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Abstract

Investigation of the role of saccharides and glycoconjugates in mechanisms of immune-mediated physiological and pathological conditions is a hot topic. In fact, in many autoimmune diseases cross-reactivity between sugar moieties exposed on exogenous pathogens and self-molecules has long been hinted. Several peptides have been reported as mimetics of glycans specifically interacting with sugar-binding antibodies. The seek for these glycoreplica peptides is instrumental in characterizing antigen mimicry pathways and their involvement in triggering autoimmunity. Therefore, peptides mimicking glycan-protein interactions are valuable molecular tools to overcome the difficulties of oligosaccharide preparations. The clinical impact of peptide-based probes for autoimmune diseases diagnosis and follow-up is emerging only recently as just the tip of the iceberg of an overlooked potential. Here we provide a brief overview of the relevance of the structural and functional aspects of peptide probes and their mimicry effect in autoimmunity mechanisms for promising applications in diagnostics and therapeutics.

Keywords

Peptide carbohydrate mimics; Molecular mimicry; Autoimmune diseases; Phage display; Diagnostics; Biomarkers

Highlights:

- Glycoreplica peptides are amino acid sequences able to recognize anti-glycans antibodies
- Molecular mimicry investigations could provide invaluable insights into the etiology of immune-mediated pathologies
- Phage display methodologies allow the generation of peptide libraries specifically targeting anti-glycans antibodies
- Structural analysis of mimicry helps to rationally improve the immunological features
- Carbohydrate-mimetic peptides are promising tools to be used in screening sera of patients suffering from autoimmune diseases for the early diagnosis and treatment

Introduction

Despite the tremendous ongoing research efforts in the field of immune-mediated diseases, little is known about the precise molecular mechanisms triggering the disruption of immune tolerance and misrecognition of "self" epitopes as foreign antigens [1,2]. Nonetheless, there is increasing evidence about the crucial role played by glycans and glycoproteins in the pathophysiology of several antibody-mediated diseases, and an association between alterations of the serum glycome and autoimmunity has been proposed [3]. More and more studies show the relevance of glycosylation to pathogen recognition, to the immune system control and immune cell homeostasis. Equally critical and maybe even connected is the glycans-antibodies interaction in the development of immune-diseases, including autoimmunity and cancer [4,5].

The occurrence of carbohydrate-protein interactions as the first cell surface flags of cell-cell communication in case of infections and many other processes involving the immune response plays a crucial role in the discrimination between "self" and "non-self" recognition [6].

Genetic predisposition is not sufficient alone to elicit the complex mechanisms at the base of many pathologies. It is now accepted that environmental factors such as infections have been implicated in the onset and/or promotion of aberrant immune response [7-9].

Among the various biological processes that could break the physiological balance, i.e., tolerance, resulting in autoimmunity, the most relevant one is the so-called 'molecular mimicry' effect. Many eukaryotic and prokaryotic pathogens express glycoconjugates on their surface or secreted products. Glycans expressed by pathogens can include terminal glycostructures, that mimicking human biomolecules trigger an immune response to the pathogen leading to cross-reactive antibodies. The best-studied examples of pathogen-induced autoimmune conditions are Guillain-Barré syndrome (GBS) and its variant Miller Fisher syndrome [10].

GBS patients present antibodies against gangliosides, i.e., sialic acid-containing glycolipids and major constituents of the nerve cell membrane. The molecular mimics are glycans expressed on lipooligosaccharides (LOS) of preceding infectious organisms, in particular *Campylobacter jejuni*, that can induce an antibody response to these carbohydrate antigens [11,12]. The specificity of the anti-ganglioside autoantibodies is closely related to the nature of the preceding infections in GBS. Specific anti-ganglioside GM1, GM1b GD1a, and GalNAc-GD1a antibodies are related to a GBS form affecting only motor nerves, whereas antibodies against ganglioside GQ1b are associated with the Miller Fisher syndrome. The Miller Fisher syndrome is a subform of GBS affecting predominantly the nerves that innervate muscles governing eye movements. Other identified ganglioside mimetics are sialyllactose derivatives in non-typeable *Haemophilus influenzae* and *Haemophilus influenzae* b-type [13,14].

Despite many epidemiological and animal studies supporting the mimicry hypothesis [15], the role of antiglycan antibodies and the connection with exogenous infective agents remains only speculative for most autoimmune diseases. For some of them, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), self-antigens recognized by the host immune system have been identified but the etiology has not been clarified [1,16]. However, the crucial role of infective agents in triggering autoimmunity is highly suspected, yet the lack of a cross-reactive exogenous antigen hampers the assessment of a precise response pathway [17,18]. On the other hand, in multiple sclerosis (MS) the hyperglucosylated bacterial adhesin HMW1 was found to recognize autoantibodies in the sera of patients [19]. Nevertheless, an unequivocal native epitope has not been found yet. Therefore a precise causal relationship between infections and MS must be further elucidated.

Since the cross-reactivity among exogenous/host structures that may lead to a pathological condition survives as a major cause for most autoimmune diseases, the long-term search for the attribution of precise biological roles requires collections of glycoconjugates and their corresponding glycan-binding proteins.

To investigate the molecular nature of the antibody-antigen interaction, to reveal the actual epitope and to understand the underlying mechanisms, glycoreplica peptides are priceless tools to reproduce glycans properties. Indeed, obtaining pure and homogeneous carbohydrates (or glycoconjugates) is a difficult task, sometimes a mission impossible, also because of their poor stability. Secondly, both oligosaccharides and peptides are targets of antibodies, but while the immune response stimulated by glycoconjugates is negligible and high-affinity carbohydrate-specific antibodies are not easily obtained, peptide sequences are able to elicit a stronger and more specific reaction [20]. Finally, cross-reactivity between peptides and glycans is of paramount importance to understand immune-mediated responses and molecular mimicry mechanisms.

Why glycoreplica peptides?

An epitope is defined as the portion of the antigen interacting with the paratope of the antibody. This definition is strictly operational [21] and includes the concept of cross-reacting epitopes, i.e., two different molecules recognized by the same antibody (Fig. 1). At the immunological level, the concept of “molecular mimicry”, underpinned by this definition, has been used to explain the break of immunological tolerance leading to autoimmune disorders. However, despite the description of several examples of human antibodies recognizing cross-reactive epitopes, i.e., both non-self sequences of viral or bacterial proteins and “self” peptides belonging to human proteins, few detailed molecular analysis of these “cross-reactive epitopes” have been reported. Nevertheless, several “peptide mimetics” have been used as immunological probes in diagnostic applications.

A molecule, such as a peptide, which mimics the structure of an epitope is called “mimotope”. This term was coined by H.M. Geysen in 1986 referring to peptide sequences causing an antibody response similar to the one elicited by the discontinuous protein antigenic determinant [22]. Glycans are now emerging as preferred epitopes being involved in many processes such as cell-cell communication and pathogenic recognition. Because of the intrinsic difficulty in obtaining pure carbohydrate derivatives both by isolation from a natural source and via chemical, and/or enzymatic synthesis, alternative strategies to experimentally reproduce and investigate glycan-protein interactions are challenging.

With the general term “glycomimetics” are commonly designated those synthetic molecules or constructs that have chemical structures based on natural carbohydrates, but presenting more or less significant variations, especially around the anomeric carbon and the type of linkages that constitute the backbone [23-25].

Alternatively, when a peptide scaffold alone is used to reproduce the biological activity of a glycan, no matter its complexity, the term carbohydrate-mimetic peptides (CMPs) is used as synonymous of glycoreplica peptides. The latter term, introduced by Taki [26] perfectly emphasizes the desirable simulation of immunological features of carbohydrate epitopes, despite the intrinsic chemical distance between the two families of biomolecules.

Whatever the preferred name to use is, the exploitation of peptides reproducing carbohydrate-antibody interactions holds the great potential to answer many unresolved questions in chemical immunology. Compared to glycans, peptides are more accessible to produce and to modify, i.e., efficiently managing their molecular structure to fine-tuning biological activity and stability. Rational design of mimetic molecules as antibody binders can include the incorporation of non proteinogenic amino acids and altered backbones, providing an expanding list of different peptidomimetics, such as peptoids, β-peptides, retro-inverso peptides, etc. [27-29].

The recent advances both in chemical peptide synthesis by efficient solid-phase techniques [30,31] and in biotechnological strategies to study peptide-protein interactions via phage-display libraries [32] have inaugurated a new promising era for implementation of glycoreplica peptides.

This review focuses on recent progress in peptide replica identification and use, and aims to present a brief summary of some relevant examples in which peptides are proposed for the diagnosis and/or treatment of immune-mediated pathologies in place of sugars.

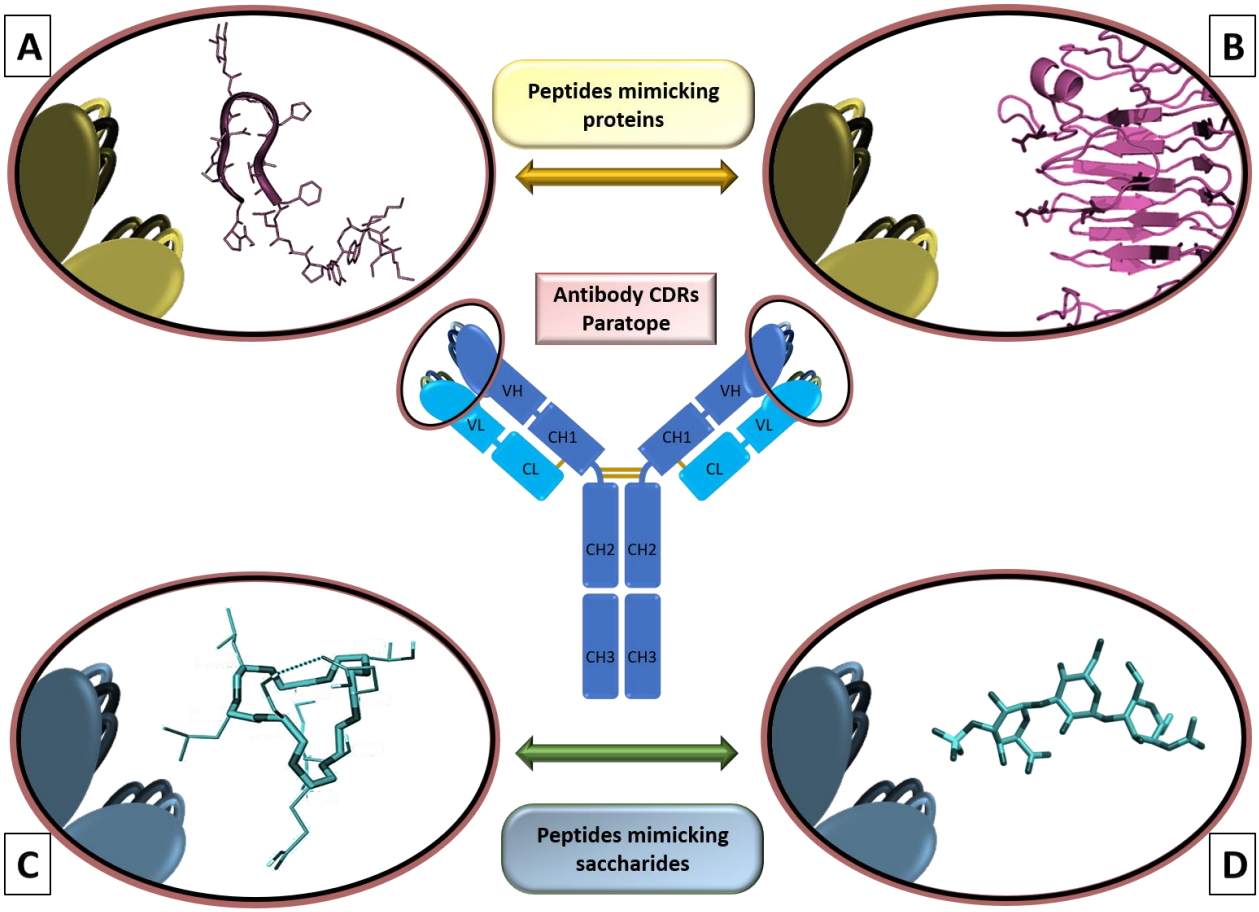

Figure 1 – Cartoon representing an antibody and its paratopes. The antibody binds the epitope antigen through the Complementarity Determining Regions (CDRs). Two structurally unrelated molecules can be recognized by the same antibody, as in the case of the synthetic probe CSF114(Glc) (A) cross-reacting with bacterial hyperglucosylated HMW1 adhesin (B) and a cyclic octapeptide (C) that binds the murine monoclonal antibody specific for HNK-1 trisaccharide (D).

Mechanism investigations and clinical relevance of molecular mimicry

The initial host response to a viral or microbial infection can alternatively produce a cross-reaction with an appropriate host-antigen, leading to a "molecular mimicry" mechanism that may degenerate in an autoimmune disease. Molecular mimicry was firstly postulated because of the evidence of existing cross-reactivities between exogenous elements and host 'self' determinants while generating monoclonal antibodies towards viral proteins [33]. Other epidemiologic and clinical evidence, such as infections often preceding autoimmune diseases or homozygote twins seldom suffering from the same autoimmune disorders, also contributed to developing the hypothesis of a mimicry mechanism. In fact, many pieces of biological evidence support the existence of a mismatch between exogenous pathogen antigens and "self" cellular components. Therefore, molecular mimicry is highly

contemplated as the primary cause for many autoimmune pathologies and is a proof-of-concept to uncover its etiologic agents [34].

Among the many pathogen-induced autoimmune diseases, only a few of them has been directly associated with bacterial glycosylation patterns, and yet for most of these the corresponding human antigen has not been depicted. Nevertheless, among the identified bacteria producing surface glycoconjugates that mimic host structures, there are many pathogenic organisms possibly involved in autoimmune diseases, such as *Campylobacter jejuni, Helicobacter pylori,* and *Haemophilus influenza* [10]. In our experience, antibodies specific to an N-glucosyl asparagine (N-Glc) glycopeptide, CSF114(N-Glc) were identified in sera of an MS patient subpopulation [35-36]. Turning our attention to non-typeable *Haemophilus influenzae*, expressing cell-surface adhesins including N-Glc [37], we started establishing a connection between *H. influenzae* infection and MS. The expression of N-Glc protein antigens using the biosynthetic machinery from *H. influenzae* and *A. pleuropneumoniae* allowed to isolate purified antibodies from MS patient sera cross-reacting with anti-CSF114(N-Glc) antibodies. Therefore, the groundwork was established for determining the nature of the molecular mimicry mechanism, and for elucidating the human protein target(s), which could be cryptic mimics recognized by anti-hyperglucosylated adhesin antibodies in Multiple Sclerosis [19].

When bacterial saccharides mimicking host peptides elicit an immune response leading to anti- host-protein antibody production, the result is the onset of autoimmunity. This effect was proven for autoimmune thyroid diseases [38]. Specifically, linear α-1-6 glucans that are produced by the probiotic bacterium *Bifidobacterium bifidum* were found to be cross-reactive with human serum thyroid peroxidase and thyroglobulin autoantibodies, providing evidence of a potential role of α-1-6 glucan that is produced by bacteria in generating autoimmune thyroid diseases [38].

Noteworthy, also in the case of SLE and AR diseases the sera antibodies were found to target exogenous agents-derived glycans [39-41].

However, despite the description of several examples of human antibodies recognizing cross-reactive epitopes, i.e., both non-self sequences of viral or bacterial proteins and self-peptides belonging to human proteins, few detailed molecular analysis have been reported. Nevertheless, several “peptide mimetics” have been proposed as immunological probes in diagnostic applications [42]. The discovery that foreign antigens and especially glycoconjugates cross-react with host elements led to the development of peptide replica. These mimetics may be very precious at three different levels:

1) to understand the etiology of the disease and to find the host epitope that causes the immune tolerance disruption;

2) as probes for diagnostic applications and to monitor the disease activity;

3) to produce peptide drugs either blocking glycan-protein interactions or stimulating the immune system for vaccine purposes.

Phage-display libraries for glycotope identification

The development of phage-display techniques can be considered the milestone in the field of epitope discovery, giving access to the high throughput production of peptide libraries to screen protein-protein interactions and antibody-antigen recognition.

In phage display, a gene encoding a specific protein sequence is artificially inserted into the phage coat protein gene, resulting in the protein or peptide expression on the outer surface of the bacteriophage. The 2018 Nobel Laureate in Chemistry George P. Smith was the first to describe this approach in 1985, when recombinant peptide sequences were produced by fusing the peptide of interest onto gene III of filamentous phage [43]. Thanks to this approach, the expression of exogenous proteins joined to coat proteins on the surface of the phage is currently the preferred method to investigate the immunogenic properties of selected putative antigenic molecules [44,45].

Bacteriophages are viruses that can affect only bacteria such as *E. coli* and nowadays several types of phage are accessible for display experiments [44]. A typical workflow of display starts with the amplification of recombinant phagemids encoding for a specific peptide sequence: once transformed into *E. coli*, the recombinant plasmids are replicated, translated and assembled into infective phages with the peptide fused to coating proteins on the surface. Phagemids displaying process gives access to a large peptide library, which is then screened for the phage binding to a target molecule such as antibodies, lectins or receptors, through a technique called biopanning [45,46]. (Fig. 2) The process consists of multiple rounds of phage binding to the molecule of interest that is stuck on a plate, washings, elutions, and reamplification in *E. coli*. During each round, specific binders are selected out from the pool and characterized through DNA sequencing to determine interacting peptide strands. Indeed, the generation of a large peptide library and efficient screening are crucial for the isolation of "hit sequences" to be used for peptide mimics of sugars [26]. Peptides selected by phage display technology often require further modifications to adjust biochemical properties and ease the analysis of binding [47].

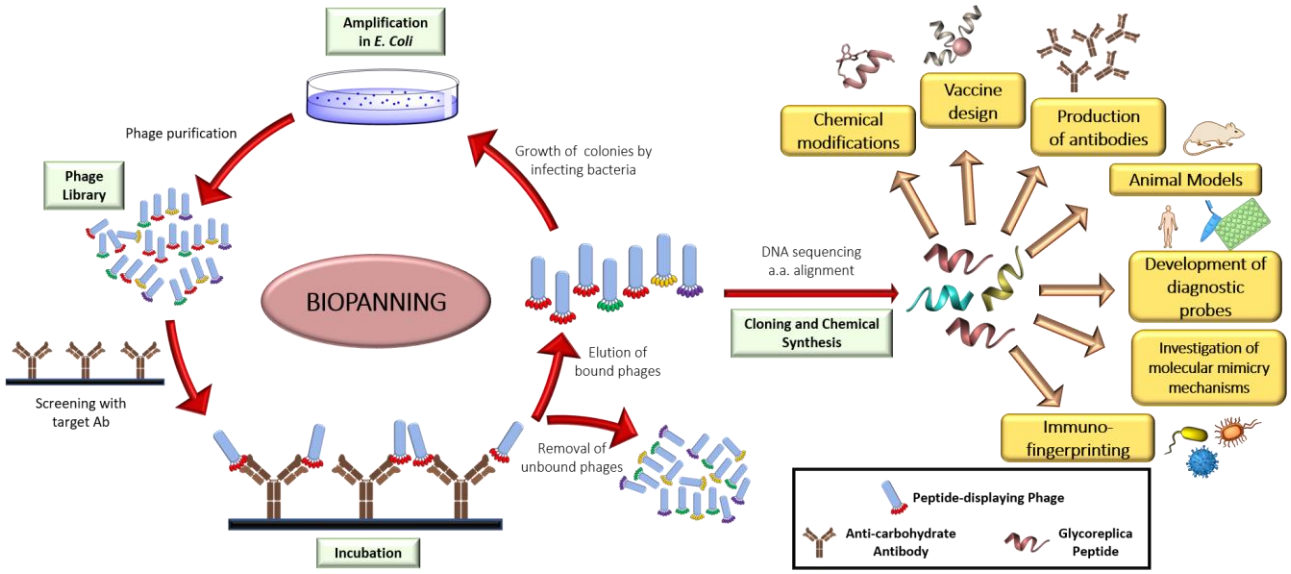

Figure 2 – Schematic representation of biopanning with phage displayed peptide libraries using immobilized anti-carbohydrate antibodies to obtain glycoreplica peptides. Selected peptide hits can be used for several applications including the screening of serum samples from patients with candidate diseases.

Structural studies

The structural determination of the binding mode at the paratope site allows the rational improvement of the immunological features. Therefore studying antibody recognition patterns is pivotal to develop more efficient glycoreplica peptide-based probes and drugs for autoimmune diseases.

Structural characterization of peptide mimicry of carbohydrates is not an easy task, primarily because of the high flexibility of both carbohydrates and peptides that hampers the achievement of high-resolution X-ray structures of complexes [42]. However, the experimental analysis carried out by X-ray or NMR techniques have elucidated that a three-dimensional structural resemblance between sugar and amino acid sequences is not particularly evident even when they bind to the same site of a protein [48-50].

Another route to unravel the driving forces and structural features in molecular mimicry is the use of *in silico* approaches such as molecular docking [42,51,52].

The mode through which the peptides are able to mimic carbohydrates is still debated, or at least experimental evidence reveals that they seldom share interacting moieties when in contact with the protein.

Noteworthy, the hydroxyl-containing amino acids serine and threonine are not the most representative residues in previously identified carbohydrate-mimetic peptides [42]. Also charged amino acids, which should be able to establish the most robust interactions through ionic bonds, and small aliphatic

amino acids, particularly alanine, valine, and isoleucine, appear in limited numbers among the previously identified peptide replica. On the other hand, aromatic moieties such as tryptophan, histidine, and tyrosine side-chains are considered much more relevant to establish Van der Waals interactions increasing the binding properties [42].

This evidence suggests that mimicry of the polyhydroxylated glycan moieties is not a requirement for a successful functional mimic. It is more likely that the three-dimensional structure in which a peptide is presented to a target and its ability to replicate the overall shape of the carbohydrate may be more important than the chemical functionalities of the peptide itself. This is the case of peptides mimicking GD2 ganglioside [48]. High-resolution crystal structures revealed that a model antibody binds the ganglioside primarily through an "induced fit mechanism", *via* an extended network of direct and solvent-mediated hydrogen bonds. Instead, the two investigated glycoreplica peptides (RCNPNMEPPRCWAAEGD and VCNPLTGALLCSAAEGD) utilize a "key and lock" interaction mechanism, establishing important hydrophobic contacts. Although the general disposition of the main chain of both peptides imitates the overall shape of the ganglioside saccharide moiety in the epitope binding cavity, the detailed interactions differ significantly among each of the three molecules (Fig. 3).

Johnson and Pinto [53] adopted the distinction between structural mimicry, due to the appropriate three-dimensional arrangement of the interacting groups on the target site, and functional mimicry, whereby the peptide utilizes an alternative binding mode *via* a different set of contacts compared to the carbohydrate. Examples of both cases are reported [53] however as long as the biophysical elicited response is the same, the proof-of-concept of glycoreplica peptides as immunological probes should not be affected by this distinction.

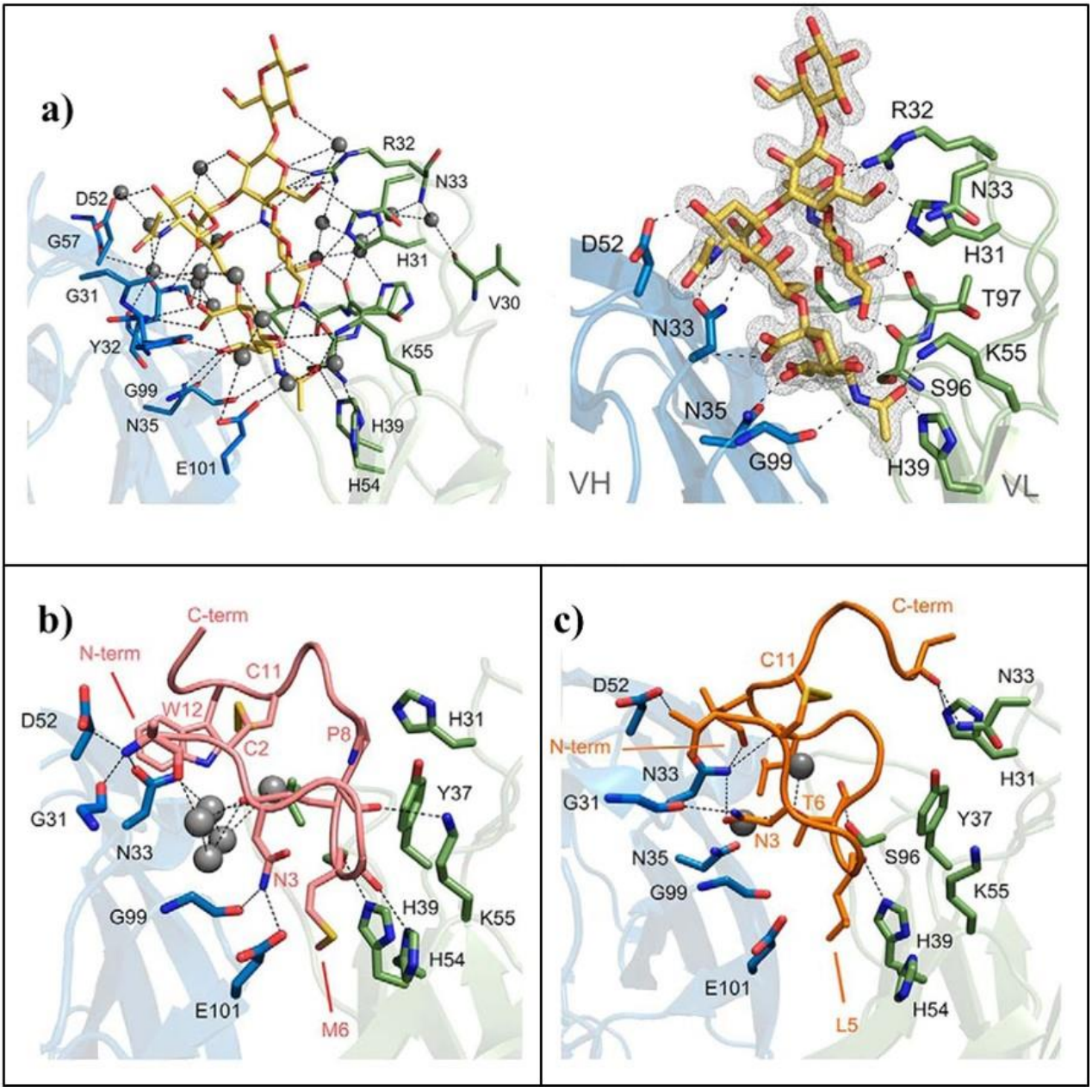

Figure 1 – Spatial views of GD2 ganglioside and peptide mimetics binding mode at the paratope of antibody 14G2a. GD2 (a, PDB code: 4TUO) binds mainly via direct and solvent-mediated hydrogen bond interactions while peptide sequences RCNPNMEPPRCWAAEGD (b, pink, PDB code: 4TUJ) and VCNPLTGALLCSAAEGD (c, orange, PDB code: 4TUL) bind through both hydrogen bond and hydrophobic interactions, although differently one from the other. The heavy chain interacting residues of the antibody are represented as blue sticks, while the ones belonging to the light chain are represented in green. Hydrogen bonds are shown as black dotted lines and water molecules as grey spheres. Adapted from [48].

Peptide replica and autoimmune diseases

Since the pioneering reports proving that not exclusively sugars are capable of binding lectins [54] and the identification of the first carbohydrate mimetic peptides [55,56], several examples of peptides interacting with lectin or anti-glycan antibodies have been described and already reviewed, mainly for antimicrobial drug discovery and vaccine development [57-59].

Most of these peptide sequences are mimetics of bacteria-derived glycans, providing a further confirmation that after an infection peptide-sugar cross-reactivity may be pivotal in the disruption of immune tolerance, resulting in the attack of the host tissues and cells by the immune system components. For example, different species of *Streptococcus* have been associated to many autoimmune disorders, such as Sydenham's chorea, PANDAS, SLE and other rheumatic diseases [107-109].

However, although in many cases peptide-sugar crosstalk has been demonstrated, not necessarily the peptide sequence appears to be related to specific human proteins. This is possibly due to the fact that peptide sequences have been selected by phage displayed peptide libraries (Table 1).

Table 1 – Examples of peptide sequences that bind anti-carbohydrate antibodies mimicking glycans, glycolipids or glycoproteins that might be related to autoimmune diseases. n.r. = not reported.

Mimetic Peptide Sequence	**Mimicked Carbohydrate or Glycoconjugate**	**Carbohydrate-related autoimmune diseases**
TPRVERN(Glc)GHSVFLAPYGWMVK [35]	Hyperglucosylated HMW1ct, adhesive protein of non-typeable *Haemophilus Influenzae*	Multiple Sclerosis [19]
FLHTRLFVSDWYHTP [60] *c*-(LSETTDL), *c*-(RTLPFS) [61] *c*-(LSETETK(Ac)DL) [62]	HNK1 - epitope	IgM monoclonal gammopathy, anti-MAG neuropathy
KWTNLPP [63]	Asialo-GM1	Multifocal motor neuropathy (MMN) [64]
IPQVWRDWFKLP [65]	GM1	Chronic inflammatory demyelinating polyneuropathy (CIDP), acute motor axonal neuropathy (AMAN), MNN and other Guillain Barré subsyndromes [64] [66] [67]
LEICSYTPDEGC, RRPKDLDKNM [68]	Neu5Gc-GM3	n.r.
WHWRHRIPLQLAAGR [69]	GD1α	AMAN and acute motor–sensory axonal neuropathy [64] [13]
CGRLHLVPDLEC [70] EDPSHSLGLDVALFM [71] LDVVLAWRDGLSGAS [72] RCNPNMEPPRCWAAEGD VCNPLTGALLCSAAEGD [73]	GD2	Chronic ataxic neuropathy [64]
LAPPRPRSELVFLSV [74]	GD3	Chronic ataxic neuropathy [64]

NMMRFTSQPPNN, NMMNTIMDPRTH [75]	LOS of non-typeable *Haemophilus Influenzae*	Multiple Sclerosis
SMYGSYN, APARQLP [76]	LOS of group B *Neisseria meningitidis*	n.r.
CSRLNYLHC [77] GGIYWRYDIYWRYDIYWRYD GGIYYRYDIYYRYDIYYRYD [78] APWLYGPA [79]	Lewis Antigen series (Le^x, sLe^x, Le^y)	n.r.
FHENWPS, FHEFWPT [80]	α-Gal antigenic epitope	Graves' Disease and "autoimmune-like" Chagas' Disease [81]
FHENWPS [82]	β-1,2-oligomannosides of *Candida Albicans*	n.r.
WENWMMGNA, FDTGAFDPDWPA [83] NPDHPRVPTFMA, LIPFHKHPHHRG [84] GEASGLCCRWSSLKGC [85] NKVIWDRDWMYP [86] CGAVIDDC [87]	Capsular polysaccharides of *Streptococcus agalactiae (Group B Streptococcus, GBS)*	n.r.
SGQARVLYSEFINAL [88] FHLPYNHNWFAL [89]	Capsular polysaccharides of *Streptococcus pneumoniae* (various serotypes)	n.r.
DRPVPY [90]	Cell-wall polysaccharide of *Streptococcus pyogenes*	n.r.
CSSVTAWTTGCG [91] DYAWDQTHQDPAK [92] RGDKSRPPVWYVEGE [93] [8]EQEIFTNITDRV [84] GFSYYRPPWIL [94]	Capsular polysaccharide of *Neisseria meningitidis*	n.r.
YKPLGALTH, KVPPWARTA [95] YLEDWIKYNNQK [49]	O-antigen of lipopolysaccharide of *Shigella flexneri*	n.r.
GTHPXL [96]	GPI-linked proteophosphoglycan antigens of *Entamoeba histolytica*	n.r.
QEPLMGTVPIRAGGGS [97]	Neutral polysaccharides of *Mycobacterium tuberculosis*	n.r.
ISLTEWSMWYRH [98]	Mannosylated Lipoarabinomannan of *Mycobacterium tuberculosis*	n.r.
CYLPFQLSC; CHPLFDARC [100]	Exopolysaccharide (EPS) of *Burkholderia pseudomallei*	n.r.
WTEIHDWEAAME [101]	Lipopolysaccharide of *Brucella melitensis* and *Brucella abortus*	n.r.
APAIPAS [102]	Lipopolysaccharide of *Vibrio cholerae*	n.r.
AEGEFSPGVWKAAFQGDKLPDPAK [103] NHNYPPLSLLTF [104] ECLLLSKYCMPS, SMCMHGGAYCFP [105]	Capsular polysaccharide of *Vibrio cholerae*	n.r.

FGGETFTPDWMMEVAIDNE SYSWMYE, GLQYTPSWMLVG [106]	Glucuronoxylomannan (GXM) of *Cryptococcus neoformans*	n.r.

However, whereas applications of glycoreplica peptides in tumor and antimicrobial therapy have been largely investigated, their direct use in the field of autoimmune diseases remains relatively unexplored. Nevertheless, in the framework of the exploitation of peptide probes to determine the presence of autoantibodies in sera, hence providing a diagnosis of an ongoing autoimmune pathology, and eventually to trap and remove pathogenic autoantibodies [110], the synthetic accessibility of peptide replica holds great potential compared to sugars. Once established the immunological connection between a peptide sequence and a glycan autoepitope, the peptide-based screening of sera would allow a much more compelling alternative for the investigation of the specific pathology.

From an immunological point of view, the trisaccharide human natural killer-1 (HNK-1) epitope, expressed in the nervous system, is an interesting carbohydrate target associated with PNS autoimmune disorders, namely peripheral demyelinating neuropathy, which relates to IgM paraproteinemia (monoclonal gammopathy). This trisaccharide HNK-1 is a unique carbohydrate epitope containing a sulfated glucuronic acid in a non-reducing terminus (Fig. 4) reacting with serum autoantibodies in anti-MAG (myelin-associated glycoprotein) IgM antibody-associated neuropathy patients and hence considered the native autoantigen [111]. Since both the chemical synthesis and the isolation from natural sources of HNK-1 appear extremely difficult and time-consuming [112], often leading to inconsistent material, the development of glycomimetics of this carbohydrate is indeed a desirable goal for diagnostic and clinical investigation. Some HNK-1 peptide mimetics were identified by screening a phage-displayed peptide library with a rat monoclonal antibody [60]. Subsequently, peptide hits optimization provided two cyclic sequences, *c*-(Leu-Ser-Glu-Thr-Thr-D-Leu) and *c*-(Arg-Thr-Leu-Pro-Phe-Ser), that exhibited enhanced functional activity as HNK-1 mimetics. In fact, these two hexapeptides were shown to bind murine anti-HNK-1 in the high micromolar range and to stimulate neurite outgrowth in mouse models [61,113]. Peptides mimicking the minimal epitope recognized by the commercially available monoclonal antibody could be used for the development of a novel and reliable diagnostic tool for anti-HNK-1 antibody identification in sera of patients affected by autoimmune neurological disorders. Therefore, further structure-activity relationship (SAR) studies, based on the measurement of binding affinities by surface plasmon resonance (SPR) were recently conducted to rationally optimize the binding affinity [62]. The optimization led to the selection of the cyclic octapeptide *c*-(Leu-Ser-Glu-Thr-Glu-Thr-Lys(Ac)-D-Leu) as the best candidate to reproduce the trisaccharide immunological features. (Fig. 5) Unexpectedly when peptide mimics are “equipped” with the native saccharide features the activity toward antibody decreases, meaning that mimicry efficiency is due to a complex mixture of precise

traits that cannot be easily predicted. However, preliminary immunoenzymatic assays indicates that patient sera do not specifically recognize the octapeptide although a larger sera screen is needed to fully assess the immunological features of the various HNK-1-replica peptides. Only mouse monoclonal antibodies are recognized [62]. Therefore, monoclonal antibodies obtained from animal models and used to select and optimize immunological probes for the detection of clinically relevant human autoantibodies could be misleading.

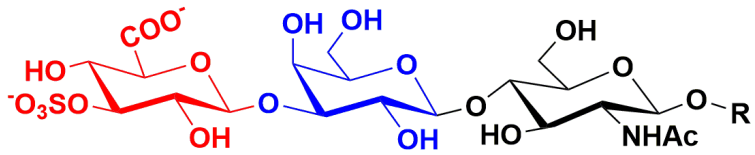

Figure 2 – Structure of the terminal trisaccharide epitope HNK-1 (HSO_3-3GlcAß1-3Galß1-4GlcNAcOR), that is expressed in structurally distinct glycans linked to R, i.e., a glycan, but also a lipid or a specific amino acid in a protein.

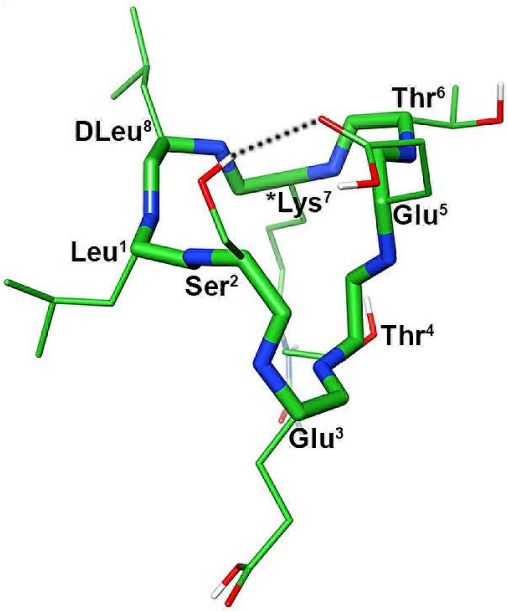

Figure 3 – NMR-derived lowest-energy conformer of cyclic peptide *c*-(Leu-Ser-Glu-Thr-Glu-Thr-Lys(Ac)-D-Leu). The black dotted line indicates the hydrogen bond between the Ser^2 hydroxy and Glu^5 carboxyl groups. C: green, N: blue, O: red , H: not shown. *Lys: acetyl-lysine. Adapted from [62].

Nucleotide mimics

It is interesting to note that not only glycans but also nucleotides (naturally containing ribose or deoxyribose) appeared to be mimicked by peptides. This ability could be also valuable in autoimmune

disease research. As a noteworthy example, a synthetic peptide isolated by the phage display technique and cross-reacting with anti-dsDNA antibodies derived from patients with SLE was identified in 1997 [114]. Antibodies to dsDNA are diagnostic for SLE, and their deposition in patients kidneys causes glomerulonephritis, because of the cross-reactivity between anti-dsDNA antibodies and renal antigens. This surrogate peptide antigen (DWEYS) functions as dsDNA mimetic and as an antagonist for pathogenic antibodies. Immunization with the more extended sequence DWEYSVWLSN was proven to increase serum anti-dsDNA antibody levels in mice, hence inducing a lupus-like autoimmune condition [115]. Autoreactive B cells were subsequently identified in a mouse model of lupus and in the peripheral blood of patients with SLE after the injection of a fluorescent derivative of this peptide, allowing visualization, characterization, and isolation of dsDNA-reactive B cells [116,117]. Moreover, some unrelated sequences were described as mimotopes of DNA and used to block autoantibody aggregation in mice models [118,119], thus confirming the great heterogeneity displayed by peptide mimic ability. The potential of peptide replica to provide diagnostic tests of SLE and to investigate the origin of anti-DNA antibodies and their interaction with polynucleotides is indeed of tremendous interest.

Conclusions

Investigating glycan-peptide mimicry (and *vice versa*) in the context of autoimmunity is an emerging topic, pointing toward the multiple roles that glycans of bacterial origin may play in autoimmune diseases.

Currently, many common autoimmune diseases have no cure. The major problem consists in the lack of an unequivocal known antigen associated to each specific disease. The identification and reproduction of conformational epitopes involved in antibody recognition is still a challenge, particularly when sugars are involved. In this direction, synthetic peptides are instrumental in developing immunosensors for biomarker detection, i.e., specific autoantibodies, with improved affinity and specificity.

From a mechanistic point of view, a significant breakthrough would be finding the immunological correlation between glycans derived from exogenous agents and displayed peptide sequences that could be ascribed to native protein structures.

For diagnostic purposes, gaining access to univocally characterized epitope-mimicking peptides will provide a crucial improvement for the screening of patient sera to detect and characterize autoantibodies.

Finally, the selective isolation of the autoantibodies will have a great impact on therapeutic applications. Although many of the reported glycoreplica peptides have been successfully tested in

animal models, to the best of our knowledge few data about their possible application in human samples have been reported. Nevertheless, developing antigen-specific therapies based on glycan mimotopes in different autoimmune diseases could contribute in the next years to stimulate the emerging field of chemical immunology. Uncovering novel epitopes and the regulatory mechanisms inducing immunity dysregulation might help ultimately to cure or even prevent autoimmune diseases.

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