbing non-integrin - DC-SIGN) and complement-related proteins (e.g. mannose-binding lectin - MBL) are functionally dependent on glycosylation. Glycans may also be ligands of human lectins such as selectins and siglecs, among others. These interactions act mostly by modulating leukocyte trafficking and other events of the immune response. Table 2 and figure 1 include examples of glycan interference in the function of specific immune receptors and other immune signalling molecules. Understanding the mechanism of how glycans modulate or fine tune the properties of immune receptors and other molecules widens our comprehension of physiological and pathological immune conditions.

Table 2 - Glycans affecting recognition by and function of immune cell receptors and other signaling molecules

Molecules	Glycan(s)	Mechanism	Reference
TLR 2	N-glycans	Biosynthesis and secretion of the receptor	[49]
TLR3 and TLR4	N-glycans	Receptor integrity and biological function	[50,51]
TCR	N-glycans	TCR clustering and signaling	[52]
TCR	O-glycans (namely GlcNAc glycoproteome)	T-cell activation and influencing TCR interactions with external galectins	[53]
DC-SIGN	N-glycans	Receptor diffusion, and modulation of the binding strength to bacterial antigen	[54]
MHC I	Sialylated N-glycans	Cell surface expression (possible effect on protein turnover)	[55,56]
MHC II	N-glycans	Recognition and binding of antigens to be presented to T cells	[57]
Immunoglobulins	Sialylated N-glycans	Recognition by FC receptors	[58–61]
Selectin ligands	Sialyl-LewisX (sLex) and 62sulfo- sLex on N- and O-glycans	Recognition by L-, E- and P-selectins,leukocyte migration/recruitment	[62–66]
Siglecs	Glycans with terminal sialic acid	Self and foreign antigens discrimination and prevention of the overactivation of the immune system	[55,67–70]

Insert Figure 1 here

2. The immunological impact of glycosylation defects – An update on CDG

CDG are metabolic genetic diseases with mostly multi-system involvement, and often debilitating clinical presentations.[15,18] These conditions frequently cause significant neurologic dysfunction and

variable impairment of other organs. Immunological involvement is present in a subgroup of CDG.[19] The rapid increase in the number of recognized CDG parallels the increasing number of CDG with relevant immunological involvement. The degree of immunological dysfunction among and within each CDG ranges from immunodeficiency phenotypes to recurrent infections without clear cellular and/or biochemical anomalies.[19] Figure 2 illustrates these CDG, their associated roles in glycosylation and their cellular location.

In this review, these CDG were grouped into two categories: (1) CDG with major (10 CDG) and (2) those with minor immunological involvement (13 CDG). These categories were defined based on the number of affected individuals, the severity of immunological manifestations and their clinical implications.

Insert Figure 2 here.

2.1. CDG with major immunological involvement

ALG12-CDG

ALG12 is responsible for adding the eighth mannose to the growing lipid-linked oligosaccharides (LLOs) during N-glycan biosynthesis. From the nine ALG12-CDG (MIM: 607143) patients reported, 6 presented with recurrent and severe infections. The cause of these immune defects are the low serum IgG levels, a hallmark of this disease (Table 3) (reviewed in [19]).

Table 3 - Immune (clinical, cellular, biochemical and others) manifestations found across CDG types with major immunological involvement.

	Clinical manifestations			Cellular alterations		Biochemical alterations	Other	
#patients	Recurrent/Severe infections	Pathogens	Autoimmune/ Inflammatory signs	Total WBC count	WBC subpopulation counts and functional parameters	Igs	e.g. vaccination response/allergies	Reference
ALG12-CDG n= 6	RTI, including pneumonia (4/6), otitis media (2/6), ear/nose infections (1/6), sepsis (1/6)	NA	NA	NA	" B cells (1/6)	" IgG (6/6), " IgM (3/6), " IgA (1/6)	Absent Ig response to diphtheria, tetanus and *Hemophilus influenzae* (1/6)	19#
ATP6AP1-CDG n=12	(1/1)	NA	NA	NA	NA	" IgG and IgA (1/1)	Failure to generate protective Igs against measles, mumps, rubella and varicella vaccine (1/1)	71
	Pneumonia and purulent otitis media (8/11), plantar abscesses and gastrointestinal infections (3/11)	NA	NA	" (6/11)	" $IgD1/CD27^+$ intermediate and switch memory cells - indicative of defective B cell differentiation (2/11)	Dysgammaglobulinemia (11/11): " IgG (9/11), " IgM (4/11), " IgA (4/11)	Some patients responded very poorly to vaccination†	72
EXTL3-CDG n=14	(2/3) Sepsis (1/3)	*Klebsiella* and *Staphiloccocus. aureus* (1/3)	Exfoliating dermatitis, blepharitis (1/3)	NA	Lymphopenia ($T^-B^+NK^+$ SCID), eosinofilia, " proliferation, " or undetectable TRECs (3/3)[a]	Hypogammaglobulinemia ' IgE (2/3)	NA	73
	(4/9) Viral upper airway infections, pneumonia (2/9), septicemia, omphalitis (1/9)	CMV, *S. aureus* (1/9)	Omenn type skin rash (2/9), eczematoid rash (1/9)	" (1/9)	Mild lymphopenia (1/9), $T^-B^+NK^+$ SCID (3/9), idiopathic CD4 lymphopenia with no naive T cells (2/9) inefficient B cell activation by T cells (1/9)	Hypogammaglobulinemia (6/9) ' IgG and IgM (1/9)	NA	74
	Pulmonary infections and dental caries propensity (1/2)	NA	NA	NA	" T cells (1/2)	" IgG and IgM (1/2)	NA	75

FUT8-CDG n= 3	Infections accompanied by respiratory failure, bronchopneumonia (1/3)	NA	Reactive airway disease (1/3)	NA	Neutropenia (1/3)	NA	NA	[76]
G6PC3-CDG	Sepsis (6/12), UTI (4/12), pneumonia (3/12), RTI (2/12), otitis, abscesses, oral ulcers, omphalitis (1/2)	NA	Panniculitis (1/12)	NA	Neutropenia (12/12) ‘ neutrophil ER stress (5/5)[b]	NA	BM maturation arrest at the stage of promyelocyte/ myelocyte (4/4)[b]	[77]
	Clinical manifestations			**Cellular alterations**		**Biochemical alterations**	**Other**	
#patients	**Recurrent/Severe infections**	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	**Reference**
G6PC3-CDG n=11[c]	Bacterial lung infections, cellulitis (1/1)	NA	Gingivitis (1/1)	NA	Neutropenia (1/1)	NA	BM maturation arrest at the promyelocyte stage (1/1)	[78]
	NA	NA	NA	NA	Neutropenia (2/2)	NA	NA	[79]
	NA	NA	NA	NA	Neutropenia (2/2)	Hypogammaglobulinemia (2/2)	NA	[80]
	Sepsis, respiratory and UTI (1/1)	NA	NA	NA	Neutropenia (1/1)	NA	BM maturation arrest at the promyelocyte/ myelocyte stage (1/1)	[81]

	Sepsis, otitis media, bronchitis, abscesses, pharyngitis, dental caries, pneumonia and chronic oral ulcers (2/2)	NA	Periodontal disease (2/2)	NA	Neutropenia (2/2) “ NK cells (2/2)	NA	Asthma (1/2), BM with “ neutrophils and ‘ mononuclear megakaryocytes, myeloid hyperplasia and vacuolization of myeloid precursors - consistent with myelokathesis (2/2)	[82]
	NA	NA	NA	NA	Neutropenia, lymphopenia and monocytosis (2/2)	NA	Myeloid dysplasia and vacuolization, thymic and erythroid hypoplasia (2/2)	[83]
	Pneumonia (2/4), otitis media (4/4), abscesses, UTI, herpetic stomatitis, sepsis (1/4)	NA	NA	“ (4/4)	Neutropenia (4/4), lymphopenia and monocytosis (1/4)	NA	BM maturation arrest with ‘ megakaryocytes (2/3), hypercellularity and myeloid hypoplasia and “ erythropoieses and hypogranulation in (1/3)[b]	[84]
	RTI and gastrointestinal infections (1/1)	NA	Recurrent proctitis and fever and oral ulcers (1/1)	“ (1/1)	Neutropenia (1/1)	NA	NA	[85]
	Pneumonia (3/5), sepsis and abscesses (2/5), oral ulcers (1/5)	*Aspergillus* (1/5)	IBD (2/5), panniculitis (1/5)	NA	Neutropenia (5/5)	NA	NA	[86]
CDG	**Clinical manifestations**			**Cellular alterations**		**Biochemical alterations**	**Other**	**Reference**
# patients	**Recurrent/Severe infections**	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	

	Sepsis, abscesses, skin, ear and bladder infections, pneumonia (1/1)	*Escherichia coli* (1/1)	Colitis, Crohn's disease, eczematous rash (1/1)	" (1/1)	Neutropenia with hypogranular neutrophils with rare döhle bodies (1/1)	" IgA (1/1)	Hypercellular BM with maturation arrest at the myelocyte/ metamyelocyte stage. During treatment there was myeloid hyperplasia, vacuolization of mature neutrophils and rare pelgroid bodies (1/1)	87
	Pneumonia (1/2), sepsis (2/2)	NA	Fever (2/2)	NA	Neutropenia (2/2)	NA	BM maturation arrest at the myelocyte stage (2/2)	88
G6PC[illegible]- [illegible]DG n=[illegible]19	NA	NA	NA	NA	Neutropenia (16/16)	NA	BM maturation arrest at the myelocyte/ promyelocyte stage (7/13), " mature neutrophils (6/13), left shifted myelopoiesis (5/13), left shifted granulopoiesis (1/13), and hypocellularity (1/13)[b]	89
	(4/5) Pneumonia, otitis media, upper RTI, abscesses (1/5)	*Aspergillus* (1/5)	Chronic gingivitis, periodontitis, granulomatous IBD (1/5)	NA	Neutropenia (5/5)	NA	Left shifted myelopoiesis (1/2)[b]	90
	(2/2) Sepsis, laryngotracheobronchitis, pneumonia, otitis media, UTI, gingivostomatitis, endocarditis (1/2)	*E. coli* (1/2)	Crohns' disease (2/2)	NA	Neutropenia (2/2)	NA	BM maturation arrest with hyperplasia of granulocyte precursors (1/1)[b]	91

	Suppurative otitis media, RTI, urinary infections, abscesses (1/1)	NA	Gastroenteritis, gingivitis, aphthous stomatitis (1/1)	" (1/1)	Neutropenia (1/1)	NA	Hypercellular BM with myeloid hyperplasia and vacuolization, pyknotic and hypersegmented neutrophils - consistent with myelokathexis (1/1)	[92]
	Clinical manifestations			**Cellular alterations**		**Biochemical alterations**	**Other**	
CDG #patients	**Recurrent/Severe infections**	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	**Reference**
G6PC3-CDG n=119	Abscesses, otitis media (1/1)	NA	Oral and genital aphthous ulcerations (1/1)	" (1/1)	Neutropenia and lymphopenia (1/1)	NA	" thymic naive CD4 cells - indicative of thymic defects (1/1)	[93]
	(2/4) Bronchiolitis, upper RTI, pneumonia, abscesses, tonsillitis, ear infections (1/4)	NA	Oral ulcer (2/4), transient presence of anti-neutrophil and anti-HLA antibodies (1/4)	NA	Neutropenia (4/4) and mild lymphopenia (2/4)	NA	NA	[94]
	Sepsis, oral candidiasis, respiratory, ear, nose and throat infections (1/1)	*Candida* (1/1)	Blepharitis, periodontal disease, oral ulcers (1/1)	NA	Neutropenia (1/1)	NA	NA	[95]
	Pneumonia (2/2), sepsis (1/2)	NA	NA	NA	Neutropenia (2/2)	NA	BM myeloid maturation arrest (2/2)	[96]
			Esophagitis, gastroenteritis, recurrent fever, colitis		Chronic neutropenia (1/1)		NA	[97]

			(1/1)					
	(2/5) Abscesses (2/5), pneumonia, otitis media, gastroenteritis, sepsis (1/5)	*Pseudomonas aeruginosa* (1/5)	Recurrent fever (3/5), aphthous stomatitis (2/5), gingivitis (1/5)	NA	Neutropenia (5/5) and monocytosis (1/5)	NA	Asthma (2/5), neutropenic BM (2/2)[b]	[98]
	Pneumonia (2/2)	NA	Gingivitis, gingivostomatitis and oral ulcers, gastroenteritis, pancolitis and IBD (1/2)	NA	Neutropenia (2/2)	NA	Recurrent fever (2/2)	[99]
CDG #patients	**Clinical manifestations**			**Cellular alterations**		**Biochemical alterations**	**Other**	**Reference**
	Recurrent/Severe infections	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	
G6PC3-CDG [illegible]	(1/1)	NA	NA	NA	Neutropenia (1/1)	NA	Hypercellular BM with " mature neutrophils and sea blue histocytes (probably derived from neutrophil destruction) (1/1)	[100]
	NA	NA	NA	NA	Cyclic neutropenia (2/2)	NA	NA	[101]
	(1/1)	NA	NA	" (1/1)	Neutropenia (1/1)	NA	Left shifted myelopoiesis with " neutrophils and ' immature forms (1/1)	[102]

	Abscesses (2/2), otitis, parotitis, aphthous stomatitis, sepsis (1/2)	*S. aureus* (2/2), *Streptococcus viridans* (1/2)	NA	NA	Neutropenia (2/2), monocytosis (1/2)	‘ IgG (1/2)	BM arrest at the promyelocyte/myelocyte stage, with hypercellular, myeloid dysplasia and “ mature granulocytes (1/2) Delayed granulocyte maturation (1/2)	[103]
	Multiple mild infections, e.g. bronchitis, otitis, pharyngitis, and gastroenteritis (14/14), stomatitis (8/14), pneumonitis (3/14), cellulitis (2/4), sepsis (1/4)	*E. coli, P. aeruginosa, Klebsiella pneumoneae,* other Gram^{-} species; *S. aureus,* and other Gram^{+} cocci[c]	Crohns’ disease (3/14)	NA	Neutropenia (14/14) Lymphopenia (3/4), “ CD4 (4/4), “ CD3 (3/4), “ B cells, ‘ NK cells, “ naive T cells (2/4)[b]	‘ IgG (14/14)	BM with dysmorphic neutrophils and megakaryocyte dysplasia (8/14)	[104]
	Otitis (3/3), RTI (2/3), neonatal sepsis, oral infection, pneumonia, skin abscesses (1/3)	*Candida* (1/3)	Aphthous stomatitis, oral ulcers, IBD, IBD-like colitis, gastritis, typhlitis and celiac disease (1/3)	NA	Neutropenia and lymphopenia (3/3), “ T and NK cells (1/3), “ B cells (2/3). “ naive T cells and “ RTEs (3/3) – points towards deficient thymic output “ proliferation after mitogen stimulation (3/3)	NA	NA	[105]
	Pneumonia, osteomyelitis, abscesses, otitis media (1/1)	NA	Gingivitis (1/1)	“ (1/1)	Neutropenia and lymphopenia with “ CD4 and B cells (1/1)	NA	BM myeloid maturation arrest with vacuolization (1/1)	[106]
	Clinical manifestations			**Cellular alterations**		**Biochemical alterations**	**Other**	
#patients	**Recurrent/Severe infections**	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	**Reference**
G6PC3-	(6/8)	NA	NA	NA	Neutropenia (8/8)	NA	BM myeloid maturation arrest	[107]

CDG n=11	Sepsis (1/8)						Neutropenia (8/8)	
	NA	NA	NA	" (1/1)	Neutropenia Some instances of " lymphocytes subsets (1/1)	NA	" myeloid/erythroid ratio, megakaryocyte and myeloid dysplasia (single nucleated megakaryocytes and mature neutrophils hypogranulation (1/1)	[108]
	Cellulitis (3/3), gastritis, colitis, pneumonia, viral infections, sepsis (1/3)	*Helicobacter. pylori, Clostridium difficile, S. aureus, P. aeruginosa,* multi drug resistant *E. coli, RSV, parainfluenza* (1/3)	Oral ulcers (2/3), IBD, ' CRP (1/3)	" (2/3)	Intermittent (2/3) and chronic (1/3) neutropenia	NA	Left shifted myeloid maturation, mild myeloid hyperplasia and megakaryocytic hyperplasia " neutrophil maturation and dysplastic megakaryocytes (1/3)	[109]
	(1/2) Paronychial nail infections, conjunctival infections (1/2)[b]	NA	Aphthae with lymphocytes and plasma cells infiltrates, IBD, erythema nodosum ' CRP (during flares) (1/2)[b]	NA	Cyclic neutropenia (1/2) Following LPS stimulation, ' IL-1² and IL-6 (2/2) ' TNF± (1/2 – the other sibling was on anti-TNF± therapy)	' IgM and IgG during flares (1/2)[b]	NA	[110]
	Ear, nose and throat infections (2/2)	NA	Aphthae (2/2), Familial Mediterranean Fever (1/2)	" (2/2)	Neutropenia, ' CD3 and " NK cells (2/2)	NA	BM myeloid arrest (2/2)	[111]
	Suppurative otitis media, purulent parotitis, oral ulcers	NA	NA	NA	Neutropenia, lymphopenia and monocytosis (1/1)	NA	BM maturation arrest at the myelocyte/ metamyelocyte	[112]

	(1/1)						stage, with toxic granulation, vacuolar degeneration and no mature granulocytes (1/1)	
CDG #patients	**Clinical manifestations**			**Cellular alterations**		**Biochemical alterations**	**Other**	**Reference**
	Recurrent/Severe infections	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	
[illegible]SCN1-CDG n=18	(1/1)	NA	NA	NA	Monocytosis and neutropenia (1/1)	Transiently " IgA	BM arrest at the promyelocytic stage (1/1)	113
	(14/14) Abscesses (9/14), upper respiratory tract (5/14) and ear, nose and throat infections (4/14), pneumonia (5/14), aphtosis, cellulitis (3/14), otitis, sepsis (2/14), omphalitis, onycholysis (1/14)	*Haemophilus influenza*, *E. coli* and *Aspergillus* (1/14)	Pneumonitis (2/14), balanitis, peridontopathy, pancolitis (1/14)	NA	Neutropenia (14/14)	NA	BM arrest (13/14), with slight dyserythropoiesis (1/14)	114
	Abscesses, pneumonia and otitis (2/2)	NA	Anti-thyroglobulin, gingivitis (1/2)	NA	Lymphopenia and " class switched B cells, " CD4 (1/2) Neutropenia and " RTEs (2/2)	" IgA (1/2), IgG (2/2) and IgM (2/2)	BM arrest at the promyelocyte-myelocyte stage with nuclear dysplasia and hypogranulation (2/2)	115
	Pneumonia, bronchitis and umbilical infection (1/1)	NA	Periodontitis (1/1)	NA	Neutropenia (1/1)	NA	BM arrest at the myelopoiesis (1/1)	116
[illegible]OGC-CDG [illegible]	Repeated sepsis (1/4)	*E. coli* (1/4)	NA	NA	' B and T cells, " lymphocytic proliferation (2/4), neutropenia (2/4)	" IgA (4/4), IgM (3/4) and IgG (2/4) " IgG half-life with " F^3 RIIa affinity (2/4)	Ineffective Ig production against measles, mumps, rubella and varicella vaccine (2/4)	19 #

PGM3-CDG n=40	Skin infections and sepsis (1/1)	*Staphyloccocus epidermidis* and coagulase negative species	NA	NA	Lymphopenia, ↓ T, B and NK cells, ↓ naïve T cells and TRECs, ↑ transitional B cells and ↓ T cell proliferation (1/1)	↓ IgA, IgE and IgG (1/1)	Hypocellular BM, defective T cell development: ↑ thymic double negative T cells (1/1)	[117]
	Respiratory tract (10/12) skin (10/12) and oral (3/12) infections, cutaneous abscesses (10/12), otitis (7/12), viral infections (3/12)	*Staphylococcus* (5/12), *Pseudomonas* (2/12), *Candida* (5/12), RSV (2/12), HPV (1(12)	Eczema often with pyodermatitis (10/12)	NA	Lymphopenia (5/8) ↓ CD3 (6/8), CD4 (8/8), CD8 (2/8), CD19 (6/8) and NK cells (2/8), reverted CD4/CD8 ratio (6/8) ↓ proliferation to mitogens (7/7), eosinophilia (5/8)[b]	↑ IgE (9/12)	Allergic manifestations (rhinitis, asthma and cow milk intolerance) (3/12)	[118]
	(1/2)				Lymphopenia ($T^-B^-NK^-$ SCID), neutropenia (2/2)		BM failure syndrome (hypocellularity) (2/2)	[119]
CDG #patients	**Clinical manifestations**			**Cellular alterations**		**Biochemical alterations**	**Other**	**Reference**
	Recurrent/Severe infections	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	
PGM3-CDG n=10	Skin (e.g. abscesses) and RTI, pneumonia, septic episodes, and viral infections (1/1)	*Candida, S. aureus*, CMV (1/1)	Severe eczema/ dermatitis, TSH receptor and TPO autoantibodies (1/1)	NA	Lymphopenia with Th2 predominance and ↑ activated effector cells, eosinophilia, deficient development based on ↓ TRECs, naive cells, RTEs and central memory cells (1/1)	↑ IgA, IgM, IgA, and, especially, IgE (1/1)	Multiple food allergies (1/1)	[120]

	(20/21) RTI, e.g. pneumonia (13/21), chronic sinopulmonary disease (6/21) and bronchitis (4/21), skin infections (10/21), namely impetigo, infected skin ulcers (2/21)), and eczema (1/21), soft tissue infections (6/21), abscesses (9/21), viral infections (10/21), severe varicella, sepsis (2/21), suppurative lesions, perichondritis, osteomyelitis (1/21), otitis (10/21), suppurative perinephritis (1/21), esophagitis (1/21), intrauterine bacterial infection (1/21)	*Candida and Staphylococcus, (6/21), S. aureus (4/21), RSV (2/21), VZV (5/21), EBV (3/21), HSV, HPV, Influenza, K. Pneumoniae, S. pneumoniae, Enterococcus cloacae, Streptococcus dysgalactiae equisimilis, P. aeruginosa hemolytic, Staphylococcc us Group A (1/21)*	Eczema (15/21), pyodermatitis (5/21), dermatitis, erythematous lesions (2/21), folliculitis (1/21)	‘ (3/18) “ (11/18)	**Leukocyte defects:**[b] Eosinophilia (9/17), neutropenia (8/18), neutrophilia (2/18)[d] **T cell defects:**[b] “ CD3 (6/8), “ CD4 (12/15), ‘ CD8 (7/7), “ naive T cells (3/7), “ RTEs (3/7), ‘ memory T cells (3/7), ‘ late effector T cells (4/7) **B cell defects:**[b] “ B cells (10/15), “ memory b cells (3/7), “$CD20^{+}CD27^{+}$ cells (7/7), ‘ transitional B cells (4/8) **Other lymphocyte defects:**[b] Lymphopenia (3/8), ‘ NK (7/14), “ NK (1/15), ‘ Th2 cells (2/), T-B-NK+ SCID (3/3) ‘ CD4/CD8 ratio (8/10), “ proliferation (6/6)	‘ IgE (17/21), IgM (3/19), IgG (6/19) and IgA (6/19) “ IgM (4/19), IgA (1/19), IgG (1/19)[b]	IgE-mediated (1/21), food (5/21) and drug (2/21) allergies Hypocellular BM failure (1/3)[b], chronic rhinitis (6/21), asthma (2/21)	19 #
-CDG	Otitis media (4/9), sinusitis (1/9), pulmonary infections (namely pneumonia) (3/9), mild to severe/chronic periodontitis (4/9), severe and/or localized cellulitis (3/9), gastroenteritis, recurrent sepsis (1/9)	Coronovirus (1/9); HSV (2/9)[e]	‘ CRP (1/9)	‘ (6/9)	‘ B and T cell (1/9), neutrophilia (5/9), “ neutrophilic mobility (2/9)	NA	Recurrent fever episodes (5/9), egg allergy (1/9), absent sLe^{x} and H antigen on erythrocytes (Bombay blood group) (7/9)	19#
CDG #patients	**Clinical manifestations**			**Cellular alterations**		**Biochemical alterations**	**Other**	**Reference**
	Recurrent/Severe infections	**Pathogens**	**Autoimmune/**	**Total**	**WBC subpopulation counts**	**Igs**	**e.g. vaccination**	

			Inflammatory signs	WBC count	and functional parameters		response/allergies	
SLC37A4 CDG n=252	(17/106) Skin (3/106) and respiratory infections (1/106)	NA	IBD (23/106), stomatitis (1/106)	NA	Neutropenia (98/106)	NA	NA	[121–135]
	(12/14) Bacterial infections (4/13), skin abscesses (3/14), sepsis (1/14)	NA	NA	NA	Neutropenia (13/14)	NA	NA	[136–139]
	NA	NA	NA	NA	" neutrophilic function and neutropenia (29/29)	NA	NA	[140,141]
	(57/57) Ear, nose, and throat infections (33/57), RTI (24/57), pyogenic skin infections (21/57), UTI (12/57), gastrointestinal infections (10/57), deep abscesses (3/57), perioral infections (37/57), perianal infections (27/57)	NA	IBD (10/57)	NA	Neutropenia (54/57) " neutrophilic function (18/57)[b]: " chemotaxis (3/18), oxygen consumption (8/18), superoxide production (4/18), calcium mobilization, and deoxyglucose uptake (2/18)[b]	NA	NA	[142]
	Bacterial infections (1/1)	NA	Gingivitis, autoimmune hypothyroidism, IBD and myasthenia gravis (1/1)	NA	Neutropenia (1/1)	NA	NA	[143]
	Pyogenic skin infections and abscess formation (1/1)	NA	NA	NA	Neutropenia (1/1)	NA	Frequent fevers (1/1)	[144]
	Severe infections (4/7), pneumonia (2/7), septic	*S. pneumonia* (2/7); *S.*	IBD (5/7)	NA	Neutropenia[†]	NA	NA	[145]

	arthritis, renal abscess (1/7), gastroenteritis (3/7)	*aureus* (2/7); *E. coli* (1/7); *Salmonella* (2/7)						
	Generalized pustular skin eruptions (1/1)	NA	NA	NA	Neutropenia with neutropenic BM (1/1)	NA	NA	[146]
	Urinary infections (1/4)			" (1/4)				[147]
CDG	**Clinical manifestations**			**Cellular alterations**		**Biochemical alterations**	**Other**	**Reference**
ts	**Recurrent/Severe infections**	**Pathogens**	**Autoimmune/ Inflammatory signs**	**Total WBC count**	**WBC subpopulation counts and functional parameters**	**Igs**	**e.g. vaccination response/allergies**	
	Respiratory and gastrointestinal infections (1/1)	NA	Stomatitis aphtosa, chronic ileum inflammation with ' neutrophil chemiotaxis, autoimmune thyroiditis, autoimmune GHD (1/1)	NA	Neutropenia (1/1)	NA	NA	[148]
	Sepsis (1/1)	NA	NA	NA	Neutropenia (1/1)	NA	Hypercellular myelopoiesis (1/1)	[149]
	Esophageal infection, skin infections, acute otitis media, pneumonia (1/1)	*Candida* (1/1)	NA	NA	Neutropenia (1/1)	NA	Neutropenic fevers (1/1)	[150]
	Ear and bladder infections, abscesses, bronchiolitis and pneumonia (1/1)	NA	Chronic gingivitis, IBD (1/1)	NA	Neutropenia (1/1)	NA	NA	[151]
	Bacterial infections (28/28)	NA	IBD (6/28)	"	Neutropenia (28/28)	NA	NA	[152]

[#] All references included in the previous review on immunological defects in CDG[19] have been reanalyzed and the information considered relevant for this update has been included. For simplicity purposes, some references were then substituted by Monticelli et al 2016. In these cases, only the total number of patients with immunological involvement (and not the total number of patients with a specific CDG) are included.

[†] The exact number of patients was not disclosed in the paper; [a] Impaired proliferation was recognized but the affected subpopulations were not disclosed; [b] Only a subgroup of the patient sample reported was tested/described for the specified parameter; [c] The number of patients infected with the pathogens is not disclosed; [d] In two patients, neutrophils partially normalized during infections; [e] In SLC35C1-CDG patients, most often no pathogens could be identified during infection/fever episodes; In Lam et al 2000, three patients are described with gene analysis results, but in terms of clinical phenotype, only one patient is detailed.

" - Low or decreased; ' High or increased; BM – Bone marrow; CMV – *Cytomegalovirus*; CRP – C-reactive protein; EBV – Epstein-Barr virus; GHD – Growth hormone deficiency; HPV - Human papillomavirus; HSV – Herpes simplex virus; IBD – Inflammatory bowel disease; Ig – Immunoglobulin; LPS – Lipopolysaccharide; NA – Not available; RSV - Respiratory syncytial virus; RTEs – Recent thymic emigrants; RTI – Respiratory tract infections; SCID – Severe combined immunodeficiency; TPO - Thyroid peroxidase; TSH - Thyroid stimulating hormone; UTI – Urinary tract infection; VZV - *Varicella zoster virus*

ATP6AP1-CDG

ATP6AP1 is the first accessory subunit of the proton-transporting vacuolar (V)-ATPase protein pump. *ATP6AP1* disruption causes immunodeficiency syndrome 47 (MIM: 300972), defects in N-glycosylation, and often in mucin-type O-glycosylation.[72] The 12 patients reported present recurrent bacterial infections, inadequate vaccination response and various laboratory abnormalities (Table 3). The virtual inexistence of viral infections in ATP6AP1-CDG can be partly explained by the recently described ATP6AP1 role in promoting infection and replication of HIV and rhinovirus. ATP6AP1 is closely associated with the transmembrane protein RNASEK, required for the replication of several viruses, and both proteins appear to co-regulate each other.[153,154] Whether the glycosylation defect plays a role in the impairment of viral replication remains to be studied. However, the predominance of bacterial infections and overall immunological alterations in ATP6AP1-CDG are possibly not only due to defective glycosylation but also to other factors, such as intracellular pH dysregulation.

EXTL3-CDG

EXTL3 is a glycosyltransferase involved in the transfer of GlcNAc to glycosaminoglycan chains and the heparan sulphate biosynthesis. EXTL3 deficiency results in an immune, skeletal and neurodevelopmental disorder (MIM: 617425). Fourteen patients have been reported with 9 presenting immunodeficiency.[75] The spectrum of immunological alterations is extensive, ranging from a patient with oral candidiasis, with otherwise normal immunological function, to severely immunocompromised patients with lethal infections (Table 3). Remarkably, Ig counts spontaneously normalized and an effective vaccination response was achieved in one patient. However, T cell deficiency remained unresolved.[73] Globally, EXTL3-CDG patients have T cell immunodeficiency with abnormalities in terms of proliferation, differentiation and maturation, probably caused, at least in part, by impaired thymic T cell development. Accordingly, in an EXTL3-CDG zebrafish model, significant alterations in thymus development were observed and rescued upon injection of wild-type human EXTL3 mRNA.[73] The absent EXTL3 expression in normal circulating T cells, while the protein is expressed in hemato- and/or lymphopoietic cells, further supports an early developmental defect.[74] However, why some patients fail to present signs of immunodeficiency or progressively improve over time is still to be determined.

FUT8-CDG

FUT8 is a fucosyltransferase involved in core-fucosylation. This enzyme transfers fucose from GDP-fucose to the first GlcNAc residue of N-linked complex glycopeptides. FUT8-CDG (MIM: 618005) is a newly described disease with only three reported patients. The immune phenotype of these patients is mainly restricted to the lungs (Table 3).[76] *Fut8* knock-out mice present similar emphysema-like lung abnormalities as well as a dysregulated TGF-² 1 receptor activation and signalling, and downregulation of other proteins (e.g. epidermal growth factor receptor and integrin).[155] TGF-² 1 deficiency may promote a proinflammatory microenvironment and might also affect lung-immunity in humans. Reinforcing these hypothesis are: (1) heterozygous *Fut8* knock-out mice display an increased number of inflammatory cells in the lungs and (2) in chronic obstructive

pulmonary disease, decreased FUT8 serum levels are related to disease exacerbations and progression.[155,156]

Since TGF-² 1 supplementation in *Fut8* deficient mice rescued the emphysema-like phenotype[157], it suggests supplementation with this cytokine as a potential treatment for patients. Remarkably, *in vitro* and *in vivo* studies, have shown that *FUT8* depletion has a greater and more generalised impact on the immune system components and response.[157,158] *Fut8*$^{-/-}$ mice exhibited an imbalanced immune cell count with high levels of eosinophils and monocytes, as well as an affected response to bacterial lipopolysaccharide (LPS) stimulation with decreased IFN-² production.[159–161] FUT8 defects also trigger profound alterations in B and T cells, as well as in the communication between these cell types. B cell anomalies include: lymphopenia, hypogammaglobulinemia, impaired pre- and IgG-BCR antigen recognition, assembly and lipid raft association.[157,159,160] Concomitantly, *Fut8* defective mice showed inefficient immunization.[157,158] Furthermore, a genome wide association study has unravelled *FUT8* as a gene of interest in IgG glycosylation, indicating that FUT8 might also have a direct impact on antibody function.[162] T cell abnormalities comprise: failure to transport TCRs to membrane lipid rafts, reduced $CD4^+$ T cell activation, lower production of Th1 and Th2 cytokines with concomitant decrease of the T cell regulatory protein Foxp3.[158,163] Thus, FUT8 impairs the communication between B and T cells, being a crucial player across all immune mechanisms both *in vitro* and *in vivo*.[158]

G6PC3-CDG

Deficiencies in G6PC3 impair the hydrolysis of glucose-6-phosphate to glucose. Patients' neutrophils have both truncated and galactose-defective N- and O-glycans.[86] *G6PC3* mutations cause severe congenital neutropenia (SCN) 4 (MIM: 612541) and Dursun syndrome (MIM: 612541).[83] The 119 G6PC3-CDG patients identified present with a wide range of immunological clinical manifestations and cellular/biochemical alterations (Table 3).

The molecular pathophysiology of G6PC3 deficiency seems to be multifactorial. Regarding the neutropenic phenotype, two mechanisms may be suggested: (1) increased ER stress and apoptosis through Gsk3² (protein kinase with a known role in immunity) hyperactivity and degradation of MCl1 (anti-apoptotic protein) have been registered in patients' cells, which may contribute to increased neutrophil death and; (2) both human and mouse models show abnormal neutrophil retention in the bone marrow due to high cell surface expression of the chemokine receptor CXCR4.[77,82,86,90] Accordingly, granulocyte colony-stimulating factor (G-CSF) therapy diminished CXCR4 levels and improved neutrophil counts.[82] Concerning neutrophil dysfunction observed in these patients, it has been shown that the ER stress may be promoted by decreased neutrophil respiratory burst levels due to NADPH aberrant glycosylation, resulting in impaired bactericidal capacity. A more thorough comprehension of these mechanisms is needed to identify other potential therapeutic targets.

JAGN1-CDG

The JAGN1 protein resides in the ER membrane and is involved in the organelle's vesicular-mediated transport, which is important for neutrophil differentiation and function. JAGN1 deficiency

affects glycoprotein processing and/or trimming, resulting in altered neutrophil N-glycosylation. Specifically, there is an increase in Gal-±-1,3-Gal–terminated triantennary glycans and a decrease in biantennary glycans with end-standing sialic acid.[164] Hence, mutations in *JAGN1* result in SCN (MIM: 616022). *JAGN1* deficiency also results in aberrant glycosylation of key molecules involved in neutrophil migration, adhesion and cytotoxicity (i.e. CD177, CD11b, CD18, neutrophil collagenase, matrix metalloproteinase 9, lactoferrin, lipocalin 2, haptoglobin and neutrophil granule protein).[164] Moreover, there seem to be diminished levels and activity of myeloperoxidase (enzyme released by neutrophil granules) in neutrophil extracellular traps, hindering the capacity to eliminate extracellular pathogens.[116] Thus, *JAGN1* deficiency might impact the immune response and lead to increased susceptibility to infection by disrupting both cell differentiation and development, as well as neutrophil migration and function.

MOGS-CDG

MOGS is the first enzyme involved in the processing of N-linked oligosaccharides. This enzyme trims the distal ±-1,2-linked glucose residue from the Glc_3-Man_9-$GlcNAc_2$ oligosaccharide precursor. MOGS-CDG (MIM: 606056) is a paradoxical case of immunological dysfunction. While being associated with an immunodeficiency phenotype (Table 3), MOGS-CDG patients present an increased resistance to viruses with glycosylated envelopes (reviewed in [19]).

PGM3-CDG

PGM3 codes for a member of the phosphohexose mutase family. The encoded protein mediates the interconversion of GlcNAc-6-P into GlcNAc-1-P, critical for the synthesis of UDP-GlcNAc. PGM3-CDG (MIM: 615816) is an immunodeficiency disorder characterized by recurrent bacterial and fungal infections, often associated with increased levels of IgE.[118,120,165] IgE glycosylation was assessed in two patients but no significant differences with controls were found.[168–170] UDP-GlcNAc deficiency due to *PGM3* mutations affects O-GlcNAc glycosylation upon T cell activation. This is important for T cell proliferation and cytokine production through the regulation of the O-GlcNAc NFAT (modified nuclear factor of activated T cells) and NF-º B mediated signalling.[53,166,167] Thus, immunological manifestations in PGM3-CDG may be due to altered cell signaling, leading to impaired Th2 phenotypes.

SLC35C1-CDG

Leukocyte adhesion deficiency type II (LAD II) (MIM:266265) is due to mutations in *SLC35C1*. These cause defects in the transport of GDP-fucose from the cytoplasm to the Golgi lumen where it is used as a substrate for fucosylation. Impaired and/or absent fucosylation negatively impacts the biosynthesis and function of selectin ligands (e.g. Le^x and sLe^x) and of various fucosylated proteins.[171,172] The clinical, biochemical and cellular consequences observed in these patients are listed in Table 3. Noteworthy, this immune deficiency can be partially corrected by fucose supplementation. (Marquardt et al. 1999; reviewed in Monticelli et al. 2016).

SLC37A4-CDG

This CDG corresponds to glycogen storage disease type Ib and Ic (MIM: 232220 and 232240, respectively). The *SLC47A4* encodes a glucose-6-phosphate transporter, which carries glucose from the cytoplasm into the ER lumen. A defective glucose-6-phosphate transport decreases both gluconeogenesis and glycogenolysis, resulting in the accumulation of glycogen in the liver and kidneys. Moreover, truncated N-glycans and lower galactosylation levels have been found in patients' neutrophils, affecting NADPH oxidase components similarly to what has been described for G6PC3 deficiency.[86,151] Besides the common features of glycogen storage disease type I, neutropenia and/or neutrophil dysfunction are found in most SLC37A4-CDG patients. Consequently, these patients are prone to recurrent infections. Immunological findings are described in Table 3. Human and mouse models suggest that behind the neutrophil shortage and dysfunction caused by SLC37A4 impairment there is the increment of oxidative stress, which leads to increased apoptosis of differentiated neutrophils.[173,174] This is further supported by the beneficial effect of antioxidant therapy.[145] Additionally, the transporter defect causes an energy impairment characterized by reduced levels of blood glucose, lactate, ATP, and NADP. This results in low respiratory burst, impaired calcium import and sequestration as well as chemotaxis defects, probably contributing to neutrophil dysfunction.[175,176] Galactose supplementation in a patient led to significant clinical improvement which suggests that the glycosylation defects play a significant immunopathological role.[151]

2.2. CDG with minor immunological involvement

ALG1-CDG

ALG1 catalyzes the addition of the first mannose in the biosynthesis of LLOs. Nearly 60 ALG1-CDG (MIM: 608540) patients have been reported.[177] In a cohort study with 39 patients, 28 % presented immunological involvement, including recurrent and/or severe infections and fever episodes (Ng et al. 2016). Hypogammaglobulinemia was identified in some patients, likely secondary to protein loss due to nephrotic syndrome.[19,178,179]

ALG14-CDG

ALG14 forms a heterodimer with the catalytic subunit ALG13 and acts early in the LLO biosynthetic pathway, promoting the addition of a second GlcNAc to form $GlcNAc_2$-PP-dolichol. *ALG14* mutations have been associated with both limb-girdle and myasthenia phenotypes. Only 7 patients have been diagnosed with the latter. Among them, two sisters were described to have autoimmune myasthenia gravis.[180] A more in-depth delineation of the myasthenia gravis phenotype, particularly at the cellular and molecular levels, is warranted to guide diagnosis and treatment. In this sibling pair, acetylcholine receptor and MuSK antibodies were not found. Nonetheless, *in vitro* studies showed that *ALG14* silencing leads to defective cell-surface expression of acetylcholine receptors.[180]

ATP6AP2-CDG

ATP6AP2 is an accessory subunit of the V-ATPase protein pump. It also functions as the renin and prorenin cellular receptor and has been implicated in X-linked parkinsonism with spasticity (MIM: 300911) and Hedera type syndromic mental retardation (MIM: 300423). Its impact on glycosylation is not clear, but this protein seems to play a role in the ER-Golgi trafficking of glycosylation enzymes. Recently, *ATP6AP2* mutations have been found in three patients with a new phenotype resembling ATP6AP1-CDG. The patients experienced recurrent infections (e.g. localized respiratory tract infections, peritonitis and sepsis). Immunological investigations showed decreased Ig counts (restricted to IgG deficiency in one patient) and altered T cell counts (low levels of $CD4^+$ and increased levels of $CD8^+$ lymphocytes).[181] Remarkably, in a mouse model of *Atp6ap2* deficiency, leukocyte depletion with pronounced lymphopenia and increased TNF± production were reported. The leukopenia was suggested to stem from defective haematopoiesis derived from abnormal WNT signalling.[182]

ATP6AP2 is highly expressed in human monocytes, T and NK cells. These cells also released pro-inflammatory cytokines (e.g. IL-6 and IFN-³) following renin stimulation. In patients with autoimmune glomerulonephritis, ATP6AP2 is expressed in infiltrating lymphocytes and macrophages around glomeruli, promoting a pro-inflammatory signaling through the MAP/ERK/COX-2 pathway.[183] Concordantly, *in vitro* models of renal fibrosis showed ATP6AP2 expression to contribute to a pro-inflammatory environment by inducing TGF-² 1 expression.[184] Conversely, in IgA nephropathy (IgAN), kidney fibrosis was correlated with decreased ATP6AP2 levels. Nevertheless, higher ATP6AP2 expression was associated with greater IgA deposits and more severe phenotypes.[185] Additionally, in Graves' disease, a thyroid-specific autoimmune disease, ATP6AP2 has been reported to be a potential disease and therapeutic biomarker.[186]

C1GALT1C1-CDG

C1GALT1C1 is a molecular chaperone required for the folding, stability and function of the enzyme catalyzing the formation of the T antigen. Hypogalactosylation (exposing GalNAc residues of which some are also sialylated), converts this antigen into the (sialyl-)Tn antigen. *C1GALT1C1* mutations cause Tn polyagglutination syndrome (MIM: 300622). This is an autoimmune condition implicated in haemolytic anaemia, leukopenia, thrombocytopenia, myelodysplasia, IgAN, Henoch–Schönlein purpura and ulcerative colitis.[187–189] Of note, in IgAN, decreased *C1GALT1C1* expression (independent from mutations) and increased O-glycan defective IgA have been found. Molecular and cellular mechanisms reported to lessen *C1GALT1C1* expression include T lymphocyte subgroup drifting and IL-17, TGF-² 1 and IL-4/STAT6/HIPK2-mediated gene downregulation, among others.[190–196] This is evidence that an anomalous B/T cell response is the underlying mechanism interfering with *C1GALT1C1* expression and mediated O-glycosylation. In fact, Tn antigen presentation to T cells has been found to be preferentially done through the binding to C-type macrophage galactose-type lectin. This leads to a subsequent downstream activation of the ERK-calcineurin axis and downregulation of C1GALT1C1 expression.[197] Upper respiratory tract infections and tonsillitis are also among the clinical manifestations seen in IgAN patients. Infections are correlated with disease onset or exacerbations.[191,198] Interestingly, *C1GALT1C1* levels are further diminished with more pronounced glycosylation defects detected in IgA1-positive a human B lymphoma cell line and in an IgAN patient blood and tonsillar lymphocytes upon LPS, ±-hemolytic

Streptococcus and *Helicobacter pilori* stimulation.[193,198–200] This suggests that pathogens themselves play a role in the modulation of the host glycosylation to facilitate invasion.

COG6-CDG

COG6 is a subunit of the conserved oligomeric Golgi complex. It is involved in the maintenance of the homeostasis of the Golgi apparatus, and, consequently, in the Golgi-dependent glycosylation machinery. Eighteen COG6-CDG (MIM: 614576) patients have been reported. Recurrent infections are the most prevalent immunological feature.[19] Recently, a patient with recurrent pneumonia and bilateral lung inflammation was reported. The patient also had a widespread skin rash and upon administration of an oral polio vaccine, he spiked a 40 ºC fever (possibly triggered by hypohydrosis).[201] Noteworthy, another patient showed secondary hemophagocytic lymphohistiocytosis with enlarged lymph nodes, dental caries, urinary infections, low NK cell counts and cytopenia. Interestingly, pancytopenia has been noted in 5 other patients.[202] Less frequent immunological alterations include B/T cell and neutrophil dysfunction, monocytosis, deficient polysaccharide antibody response, and combined immunodeficiency.[19] An *in-silico* genome-wide linkage study has identified a polymorphism responsible for regulating *COG6* expression in monocytes. This polymorphism correlated with both rheumatic arthritis and psoriasis susceptibility and was recognized as a predisposing genetic locus for systemic lupus erythematosus.[203,204] Both studies highlight the importance of *COG6* in immune-mediated disorders.

DOLK-CDG

DOLK is an enzyme of the biosynthetic pathway of Dol-P-Man, a sugar donor required for C- and O-mannosylation, N- and O-linked glycosylation of proteins and glycosylphosphatidylinositol anchors. The immunological phenotype seen in DOLK-CDG (MIM: 610768) is mainly characterized by recurrent, severe and sometimes fatal infections.[19,205] Biochemical and cellular data explaining the immune dysfunction are lacking.

GALNT3-CDG

GALNT3 belongs to a family of GalNAc-transferases and catalyzes the transfer of a GalNAc to a Ser or Thr on the receptor protein, the first step of O-linked oligosaccharide biosynthesis. *GALNT3* mutations are among the causes of hyperphosphatemic familial tumoral calcinosis (MIM: 211900). In a subset of GALNT3-CDG patients, auto-immune and inflammatory manifestations have been found.[19,206] In a cohort of 7 patients, 5 showed diaphysitis, 4 had signs of ectopic calcifications and chronic inflammation, while three exhibited manifestations of overwhelming systemic inflammation. The latter included intermittent fever, cutaneous calcinosis with inflammatory reaction and chronic fatigue, increased C-reactive protein levels and high erythrocyte sedimentation rate. Two of these patients also had anemia, consistent with inflammation-mediated chronic disease and thrombocytosis, while leukocytosis was detected in one patient.[206]

MAN1B1-CDG

Genetic defects in *MAN1B1* affect the trimming of one ±-1,2-linked mannose from the oligosaccharide $Man_2GlcNAc_2$ in the Golgi apparatus. Despite the abnormal N-glycosylation of patients' Igs and serum proteins, no clinical evidence of immunological manifestations in MAN1B1-CDG patients exists.[19] Very recently, mutations in another mannosidase -*MAN2B2*- have been linked with a new CDG characterized by immunodeficiency (personal communication). Altogether, these reports suggest a possibly relevant immune role of the glycan trimming pathway.

MGAT2-CDG

MGAT2 catalyzes the addition of the second GlcNAc to complex (trimannosylcore) N-glycans in the Golgi apparatus. MGAT2-CDG (MIM: 212066) has been reported in 12 patients (with 9 belonging to the same large kindred). Detailed immunological assessment is only available for one patient, who presented both recurrent respiratory and urinary tract infections associated with low IgG levels, decreased total hemolytic complement assay, C2, C3a, and increased C3d.[19,207] Another patient has been diagnosed with scalp psoriasis.[208]

Myeloid lineage cells and purified *Mgat2* null DCs derived from mice displayed absent polysaccharide A presentation and activation of T cells.[209,210] Interestingly, a subset of these mice presented an autoimmune phenotype triggered by glycosylation alterations at erythrocytes' surface. These deficiencies caused an IgM-mediated naive T cell depletion, low cytokine production and hindered IgG-mediated response upon polysaccharide conjugate vaccination.[211]

PMM2-CDG

PMM2 catalyzes the conversion of mannose-6-phosphate into mannose-1-phosphate, a crucial step in the synthesis of dolichol oligosaccharides. PMM2-CDG (MIM: 212065) is the most common N-glycosylation disorder. Some patients present immunological dysfunction. A recent study has identified viral infections as triggers of stroke-like episodes in 42 % of PMM2-CDG children (3/7).[212] This observation matches the previous finding that approximately half of all stroke-like episodes in PMM2-CDG are accompanied by infections and/or fever episodes.[19] Recurrent infections in PMM2-CDG appear to be mainly restricted to infancy/early childhood. Recently, two patients who suffered from recurrent respiratory infections and bacterial sepsis during infancy were reported.[213,214] Infections can be very severe, sometimes leading to death.[214] Regarding cellular and biochemical anomalies contributing to the clinical phenotype, hypogammaglobulinemia, T lymphopenia and reduced neutrophil chemotaxis have been reported. Altered responses to vaccination have occasionally been described.[19,213,214]

SLC39A8-CDG

SLC39A8 is a solute carrier, acting as a manganese and zinc transporter. *SLC39A8* deficiency has a secondary impact on glycosylation due to the dependence of ² -1,4-galactosyltransferases on Mn^{2+}.

Hence, the severity of Mn^{2+} deficiency is directly linked to the degree of glycosylation impairment, converting SLC38A8-CDG into a multi-system disease characterized by trace element and galactosylation defects.[37,215] Thus, the immune anomalies seen in these patients are most probably multi-factorial. From the 12 reported patients, 6 presented glycosylation anomalies and three of these experienced recurrent infections.[215–217] Notably, the Ala391Thr *SLC39A8* missense variant was correlated with Crohn's disease and microbiome composition, immune related traits and growth.[218,219] Stimulation of various mouse tissues with proinflammatory molecules led to a general increase in the levels of Slc39a8.[220] In *Slc39a8* hypomorphic mice, hematopoiesis was impaired, which led to severe anemia, but also to abnormal myeloid cells.[221,222] Gene expression studies performed in this animal model revealed an association between *Slc39a8* deficiency, innate immunity defects and altered response to inflammatory signals.[222] The array of clinical and biochemical/cellular alterations reported in SLC39A8-CDG patients and models warrants further immunophenotyping and examination of the glycosylation status.

VPS13B-CDG

Mutations in *VPS13B* are responsible for Cohen syndrome (MIM: 216550). *VPS13B* is thought to encode a Golgi transmembrane protein involved in glycosyltransferases and glycans vesicle-mediated transport. In fact, a pattern of agalactosylated and asialo-fucosylated structures, indicative of a N-glycan maturation defect, was found in serum proteins of these patients.[223] Intermittent congenital neutropenia is a hallmark of this disease (detected in 168/224 VPS13B-CDG patients).[223–247] One patient had leukopenia without neutropenia whilst another had pancytopenia.[232,248] Decreased neutrophil counts often coincide with infections, namely recurrent aphthous oral ulcers, gingivitis, otitis, rhinopharyngeal, pulmonary (e.g. pneumonia, bronchitis), skin (e.g. cellulitis) and urinary tract infections.[225,232,238,239,242,245,246] The link between defective glycosylation and clinical manifestations in these patients remains unidentified. VPS13B seems to have a major role in the Golgi integrity and trafficking, specifically, in the endosomal-lysosomal pathway. Therefore, the glycosylation defects may be secondary to Golgi disruption. Indeed, the early endosome antigen and the lysosomal-associated membrane protein 2 were underglycosylated in patients' fibroblasts.[223,249]

3. General immunological diagnosis and monitoring recommendations

Taking into consideration all the clinical, biochemical and cellular alterations mentioned above, we recommend that full and detailed leukocyte and Ig counts should be routinely performed in CDG with immunological involvement. When there is evidence suggestive of other immune dysfunctions, further tests ought to be done. For instance, vaccination response (by measuring protective antibody titers) should be determined in ALG12-CDG, ATP6AP1-CDG, COG6-CDG, MGAT2-CDG, MOGS-CDG and PMM2-CDG. Immune cells/proteins functional characterization assays should be performed in some CDG, such as:

- neutrophil respiratory burst levels in G6PC3-CDG and SLC37A4-CDG, neutrophil migration in SLC35C1-CDG and JAGN1-CDG, and neutrophil reactive oxygen species, and chemotaxis assessment in SLC37A4-CDG;

- lymphocyte phenotyping to assess cell differentiation, development and proliferation (e.g. T-cell proliferation and differentiation in PGM3-CDG and T-cell development in EXTL3-CDG) and
- Ig isotyping (advised in all CDG with immunological involvement) / analytical assays (e.g. half-life and Fc receptor affinity in MOGS-CDG).

In CDG with inflammatory episodes/symptoms, namely GALNT3-CDG, CRP, erythrocyte sedimentation rate and cytokine panels should be frequently monitored. Organ-specific examination should be undertaken. Particularly in the case of FUT8-CDG, patients should be attentively monitored for lung function, physiology and infections, whilst both G6PC3-CDG and SLC37A4-CDG patients should be tested for IBD. Bone marrow examinations should be done for G6PC3-CDG, JAGN1-CDG, PGM3-CDG and SLC37A4-CDG.

Standardized and systematic registration of infection history and infectious agents would be extremely useful to clarify the immunological involvement of some CDG, especially of those in which recurrent infections are the sole (reported) immunological manifestation. In MOGS-CDG, comprehensive studies of disease-causing pathogens and pathogen-resistance mechanisms are needed.

Autoantibody measurement should be carried out for C1GALT1CT-CDG (analysis of anti-Tn antibodies) and could be considered when other immunological tests/markers fail to explain the clinical features.

It is also highly recommended – particularly to better unravel the role of glycosylation in the immune-related mechanisms/dysfunctions – to assess the glycosylation status of immune cells and proteins (e.g. IgG in FUT8-CDG or IgA in C1GALT1C1-CDG).

4. Immunomodulatory therapeutic avenues in CDG

Diverse therapeutic approaches for CDG are currently at several phases of development and testing. These include dietary supplementation, gene therapy, pharmacological chaperones, drug repurposing and organ transplantation. Since the therapeutic benefit of these treatments remains largely undetermined, very few of these therapeutics are approved specifically for CDG.[250] In general, the standards of care for CDG patients are limited to preventive and management interventions, also concerning immunological complications.

Regarding infection treatment, intravenous (IV) Ig administration has resulted in clinical improvement in many CDG patients.[19,71,72,75,178,181] Nevertheless, patients with hypogammaglobulinemia refractive to Ig therapy have been reported.[113,115,117,165] Antibiotics– via IV or oral administration – have also been extensively used both as a preventive and curative measure. In general, a good response to antibiotic therapy (alone or in combination with Igs replacement therapy) has been observed.[81,85,91–93,104,105,109,117,119,120,148–151,165,202]

Concerning autoimmune and inflammatory manifestations, anticholinesterase inhibitors and steroids have had fluctuating beneficial effects on CDG patients presenting with autoimmune myasthenia gravis.[143,180] Anti-inflammatory and immunosuppressive agents have been employed to manage IBD in G6PC3-CDG.[87,93,105,110,143] Interestingly, in two GALNT3-CDG patients with generalized inflammation and foamy macrophages a treatment regimen with IL-1 antagonists resulted in decreased inflammatory markers, reduced tumour burden and improved general health in both

subjects. Hence, inflammation markers (e.g. CRP levels and macrophage infiltration) should be monitored and targeted anti-inflammatories considered as an adjuvant treatment.[206] Noteworthy, the immunosuppressant drug mycophenolate (Mofetil) – used as an IgAN therapeutic agent – and alternative plant-based medicines have both been described to upregulate *C1GALT1C1* expression and to correct the IgA O-glycosylation profile, with clinical benefit documented for the former.[199,251] Even though neither of these compounds have been reported in the treatment of C1GALT1C1-CDG, their mechanism of action suggests potential therapeutic interest.

Therapeutic strategies to correct immune cell counts and/or function include leukocyte transfusion, steroids and G-CSF.[89,123,165] Simultaneous treatment with G-CSF and antioxidant therapy (i.e. vitamin E) in SLC37A4-CDG patients diminished infections and improved neutrophil counts.[143,149] Nevertheless, therapy resistance, lack of compliance, inconsistent effectiveness and/or side-effects (e.g. splenomegaly, giant cell tumours and acute myeloid leukemia following high doses of G-CSF) have been reported in these patients. [90,104,105,113,115,116,139]

In severe immunodeficiencies, hematopoietic stem cell transplantation (HSCT) has been performed.[75,104,113,115,250,252] HSCT has resolved infections in a JAGN1-CDG patient, recovered normal T-cell development in an EXTL3-CDG patient and is now an approved therapy for PGM3-CDG in the USA, due to its outstanding clinical results in some patients.[75,113,115,250]

Nutrient supplementation with sugar precursors (e.g. galactose, fucose, glucose) has been attempted. In many cases, it was unsuccessful, as for instance the supplementation with UDP-GlcNAc in PGM3-CDG. In other cases, there was no correlation with the reversion of immunological manifestations, as exemplified by the mono- and/or combined supplementation of galactose, Mn^{2+} and uridine in SLC38A8-CDG.[37,216,250] The exceptions are: (1) fucose administration in SLC35C1-CDG patients with well-defined mutations which significantly improved leucocyte migration defects, leukocytosis and resolved infections; and (2) galactose supplementation in combination with G-CSF in a SLC37A4-CDG patient that ceased infections and abscesses. These targeted approaches underpin the glycosylation defect as the underlying cause of the immune-related alterations observed in patients and reinforce the necessity of targeted and more effective immune-therapies for CDG patients.[151,171]

5. Discussion

To set up a proper response, the immune system needs to first recognise, then to identify, distinguish antigens (e.g. self from non-self) and respond accordingly. Equally important is the regulation and subsidence of the immune response to avoid exacerbations and collateral damage. To efficiently perform all these actions, the immune system and response rely on numerous cell-cell and receptor-ligand interactions mediated through proteins and/or glycans. The existence of an almost infinite possibility of clinical outcomes in CDG patients highlights the complexity of the glycan function in the immune response.

Glycosylation and glycans in the immune system rest on three main pillars: ubiquity, diversity/content and context. All these variables can influence the functionality of immune organs,

cells and molecules. Glycans are practically present in all components of the immune system.[23–32] However, their distribution is not random. In fact, each cell type and molecule has its own glycan signature according to its function and activation status.[253]

Although this review has focused on how immune response is impaired by glycosylation defects, glycan content does not always follow the rule "the more, the merrier". One example is the fucose content which as mentioned above, in FUT8-CDG and SLC35C1-CDG, has been shown to be critical for a number of immune functions. Yet, lower fucose content in the IgG Fc region can increase antibody-dependent cellular cytotoxicity and receptor Fc³ RIIIa binding capacity, which are of major relevance for therapeutic antibodies.[254,255] Additionally, a depletion of core-fucose content may change the proteins' hydrophilicity and automatically affect their cellular localization.[163] Illustrative cases of immune alterations due to increased fucosylation are: a) the discovery of the hyperfucosylated surfactant protein D – a protein that impacts the immune response of the respiratory tract– as a biomarker of chronic obstructive pulmonary disease; and b) the association between TCR and other T cell proteins core-fucosylation, inflammatory cytokines production and colitis onset in mice and humans.[163,256]

As for the third pillar, the immune response can greatly vary depending on the immunological context. In the cancer microenvironment, blocking core-fucosylation induces a T cell response. In contrast, in colitis and systemic lupus erythematosus increased core-fucosylation is accompanied by an increased T cell activation and response.[158,163,257] However, in both cases, core-fucosylation inactivation may be a potential new therapeutic avenue. The essential character of the immunity context can be further illustrated by the example of FUT8-CDG. In these patients, immunological involvement appears to be mainly restricted to the lungs.[76] Furthermore, *Fut8* null mice exhibit a pro-inflammatory lung environment, whereas the systemic immune response seems to be somehow compromised or attenuated.[155,158,160] *ATP6AP1* deficiency is associated with immune-related complications that include recurrent bacterial infections. However, in the context of periodontitis, *ATP6AP1* silencing resulted in decreased inflammation with diminished infiltration of T-cells, DCs, macrophages and expression of pro-inflammatory cytokines in the periodontal lesion.[258] Other variables influencing and influenced by glycans are the developmental stage, protein/cell activation status and aging.[53,74,197,259]

The paradoxical activity of glycans in the immune response can be clearly demonstrated by their ambiguity in host-pathogen interaction and recognition. If glycan deficiency can weaken the immune system, alter cellular production/function and even microorganism recognition, it may also render individuals more resistant to infectious agents. This happens when internalization is highly dependent on the host cells' glycan content.[35,153,154] Although the clinical consequences remain elusive, GALNT3-CDG disease models show that this gene may have a dual role in IAV early infection. On the one hand, *GALNT3* expression inhibited IAV replication. Accordingly, higher viral titers in the lungs of *Galnt3* knockout mice and *GALNT3* knockdown cells were observed. On the other hand, increased GALNT3 expression paradoxically initiated mucin-type O-glycosylation, facilitating IAV replication.[260,261] Among other CDG-related genes, varying degrees of pathogen resistance (mainly of viral origin) have been reported.[38,39,44,153,154] Despite intensive studies on viral infectivity and recognition of host glycans, the underlying molecular mechanisms and exact pathophysiological consequences in CDG remain largely unidentified.[35,43,262] Hence, it would be

helpful to know if there is an infectious agent(s) preponderance among CDG. This would help to establish appropriate care for these patients. In a broader perspective, it might provide clues for the development of anti-infectious approaches for other human diseases.

The current treatment approaches to address immunological dysfunction in CDG are mainly limited to prevention (e.g. Ig administration in case of hypogammaglobulinemia) or management strategies (e.g. G-CSF to treat neutropenia or antibiotics for recurrent infections). The only available targeted therapies directed at correcting immunological dysfunction in CDG are fucose supplementation exclusively in SLC35C1-CDG and HSCT in PGM3-CDG patients (the latter approved in the USA). However, proof-of-concept studies suggest that galactose supplementation and HSCT may be beneficial for other CDG.[75,113,115,151,171,250]

Although there is a clear cause-effect mechanism linking glycosylation defects and immune dysfunction in some CDG, the complexity and multi-organ involvement often observed in these disorders foster other possible mechanisms for such alterations. It cannot be excluded that in some CDG patients, immunological manifestations may arise as collateral complications (i.e. hypogammaglobulinemia secondary to nephrotic syndrome) or have a multifactorial origin.[178] For instance, in SLC39A8-CDG, intracellular Mn^{2+} levels are decreased which impacts the galactosyltransferase activity. Hence, there may be a cumulative and/or a synergistic phenomenon, since Mn^{2+} has a known role in immunity. Supporting this hypothesis is TMEM165-CDG (MIM: 614727) also accompanied by abnormal Mn^{2+} metabolism and hypogalactosylation. Immune-related manifestations in TMEM165-CDG patients appear to be more heterogeneous and rarer than in SLC39A8-CDG. Among the 10 reported TMEM165-CDG patients, unexplained, recurrent fevers happened in 4 patients and two died from septic shock. There are also single reports of food allergy, infection leading to respiratory insufficiency, and blepharitis.[263,264] Contributing to the complexity of the putative glycosylation-immune system interplay is the fact that glycosylation effectors themselves are also glycosylated. Of note, SLC39A8 itself has been reported to be heavily glycosylated upon activation.[265] This potentially creates a glycosylation loop that might contribute to phenotypic diversity.

The clinical impact of the immune system may also be underestimated in CDG. Immune dysfunction has been associated with other clinical signs, such as stroke-like episodes and pericardial effusion in PMM2-CDG.[212,266] Even though immunological dysregulation has been found only in a subset of CDG patients, tissue/organ-specific immunologically-governed alterations might be more common and clinically relevant in CDG than anticipated. Reported pulmonary manifestations and TGF-² 1-driven mechanisms in patients and animal models argue that this could indeed be the case in FUT8-CDG.[76,155].

Illustrative examples of immunological heterogeneity are FUT8-CDG and SLC35C1-CDG. Both play crucial roles in core-fucosylation[267,268], but their immunological phenotypes are quite dissimilar. Whilst SLC35C1-CDG patients show a more systemic immunological dysfunction (including neutropenia, inability to generate pus, marked leukocytosis, eosinophilia, and decreased number of B lymphocytes), FUT8-CDG patients exhibit a more lung-specific immune phenotype. Nevertheless, congenital neutropenia was also reported in a FUT8-CDG patient. Moreover, FUT8-CDG animal

models have shown generalized immunological alterations, namely T and B cell dysfunction resembling the clinical presentation of SLC35C1-CDG patients. An important factor that might be contributing to this significant clinical discrepancy is the low number of patients reported. Only 3 FUT8-CDG patients have been identified so far, whereas at least 9 SLC35C1-CDG patients are known. These patients may be unrepresentative of the clinical presentation of this condition. This is another reason why immunological anomalies may be underrepresented and undervalued in CDG. Another possible explanation, which requires further investigation, is that FUT8 might be particularly important in the lungs. Evidence substantiating this hypothesis are: a) FUT8 was found to be highly expressed in the lungs[269]; b) the family of human fucosyltransferases (FUTs) includes a total of 13 enzymes. Some of these FUTs are involved in the biosynthesis of (s)Lex and H antigens (FUT3–MIM: 111100, FUT4-MIM: 104230, FUT5–MIM: 136835, FUT6–MIM: 136836, FUT7–MIM: 602030). Therefore, these enzymes may compensate for the lack of FUT8 activity in other tissues and cells. Contrastingly, SLC35C1 appears to be the most relevant human GDP-fucose transporter (with high expression in the bone marrow and the immune system.[270] This invalidates the activation of compensatory mechanisms/transporters and can explain the more severe and generalized immunological involvement seen in these patients. Putative explanations and hypothesis apart, these paramount clinical differences reinforce another puzzling question: "Why are there such great immune-related clinical disparities across CDG involved in the same glycosylation pathway?". Our hypothesis may offer some solace but fails to adequately answer this question.

This multitude of contributing factors, in addition to possible compensatory mechanisms are reflected in the spectrum of immune complications, age-related incidence or spontaneous clinical improvement registered in some CDG.[73]

All in all, the ultimate question persists: "If glycans are so important for immunity, why is immunological involvement not more common within CDG?". A few light-shedding assumptions can be advanced:

- Since total glycosylation abrogation is incompatible with life, patients still retain residual levels of glycosylating capacity, which may prevent the development of more severe phenotypes, preserving reasonable immune responses; [260]
- The possible development of bioequivalence glycosylation mechanisms which create alternative pathways, allowing to achieve similar functionality;[271]
- The differential enzymatic expression and existence of cell/tissue/organ specific defects which leads to site-specific anomalies, thus restricting systemic and more severe phenotypes.[155]

Immunological involvement in CDG reflects the complexity of the immune system, the heterogeneity of this disease group as well as the diversity of the glycome.

6. Conclusion

The pivotal, multi-faceted roles of glycans and glycosylation in the immune system are undeniable. From pathogen-host interaction, to lymphocyte and antibody production and function; from TLR to BCRs, from innate immunity to adaptive response, glycans are everywhere.

CDG are a proof of this connection and can therefore be a model for better understanding both basic and clinical mechanisms involved in the immune response. This may be transposable to other human diseases with glycosylation and immunological alterations, such as cancer, autoimmune diseases, Parkinson disease, among others. Uncovering common genes, proteins, pathways and clinical manifestations will certainly accelerate and improve diagnosis, management and potential therapies.

List of Figures

Figure 1 - Title: Innate and adaptive immune mechanisms. Examples of glycans affecting the function of immune cell receptors and other signaling molecules. **Legend:** Graphic representation of some innate and adaptive immune response mechanisms and illustrative examples of the influence of glycans in the function and recognition by immune cell receptors and other signaling molecules. A) Innate and adaptive immune response mechanisms, cells, proteins and other components; B) N-glycosylation-mediated T-cell receptor (TCR) clustering and signaling; C) N-glycosylation impact on Toll-like receptor synthesis, expression, integrity, and activity; D) DC-SIGN N-glycosylation status affects organisation, membrane diffusion and pathogen recognition; E) (De)Sialylation of MHC-I molecules disturbs their cell surface stability; F) MHC-II N-glycosylation status impacts the its capacity to recognize presented peptides.

Figure 2 – Title: Glycosylation steps in which defects have been reported with immunological consequences. **Legend:** Graphic representation of some. The figure is divided into three parts: A) N-glycosylation, B) O-glycosylation (more specifically O-GalNAc glycosylation) and C) glycosaminoglycan synthesis (particularly heparin and heparan sulfate biosynthesis). Defects potentially affecting multiple glycosylation pathways (ATP6AP1, ATP6AP2, COG6, G6PC3 and SLC35C1) were included in figure A, and several steps and enzymes have been omitted for simplicity of representation. For the same reason, a simple and unbranched core 1 O-glycan is represented in figure B. Only enzymes described in the text are depicted.

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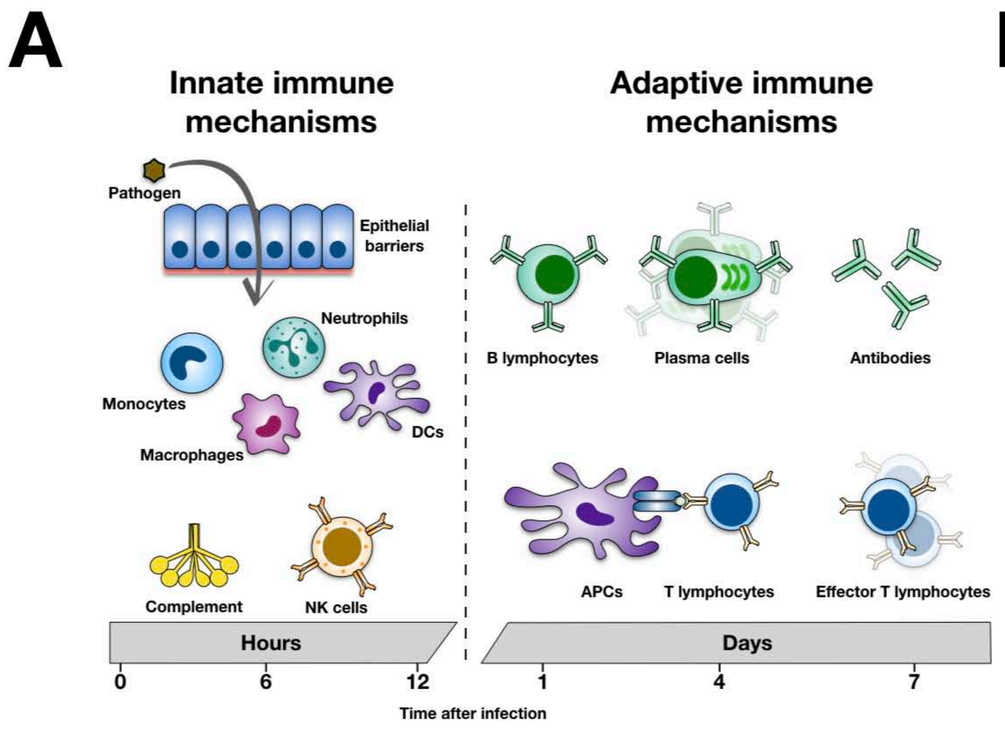

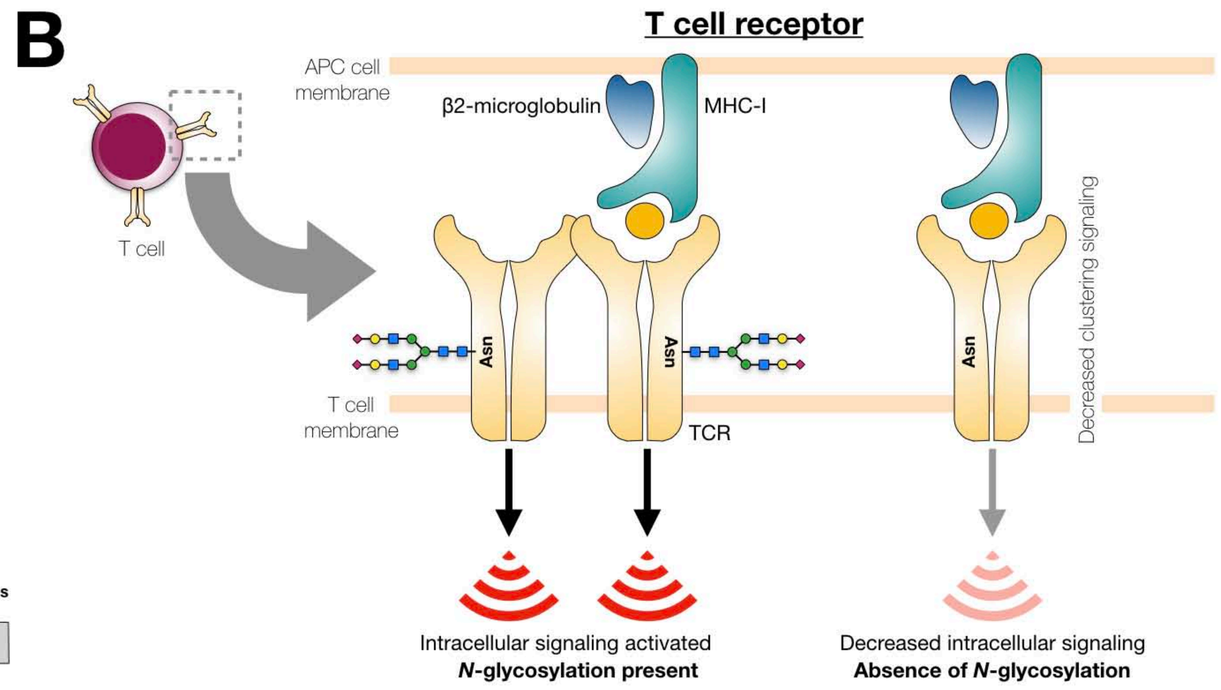

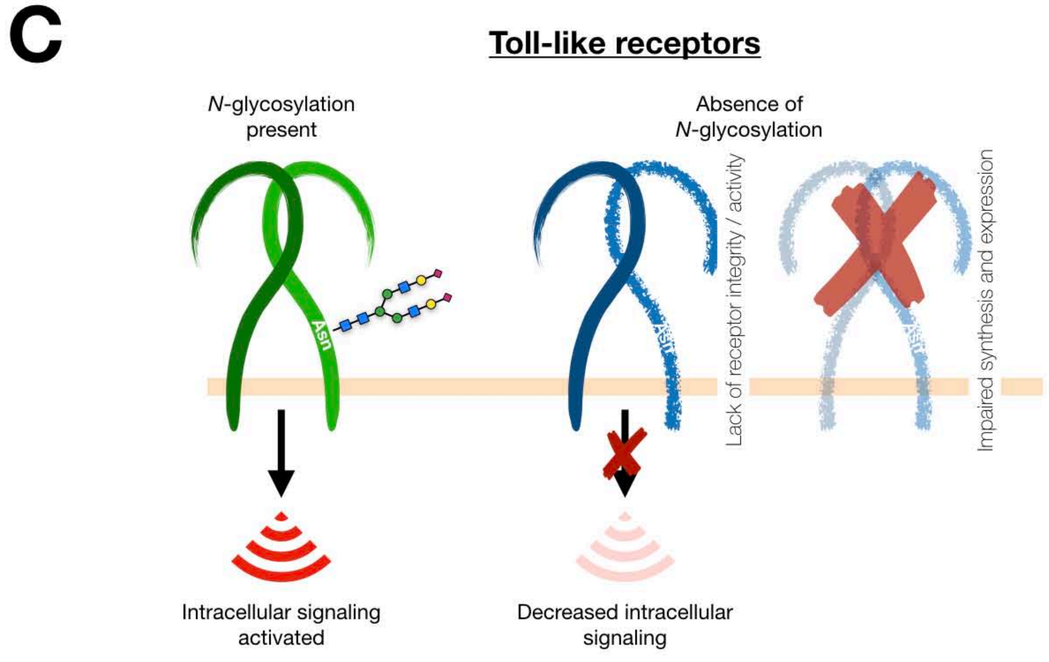

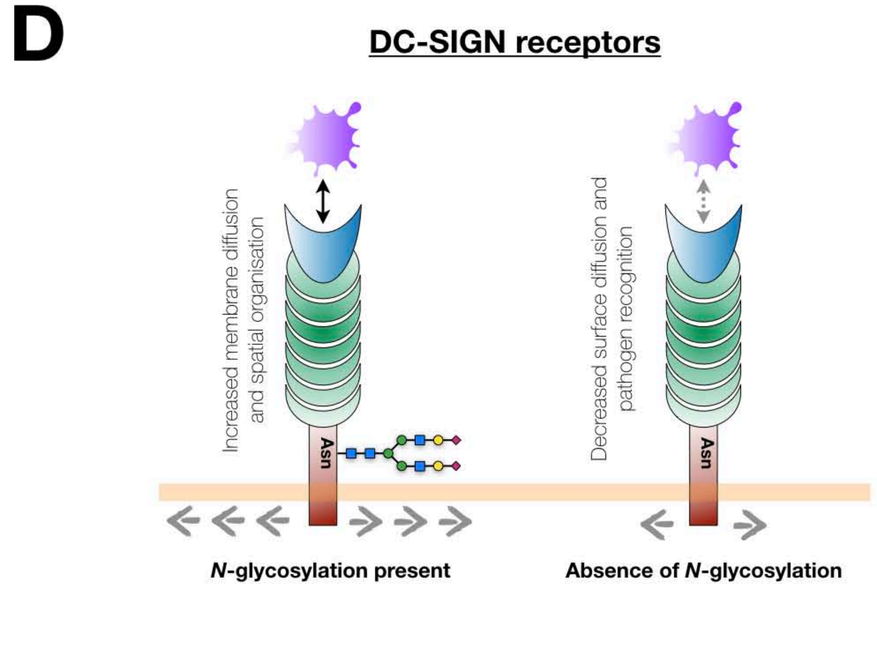

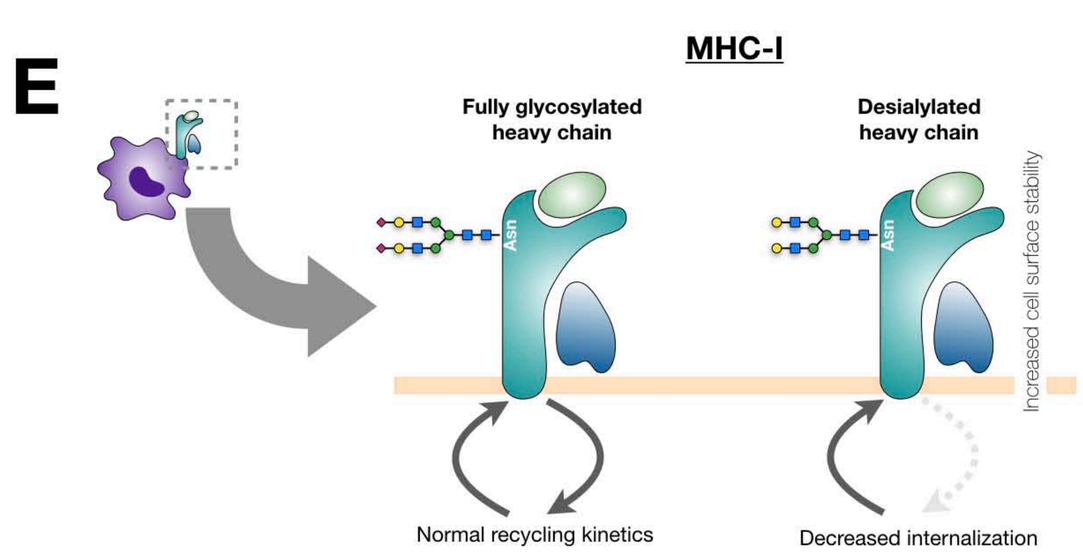

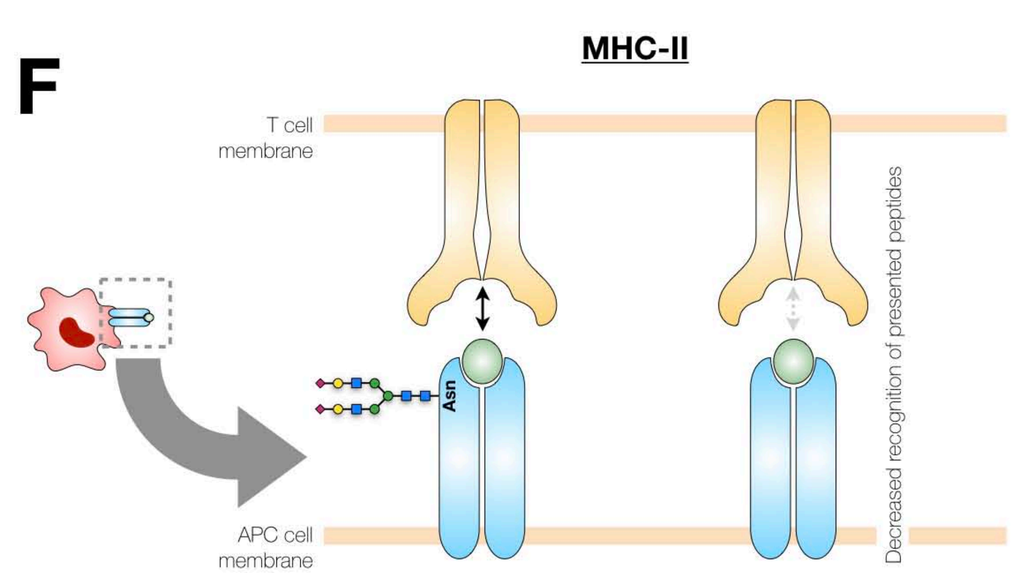

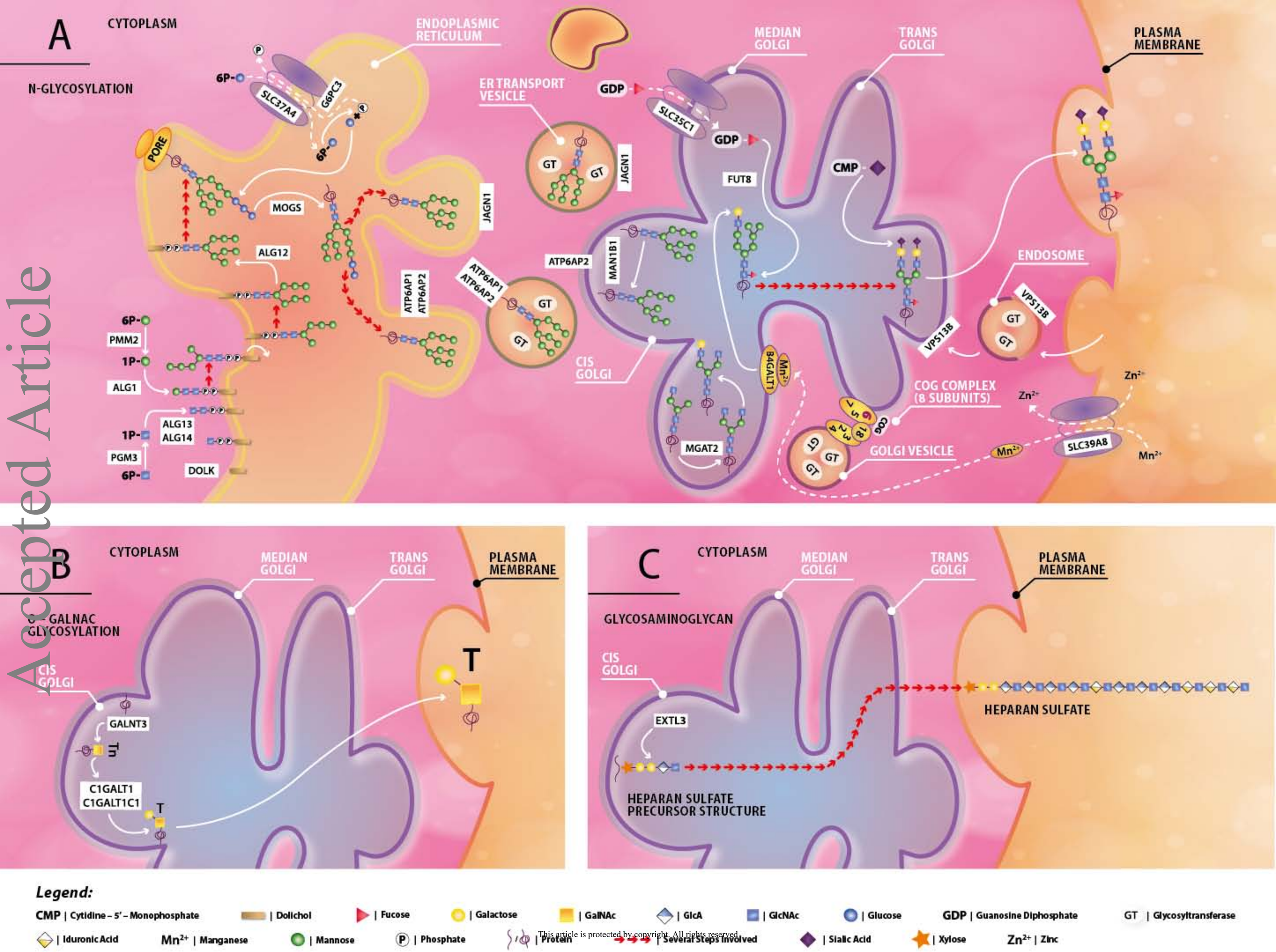

Legend:

CMP | Cytidine – 5' – Monophosphate | Dolichol | Fucose | Galactose | GalNAc | GlcA | GlcNAc | Glucose | **GDP** | Guanosine Diphosphate | GT | Glycosyltransferase | Iduronic Acid | Mn^{2+} | Manganese | Mannose | (P) | Phosphate | Protein | Several Steps Involved | Sialic Acid | Xylose | Zn^{2+} | Zinc