N-glucosylated peptides of NogoR and OMGp proteins cross react with anti-hyperglucosylated adhesin of non-typeable *Haemophilus influenzae* antibodies in Multiple Sclerosis

Quagliata, Michael[1,2], Feliciana, Real-Fernandez[1,3], Francesca, Nuti[1,2], Rovero, Paolo[1,4], Papini, Anna Maria[1,2]

1 Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology (Peptlab), University of Florence
2 Department of Chemistry "Ugo Schiff", University of Florence (DICUS), Via della Lastruccia 3, 50019, Sesto Fiorentino, Italy
3 Institute for the Chemistry of Organometallic Compounds (CNR-ICCOM), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy.
4 Department of Neurosciences, Psychology, Drug Research and Child Health Section of Pharmaceutical Sciences and Nutraceutics (NeuroFarBa), University of Florence,Via Ugo Schiff 6, 50019, Sesto Fiorentino, Italy

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS) whose etiology, up to date, still remain unclear. Genetic and environmental factors can contribute to this complex disease. Bacterial and/or viral infections are more and more accepted to be involved in triggering the immune response and therefore disease onset. In fact, a molecular mimicry mechanism between bacterial and myelin proteins could explain the aberrant immune response leading to non-self-recognition of self-epitopes in myelin proteins. Moreover, bacterial infections could promote aberrant modifications of CNS myelin proteins causing disruption of self-tolerance. In the case of MS our group has been investigating for more than 15 years the involvement of *N*-glucosylation of asparagines (N-Glc). Recently, we have shown that the hyperglucosylated protein HMW1ct($Glc_{7,8,9}$), expressed by non-Typeable Haemophilus influenzae (NTHi) has up to 9 beta-turns exposed with the N-Glc motif and recognizes specific antibodies in MS patient sera. These antibodies cross-react with a structure-based designed N-Glc beta-turn synthetic antigenic probe termed CSF114(N-Glc). By a bioinformatic approach, peptides of myelin proteins i.e., Factor associated with neutral sphingomyelinase activation (FAN), Oligodendrocyte Myelin Glycoprotein (OMGp), and Nogo Receptor (NogoR) were selected because of structural and sequon homology with CSF114(N-Glc). We present herein that N-Glc peptides [N^{641}(Glc)]FAN(635−655), [N^{179}(Glc)]NogoR(173−191), and [N^{192}(Glc)]OMGp(186−204) recognise anti-hyperglucosylated adhesin antibodies. Interestingly, monoglucosylated peptides [N^{179}(Glc)]NogoR(173−191) and [N^{192}(Glc)]OMGp(186−204) were demonstrated to inhibit anti-hyperglucosylated adhesin antibodies (IC_{50}= $2.7\cdot10^{-7}$M and $1.0\cdot10^{-6}$M respectively), despite the protein bears up to nine N(Glc) minimal epitopes. This first example of short monoglucosylated peptides of NogoR and OMGp (involved in CNS regeneration) is in agreement with molecular mimicry between bacterial and myelin proteins. Moreover, the aberrant glucosylation motif fundamental for antibody recognition, further supports the hypothesis of NTHi bacterial infection stimulating and perpetuating autoimmune response in Multiple Sclerosis.