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Glycopeptides as synthetic antigenic probes for MOG Antibody-Associated Disease

UNIVERSITÀ DEGLI STUDI FIRENZE

PeptLab INTERDEPARTMENTAL RESEARCH UNIT OF PEPTIDE AND PROTEIN CHEMISTRY AND BIOLOGY

DICUS DIPARTIMENTO DI CHIMICA "UGO SCHIFF"

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Introduction

Myelin-oligodendrocyte glycoprotein antibody-associated disease (MOG-AD) has been recently identified as an antibody-mediated demyelinating autoimmune disorder of the CNS, distinct from multiple sclerosis (MS) of which it was long considered a subtype, due to some clinical and radiological overlaps. However, a specific diagnosis is essential due to the different clinical features, treatment considerations, and prognoses compared to MS[1].

Autoantibodies targeting myelin-oligodendrocyte glycoprotein (MOG), in particular anti-MOG IgGs, are considered biomarkers of the disease. Cell-based assays are (CBAs) the recommended method for testing anti-MOG antibodies, according to international consensus[2]. Despite being considered the gold standard, CBAs have not yet been standardized, and low positive results show poor agreement. Moreover, CBAs are time-consuming and require expertise both in the interpretation of results and in working with cells[3].

Conversely, **ELISAs** are user-friendly, cost-effective, and, with the potential to be automated, are ideal for simultaneous screening of larger numbers of patients[3]. However, ELISAs using linear or refolded recombinant full-length MOG have thus far given inconsistent results.

Myelin-oligodendrocyte glycoprotein (MOG) is a 218 amino acid glycoprotein (molecular mass 26–28 kDa). MOG is uniquely expressed on the surface of the myelin sheath, specifically on oligodendrocytes membrane, in the central nervous system (CNS), but it is a quantitatively minor component of myelin (0.05%).
The biological role of MOG is still unknown. The N-terminal region of MOG forms an Ig-V fold consisting of two antiparallel β-sheets. It also presents one N-linked glycosylation site on asparagine 31[4].
MOG is known to be an encephalitogenic protein that can elicit a demyelinating immune response in numerous experimental models of inflammatory demyelinating diseases.
Conflicting results have been obtained when using full-length MOG in ELISAs to test MS patient sera.

Myelin-oligodendrocyte glycoprotein
PDB: 1PKO

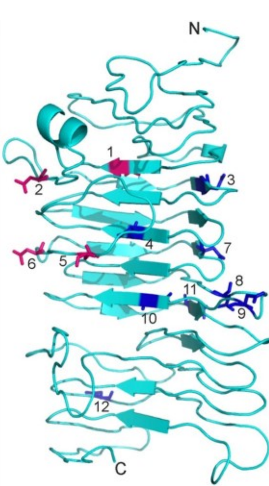
Protein **post-translational modifications** (PTMs), such as phosphorylation, glycosylation, citrullination, and ubiquitination, are essential for increasing proteome diversity and regulating protein activity, structure, localization, and interactions, thus influencing numerous vital biological processes. However, dysregulation of the spontaneous or enzymatic reactions that generate PTMs, triggered by processes such as inflammation, can cause a lack or abundance of modifications that lead to the generation of neoantigens, that can be targeted by antibodies, generating an autoimmune response[5,6].

In this context, recombinant or extracted protein antigens often used in immunoassays may not reproduce the correct modifications, as well as the correct folded conformation, essential for antibody recognition.

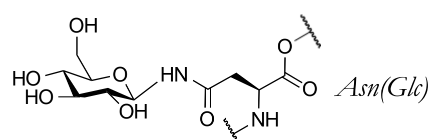
On the other hand, synthetic **peptides** can be produced as specific chemical entities in high quality and quantity through standard and reproducible solid-phase synthesis protocols. SPPS also enables the straightforward introduction of modifications in the sequence to mimic the native epitope, using non-standard, modified amino acids corresponding to the target PTM.

Non-typeable *Haemophilus influenzae* adhesin

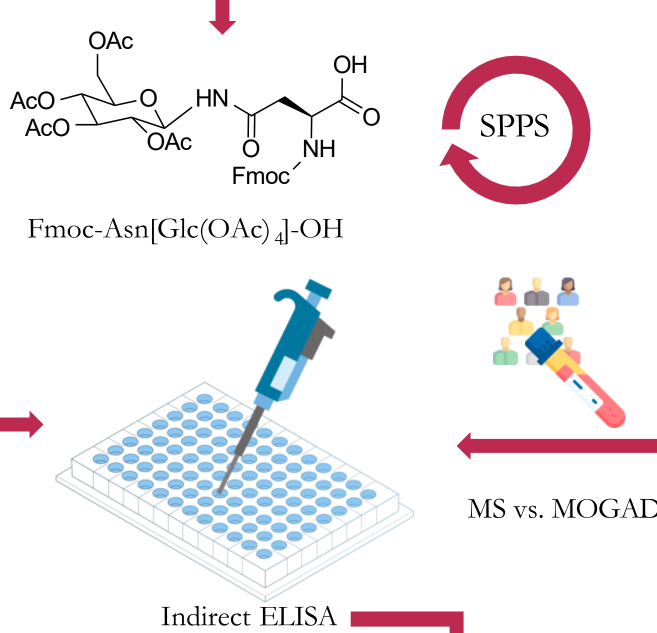
The Asn(Glc) modification is virtually absent in humans but is found in prokaryotes in surface proteins such as adhesins. It was found that a hyperglucosylated protein domain from adhesin protein of nontypeable *Haemophilus influenzae* (NTHi) termed HMW1, is preferentially recognized by antibodies from sera of an MS patient subpopulation, supporting the hypothesis of the involvement of an infection by an exogenous pathogen in MS pathophysiology[7].



I-TASSER model of HMW1ct



Aim of the work



	Peptides	Sequence
1	hMOG(25-55)	RISPGKNATGMEVGWYRPPFSRVVHLYRNGK
2	[N[31]]hMOG(25-55)	RISPGKN[31](Glc)ATGMEVGWYRPPFSRVVHLYRNGK
3	[N[53]]hMOG(25-55)	RISPGKNATGMEVGWYRPPFSRVVHLYRN[53](Glc)GK
4	[N[31],N[53]]hMOG(25-55)	RISPGKN[31](Glc)ATGMEVGWYRPPFSRVVHLYRN[53](Glc)GK

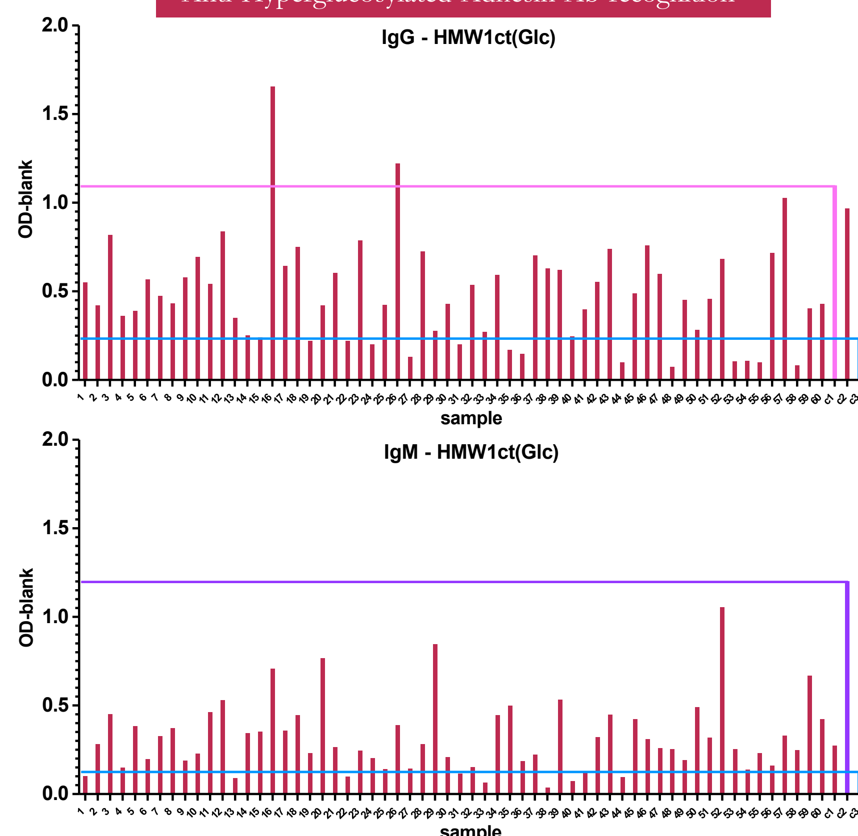
Cleavage, purification and characterization of peptides

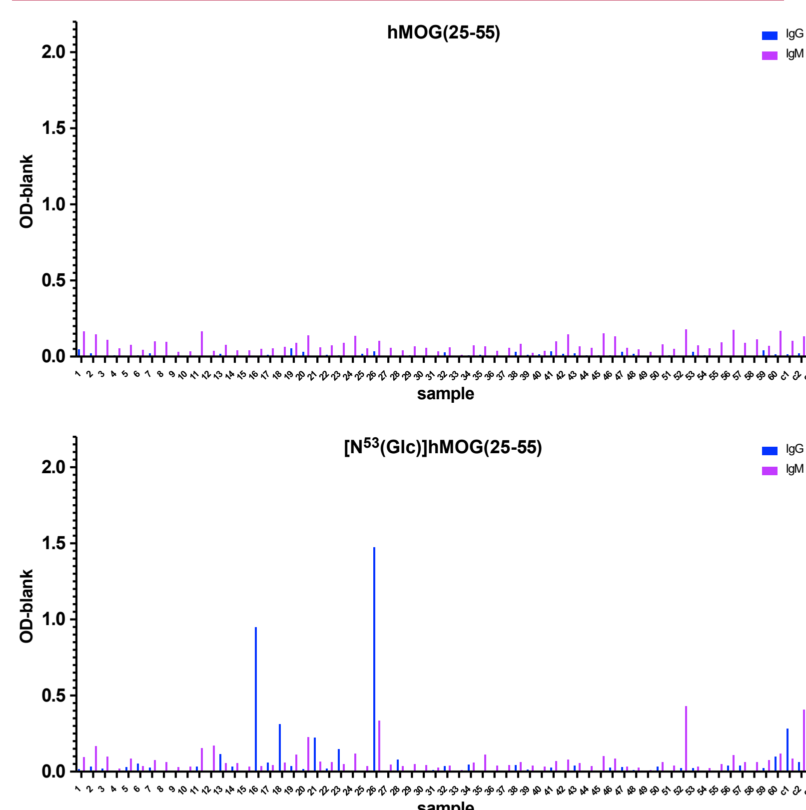
Peptides	HPLC R_f (min)	HPLC purity (%)	ESI-MS (m/z) found (calcd)
1	4.28	>95	1192.89 (1192.63)
2	3.88	>95	1245.69 (1245.98)
3	3.62	>95	1245.56 (1245.98)
4	3.75	>95	1300.90 (1300.99)

Peptide characterization by RP-HPLC-MS on Waters Alliance™ Chromatography system (Separation Module 2695, Detector Diode Array 2996) equipped with an online single quadrupole ESI-MS Waters Micromass ZQ using a Supelco BIOshell A160 Peptide C18 (10 cm x 3.0 mm, 2.7 µm) column. Analytical HPLC flow at 0.6 mLmin^{-1}; temperature 35 °C. Eluents: 0.1% (v/v) TFA in H_2O (A) and 0.1% (v/v) TFA in ACN (B), λ 215 nm. 10-90% gradients of B solvent were performed in 5min. ESI-MS: detected as $[M+3H]^{3+}$.

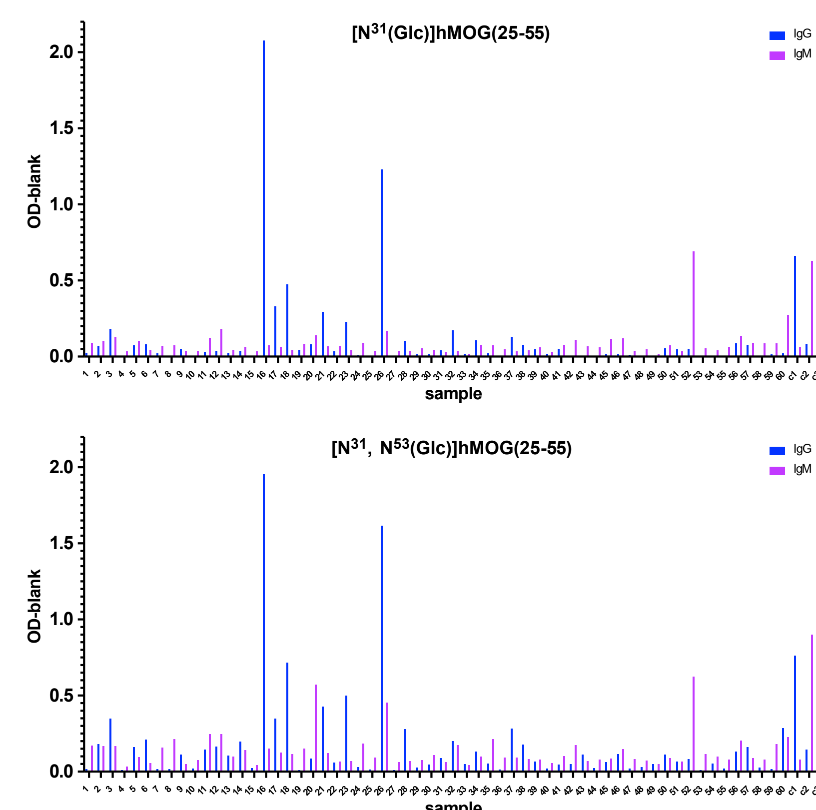
Preliminary results on MS patient sera

Anti-Hyperglucosylated Adhesin Ab recognition





Role of N-glucosylation in Ab recognition



Conclusions and future perspectives

- ✓ Confirmed preferential recognition of N-glucosylated human MOG peptides over the non-glucosylated ones in a subpopulation of a new cohort of MS patients, from which MOG-AD patients are excluded according to recent classification criteria.
- ✓ Confirmed recognition of hyperglucosylated adhesin of non-typeable *Haemophilus influenzae* in a new cohort of MS patients, from which MOG-AD patients are excluded according to recent classification criteria.

- ► Comparative evaluation of the results obtained with MOG-AD patient serum following equal protocols to study the role of N-glucosylation in antigen-antibody recognition and optimize the synthetic peptide antigenic probes for specific recognition of MOG-AD patients.
- ► Comparative evaluation of the results obtained with MOG-AD patient serum following equal protocols to determine whether non-typeable *Haemophilus influenzae* could be involved in MOG-AD pathogenesis.

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Acknowledgments

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