Imperative role of glycosylation in human MOG-HLA interaction: molecular insights of MOG-Ab associated demyelination

P. Jayananth, R. Madhumitha and L. Ramya

Computational and Molecular Biophysics Laboratory, School of Chemical and Biotechnology, SASTRA Deemed University, Thirumalaisamudram, Thanjavur, Tamilnadu, India

Communicated by Ramaswamy H. Sarma

ABSTRACT
Myelin oligodendrocyte glycoprotein is a transmembrane protein found on the outer lamella of the myelin sheath. The autoimmune attack on the MOG leads to demyelination which differs from normal multiple sclerosis. MOG has three epitope regions MOG\textsubscript{1-22}, MOG\textsubscript{35-55}, and MOG\textsubscript{92-106} in the extracellular region, and the crucial MOG\textsubscript{35-55} epitope and Human Leukocyte Antigen (HLA) interaction is the initial step for autoantibody generation. To study the effective role of glycosylation in MOG-HLA interaction, we performed molecular dynamics simulations of the complex where HLA interacts with three MOG epitopes both in the absence and presence of glycan. The results projected that the epitope MOG\textsubscript{1-22} is decisive for the HLA interaction in the absence of glycan and HLA interacts with the epitope MOG\textsubscript{35-55} irrespective of glycan existence. The residues Arg\textsubscript{9}, Arg\textsubscript{46}, and Arg\textsubscript{66} were found to interact strongly with HLA even in the presence of glycan. The glycan increased the flexibility of hMOG and enhanced the interaction of MOG with water molecules.

Abbreviations: MOG: Myelin Oligodendrocyte Glycoprotein; GlcNAc: N-Acetylglucosamine; CNS: Central nervous system; TCR: T-Cell Receptor

1. Introduction
Myelin sheath is the extension of the plasma membrane placed in a regular segment of the axon. Myelin is a protecting layer that surrounds the neurons and functions as the insulator to improve the stimuli’ speed. It is composed of protein and fatty substances (Matthieu, 1993). This allows the electrical impulses to transmit rapidly and efficiently along with the nerve cells. Myelin has a highly complex structure yet it has compact circuitry. Cognitive processes like learning, language, intelligence require plasticity in the Central Nervous System (CNS). The role of the myelin sheath is essential in-circuit plasticity (Williamson & Lyons, 2018). Damage in the myelin slows down impulse transmission which will cause neurodegenerative diseases (Yannakakis et al., 2016). Inflammatory demyelination occurs when immune systems attack the Myelin Oligodendrocyte...
glycoprotein (MOG). Several types of demyelination like Multiple Sclerosis, Optic neuritis, and acute-disseminated encephalomyelitis targeted the central nervous system. MOG is a glycoprotein and found on the external lamella of the myelin sheath and this is confined only to CNS. Hence, the initial studies considered the MOG-antibody-induced demyelination leads to multiple sclerosis (Love, 2006; Yannakakis et al., 2016). In multiple sclerosis (MS), the signs of B-cell activation occur predominantly within the CNS, whereas MOG-Ab associated CNS demyelination is characterized by CNS-directed antibodies produced in the periphery. It was observed in the serum of patients with MS, anti-MOG Ab was negative while it is positive in MOG-Ab associated demyelination. The main difference between them was actually from the histopathological findings (Weber et al., 2018).

MOG comprises only 0.01%–0.05% of the myelin proteins. The neural disease is caused by the formation of the trimolecular complex of the T-cell receptor (TCR), MOG, and Human Leukocytes Antigen (HLA) which in turn leads to demyelination (Koukoulitsa et al., 2020). The human MOG gene is located at chromosome 6 in the HLA gene. The functional complex of the T-cell receptor (TCR), MOG, and HLA consists of two chains (HLA-DR) was retrieved from RCSB (PDB ID: 1YMM). HLA consists of two chains α and β in that β chain had missing residues from 105–113 which was modeled using Charmm-Gui (Wu et al., 2014). The HLA was docked with the modeled hMOG at each of its 3-epitope regions using the Rosetta server (Lyskov & Gray, 2008). This was performed to identify the HLA specific epitope among the three existing epitopes (1–22, 35–55, 92–106) of hMOG. The top 10 docked structures for each hMOG epitope-HLA were taken and the best complex structure was identified based on score and number of interactions in each epitope site. The total score may not indicate the best-docked model as the main contribution comes from the folding energies of the monomers. Thus we focused on the number of interactions and the interface score (I_sc) of the complex which represents the energy of the interactions across the interface. These scores are very much smaller than the total scores (typically −2 to −10). Here, the selected docked structures from every three sets were found to have the I_sc value −2.604, −0.083, and −2.932. Hence, we had three systems with HLA docked to MOG at three epitope sites (Figure 1). The docked hMOG-HLA complex with HLA near all three epitopes was submitted to the GLYCAMP database (http://glycam.org) and N-Acetylglycosamine (N-glycan) was added at Asn31 position of hMOG using the force field GLYCAM_06 (Kirschner et al., 2008). Thus the six complex systems of hMOG-HLA were subjected to MD simulations to study the HLA interacting key residues and dynamic behavior of hMOG. The systems were labeled as EP1, EP2, EP3 (in the absence of glycan and HLA near the three epitopes of hMOG) and GEP1, GEP2, GEP3 (in the presence of glycan and HLA near the three epitopes of hMOG), respectively.

2. Materials and methods

The three-dimensional structure of human MOG (hMOG) was modeled using homology modeling SWISS-MODEL (Waterhouse et al., 2018) with the template of mouse MOG structure (PDB ID: 1PY9) having sequence identity 90.5%. The modeled MOG conformation consists of 116 residues. The modeled structure of MOG was validated using Φ-Ψ plot shown in supplementary material Figure S1a. The program verify3D (Bowie et al., 1991) was used to determine the compatibility of the modeled structure (Figure S1b). Further, the human MOG was modeled using the Robetta server (Raman et al., 2009) and the best-modeled structure has the backbone atoms RMSD 0.58 Å with the structure modeled from SWISS-MODEL (Figure S2). The structure of the Human Leukocyte Antigen (HLA-DR) was retrieved from RCSB (PDB ID: 1YMM). HLA consists of two chains α and β that had missing residues from 105–113 which was modeled using Charmm-Gui. The HLA was docked with the modeled hMOG at each of its 3-epitope regions using the Rosetta server (Lyskov & Gray, 2008). This was performed to identify the HLA specific epitope among the three existing epitopes (1–22, 35–55, 92–106) of hMOG. The top 10 docked structures for each hMOG epitope-HLA were taken and the best complex structure was identified based on score and number of interactions in each epitope site. The total score may not indicate the best-docked model as the main contribution comes from the folding energies of the monomers. Thus we focused on the number of interactions and the interface score (I_sc) of the complex which represents the energy of the interactions across the interface. These scores are very much smaller than the total scores (typically −2 to −10). Here, the selected docked structures from every three sets were found to have the I_sc value −2.604, −0.083, and −2.932. Hence, we had three systems with HLA docked to MOG at three epitope sites (Figure 1). The docked hMOG-HLA complex with HLA near all three epitopes was submitted to the GLYCAMP database (http://glycam.org) and N-Acetylglycosamine (N-glycan) was added at Asn31 position of hMOG using the force field GLYCAM_06 (Kirschner et al., 2008). Thus the six complex systems of hMOG-HLA were subjected to MD simulations to study the HLA interacting key residues and dynamic behavior of hMOG. The systems were labeled as EP1, EP2, EP3 (in the absence of glycan and HLA near the three epitopes of hMOG) and GEP1, GEP2, GEP3 (in the presence of glycan and HLA near the three epitopes of hMOG), respectively.
All six systems were subjected to molecular dynamics simulations using the Amber18 program (Case et al., 2018). The following protocol was used for each of the six complex systems. The system was solvated in a TIP3P cubic water (Jorgensen et al., 1983) box of size 12 Å. The net charge of the system was \( \pm 18 \). The counter ions (\( 18\text{Na}^+ \)) were added to electrically neutralize the system. The system was minimized for 1000 steps of steepest descent followed by 4000 steps of the conjugate gradient method using the amberff14SB force field (Maier et al., 2015). The well-minimized structure was then heated for 50ps to reach the desired temperature of 300 K. The system was subjected to equilibration for 5 ns in the NPT ensemble. The production phase was performed for 100 ns in the NPT ensemble. The integration time step was kept at 2 fs. Trajectories were recorded at every 10 ps which yielded 10,000 conformations. All the 10,000 conformations from each of the six systems were considered for analysis using the CPPTRAJ module (Roe & Cheatham, 2013) in the AMBER18 program. MD simulations were run in triplicate which includes 18 simulations with each 100 ns run (Figure S3).

### 3. Results and discussion

The main results drawn from this study were (i) the presence of glycan increased the flexibility of hMOG in GEP1 and GEP2. (ii) The intramolecular interaction of hMOG was reduced in the presence of glycans except in the system GEP3. (iii) The glycan enhanced the interaction of hMOG with water molecules in GEP2 whereas it reduced their interaction in GEP3. (iv) The interaction of hMOG with HLA was reduced in GEP1 whereas the interaction increased in GEP3. Though it was evident that the glycan reduced the interaction of hMOG with HLA in GEP1, the average interaction energy and hydrogen bonds were double in GEP1 than that in GEP2 and GEP3. (v) From the interaction energy, hydrogen bonds interacting residues, it was apparent that the epitope MOG\(_{1-22}\) of hMOG remains crucial for HLA interaction in the absence of glycan. (vi) Even in the presence of glycan, the residues Arg9, Arg46, and Arg66 have strong hydrogen bond interactions with HLA.

### 3.1. Docking

Table 1 shows the docking score and number of interactions of HLA with hMOG in the top 10 models obtained from the Rosetta server for the systems EP1, EP2, and EP3, respectively. In the system EP1, the model with 50 interactions between HLA and hMOG with docking score \(-162.579\) was selected for MD simulation. Similarly from EP2 and EP3 systems, the models with 40 and 42 interactions between HLA and hMOG with docking scores \(-93.357\) and \(-162.733\) were selected, respectively. In Rosetta, the score is the weighted sums of energy terms which include electrostatics, van der Waals’ interactions, and statistical terms like the probability of finding the torsion angles in \( \Phi-\Psi \) space. The interaction includes close contacts and hydrogen bonds between hMOG and HLA.
3.2. Molecular insights of hMOG when HLA interacts with MOG$_{1-22}$ (systems EP1 and GEP1)

Table 2 gives the average analysis parameters for hMOG and HLA for all the six complex systems. The average backbone atoms RMSD of hMOG both in EP1 and GEP1 were 0.9 and 1.2 Å, respectively. From Figure 2a, it was evident that the presence of glycan increases the flexibility of the protein hMOG. This is in agreement with the RMSF of hMOG as the average value increased from 1.7 to 2.5 Å. Figure 2d indicates that the RMSF fluctuation of hMOG increased in the presence of glycan. The average intramolecular interaction energies of hMOG in the systems EP1 and GEP1 were $-2023.34$ and $-1925.89$ kcal/mol (Figure 3a). The glycan reduced the intramolecular interaction of hMOG till 70 ns of dynamics simulation. The average intermolecular interaction energy of hMOG with water molecules in both systems EP1 and GEP1 were $-3710.14$ and $-3828.43$ kcal/mol, respectively (Figure 3b). The presence of glycan enhances the interactions of the hMOG-water molecules during the initial 40 ns of simulation. The average intermolecular interaction energy of hMOG with HLA in both systems EP1 and GEP1 were $-359.06$ and $-329.29$ kcal/mol which indicates that the presence of glycan reduced the intramolecular interaction as well as intermolecular interaction of hMOG with HLA whereas it enhanced the interaction of hMOG with water molecules. Considering the hydrogen bond interactions, there are no significant changes observed from the average values but here also it was apparent that the glycan reduced the interaction between hMOG and HLA from 13.6 to 11.0 (Figure 4c). Table 3 gives the key residues of hMOG forming hydrogen bonds with the HLA and retained over 30% of simulation duration. Here we observed in system EP1, the residue Glu116 of hMOG not present in epitope 1 was found to have strong hydrogen bond interaction with HLA. Similarly, in system GEP1, the residue Glu65 of hMOG which is not in epitope 1 found to have interaction with Arg39 of HLA. Apart from these interactions, all the other interactions were from epitope 1 of hMOG with HLA. The numbers of interactions between hMOG-HLA were reduced in the presence of glycan. Moreover, it was observed that the residue Arg9 interacts strongly with the HLA both in the presence and absence of glycan.

Figure 5 represents the number of water oxygen in the first and second hydration shell of hMOG. The first hydration shell was calculated as 3 Å distance from hMOG and the second shell distance was from 3 to 5 Å, respectively. The number of water oxygen in the second shell was more than that in the first shell as expected. Supplementary Figure S4 gives the secondary structural regions of hMOG for each epitope regions in both EP1 and GEP1 systems. The secondary structural information of hMOG indicated that the key residue Arg9 observed in the random coil was changed to bends in the system GEP1. Similarly, the residues 41 to 46 belonging to epitope 2 were disturbed in the presence of glycan during the initial 40 ns simulation. Even though the HLA interacted with epitope 1 of hMOG, epitope 2 had residual structural changes due to the presence of glycan near the site.

The backbone atoms RMSD of the lowest intramolecular interaction energy structure of hMOG from both EP1 ($-2328.91$ kcal/mol) and GEP1 ($-2274.54$ kcal/mol) was 1.3 Å (Figure 6a). It was observed that the lowest energy structure of hMOG in EP1 has β-turns in Lys72-Glu77 whereas the lowest energy structure of hMOG in GEP1 has α-helix (Figure S7). Similarly, the residues Pro62 to Tyr64 and residues Phe89 to Asp92 were found to be in 3_10 helices in EP1 and the lowest energy structure in GEP1 has β-turns for these residues. The secondary structural region for this lowest energy conformation was calculated using the STRIDE program (Heinig and Frishman, 2004). Though the glycan induced turns for the residues Pro7 to Pro10 in epitope 1 most of the structural changes were observed outside epitope 1. From the interaction energies, it was found that the glycan reduced the intramolecular energy of hMOG and thereby leads to significant structural changes.

3.3. Molecular insights of hMOG when HLA interacts with MOG$_{35-55}$ (systems EP2 and GEP2)

From Table 2 it was apparent that there was no significant change in the average backbone atoms RMSD of hMOG in both EP2 and GEP2 systems. The average RMSF of hMOG was increased in GEP2 (4.7 Å) from 2.7 Å in EP2. Figure 2e
shows that the RMSF of hMOG increases significantly in the presence of glycan. Hence, as in GEP1, here also it was observed that the glycan increases the flexibility of hMOG.

The average intramolecular interaction energy of hMOG in both EP2 and GEP2 was $-2021.67$ and $-1981.58$ kcal/mol, respectively. The glycan reduced the intramolecular interaction of hMOG. The average intermolecular interaction energy of hMOG with water molecules in both EP2 and GEP2 was $-3589.43$ and $-4221.17$ kcal/mol. Figure 3e shows that glycan increased the interaction of hMOG with water molecules. The average intermolecular interaction energy of hMOG with HLA in both EP2 and GEP2 was $122.57$ and $172.78$ kcal/mol which again indicates that the presence of glycan enhances the hMOG-HLA intermolecular interaction when HLA interacts with epitope 2 of hMOG. Thus, glycan was found to reduce the intramolecular interaction of hMOG whereas it increases the hMOG intermolecular interaction with both water molecules and HLA. The average intramolecular hydrogen bonds of hMOG in both EP2 and GEP2 were 95.3 and 93.2 which shows that almost all the hydrogen bonds in EP2 were retained in GEP2. From Table 2 and Figure 4e, it was apparent that the glycan increased the interaction of hMOG with water molecules. The average intermolecular hydrogen bonds between hMOG and HLA in both EP2 and GEP2 were 6.3 and 5.4, respectively. From Figure 4f, it was evident that the stronger interaction between hMOG and HLA during the initial phase in EP2 was considerably reduced in the presence of glycan. From Table 3 it was found that the residue Arg66 of hMOG which is not present in epitope 2 found to have strong hMOG-HLA hydrogen bond interactions in both EP2 and GEP2 whereas the Arg52 hydrogen bond interaction with HLA in EP2 was not present in GEP2. In GEP2, the residue Arg66 outside the epitope 2 formed the hMOG-HLA hydrogen bond interactions.

The number of water molecules in the first and second hydration shell of hMOG was increased in the presence of glycan (Figure 5c-d). This is following the observation that the glycan increases the interaction of hMOG with water molecules. Taking into account secondary structural regions in both EP2 and GEP2 (Figure S5), the residues Arg41 and
Arg46 in distorted sheet regions were changed to perfect sheet regions in the presence of glycan. It was known that the residues Arg41 and Arg46 act as an anchor in the interaction with TCR (Yannakakis et al., 2016). The hMOG residues from Pro42 to Ser45 were retained as turns in the GEP2 system while it was distorted bends in EP2. Moreover, the residues adjacent to the epitope2 site from Gln57 to Ala62 formed distorted 3_{10} helices in GEP2.

The backbone atoms RMSD of the lowest intramolecular interaction energy structure of hMOG from both EP2 (−2021.67 kcal/mol) and GEP2 (−1981.58 kcal/mol) was 1.1 Å (Figure 6b). Here the lowest energy structures from both EP2 and GEP2 have no significant secondary structural changes (Figure S8).

### 3.4. Molecular insights of hMOG when HLA interacts with MOG_{92–106} (systems EP3 and GEP3)

From the average backbone atoms RMSD of the EP3 and GEP3 (Figure 2c), it was apparent that the glycan presence increases the flexibility of the hMOG. The average RMSF of hMOG in both EP3 and GEP3 was 3.1 and 1.9 Å which shows that the fluctuation of residues decreases during the presence of glycan (Figure 2f). Here vital point was that the overall molecule flexibility increased in presence of glycan whereas the fluctuation of crucial residues reduced significantly in glycan presence. This observation was quite different from those in GEP1 and GEP2.

The average intramolecular interaction energy of hMOG for EP3 and GEP3 was −1962.52 and −1998.30 kcal/mol, respectively. Figure 3g shows that the presence of glycan enhances the intramolecular interaction energy of hMOG almost throughout the dynamics simulation. The average intermolecular interaction energy of hMOG with water molecules in both systems was −4330.32 and −3735.35 kcal/mol which indicates that the glycan reduces the hMOG interaction with water molecules unlike in GEP1 and GEP2 (Figure 3h). The average intermolecular interaction energy of hMOG with HLA in both EP3 and GEP3 was −102.84 and −285.39 kcal/mol, respectively. Here the glycan enhanced the hMOG-HLA interaction to a greater extent (Figure 3i). Thus, glycan helped hMOG to increase the intramolecular interaction and intermolecular interaction with HLA and reduced the interaction with water molecules. This observation was contradictory to that analyzed in GEP1 and GEP2. Considering the hydrogen bond interactions, there were no significant changes in the average intramolecular hydrogen bonds of hMOG in EP3 and GEP3. The intermolecular hydrogen bond interactions between hMOG and water molecules were reduced in the presence of glycan (Figure 4h).
average intermolecular hydrogen bonds between hMOG and HLA in both EP3 and GEP3 were 6.1 and 11.8, respectively. Here glycan enhances the formation of hydrogen bond interactions between hMOG and HLA (Figure 4i). This is following the interaction energy calculations. It was interesting to ponder why hMOG behaves differently when epitope 3 interacts with HLA in the presence of glycan. It was noted that the hMOG residues having hydrogen bond interaction with HLA were not present in the epitope3 of hMOG and most of the hMOG residues were Arg46, Arg66, Glu36, and Glu64 in GEP3 (Table 3). This suggests that the HLA preferred to interact with hMOG epitope 2 or its adjacent region rather than the epitope 3.

Unlike in GEP2, here glycan reduced the number of water molecules in both hydration shell I and II of hMOG (Figure 5e-f). This is in agreement with energy and hydrogen bond
analysis that the glycan reduced the interaction of hMOG with water molecules. From the secondary structural analysis (Figure S6), it was observed that the residues Arg41 and Arg46 changed from sheet to distorted sheet in the presence of glycan and the residues Pro42 to Ser45 changed to distorted turns in GEP3 during the initial 50 ns of simulation. Similarly, the residues His49 to Arg52 were changed from sheet to distorted sheet during initial 40 ns in GEP3, though residues Asp102 to Tyr105 has some changes in their secondary structural regions in glycan presence, the crucial changes were observed only in epitope2 of hMOG. The backbone atoms RMSD of the lowest intramolecular energy structure of hMOG from both EP3 and GEP3 was 1.5 Å (Figure 6c).

There were no significant changes in the secondary structural regions of hMOG for the lowest energy structures as in GEP1 (Figure S9).

The final conformations (100 ns MD simulation) of the hMOG-HLA interaction for all six systems were shown in Figure 7. Table 4 shows that there were eight hydrogen bonds between hMOG and HLA in EP1 and only two of them observed in GEP1. There were three hydrogen bonds between the hMOG and HLA and new two hydrogen bonds were observed in GEP2. In the case of GEP3, five hydrogen bonds were observed between hMOG and HLA where the residues from hMOG were not from epitope 3 regions but epitope 2 of hMOG.

From the MD simulation study of the MOG-Fab complex, it was apparent that the flexibility of MOG was reduced in the presence of glycan in both unbound and bound states. The results emphasized that the glycosylation introduced additional secondary structural regions in MOG by reducing the flexibility and thereby maintaining structural stability. The glycan reduced the interaction between MOG and Fab molecule (Ramya, 2020). Here the Fab molecule interacted with the epitope 3 of MOG.

Here in the case of the MOG-HLA complex, the glycan increased the flexibility of hMOG when HLA was placed near the epitope 1 and 2 of hMOG. The increase in flexibility of MOG was highly evident from the RMSF value (Table 2) of GEP2 followed by the system GEP1. The flexibility of MOG was reduced by glycan in the system GEP3 and the average value of hMOG RMSF dropped from 3.1 to 1.9 Å. Thus in the presence
of glycan, the overall flexibility of MOG decreases when either Fab or HLA interacts with the epitope 3 of MOG.

Tselios and his coworkers (Yannakakis et al., 2016) studied the interactions of hMOG35–55 with both the HLA and TCR using molecular dynamics simulation. From the study, they emphasized that the major hydrogen bonds between hMOG35–55 and HLA DR2 involved the residues Arg41 and Arg46 of hMOG where the hydrogen bonds with occurrence more than 20% were considered. The residue Arg41 plays a crucial role in antigen identification as it interacted strongly with TCR. They found that the residues Arg41 and Arg46 of hMOG35–55 were the key residues that enhanced the interaction of hMOG35–55 with TCR. Here in our study, we found the residues Arg9, Arg46 and Arg66 interact strongly with the HLA even in the presence of glycan. The two residues identified crucial from their study Arg41 and Arg46 of hMOG35–55 (Yannakakis et al., 2016) were observed in perfect β-sheet regions in the presence of glycan. The residue Arg41 was not identified as key residue from our study and the plausible causes maybe (i) we considered only those hydrogen bonds which were retained more than 30% of the simulation (ii) we considered the hMOG with residues 2–117 and not just the epitope 2 of hMOG (iii) we added the glycan at the Asn31 position and our main focus was to identify the MOG epitope among the existing 3 epitopes crucial for HLA interaction in the presence of glycan.

The conformational changes of rat/mouse MOG35–55 were examined using both the experimental and molecular dynamics simulation studies (Ntountaniotis et al., 2017). In this study, they found that the peptide MOG35–55 changed the conformation variedly like α-helix in the presence of both trifluoroethanol and micellar SDS in NMR experiment, as β-strands in nonmicellar SDS from circular dichroism study and mostly random coils in molecular dynamics simulation from both implicit and explicit DMSO solvent environments. In our study, we observed the epitope hMOG35–55 in all the six systems to be majorly in β-sheets with β-turns in the region Pro42-Ser45 and Asn53-Gly54, respectively. This observation is similar to the experimental crystal structure.

From Table 3, it was evident that the presence of glycan reduced the interaction between HLA and hMOG when HLA was near the epitope hMOG1–22. In the case of system GEP2, the glycan has no significant role in the HLA-hMOG

| Systems | Hydrogen bonds | Systems | Hydrogen bonds |
|---------|---------------|---------|---------------|
| EP1     | ASP43.B-ARG4.C* | GEP1    | ASP43.B-ARG4.C* |
|         | ALA49.B-SER27.C* |        | ALA49.B-SER27.C* |
|         | ARG146.A-GLU116.C |       | ARG146.A-GLU116.C |
|         | ARG140.A-ARG13.C |        | ARG140.A-ARG13.C |
|         | GLU14.B-ARG9.C  |        | GLU14.B-ARG9.C  |
|         | GLU35.B-ARG25.C |        | GLU35.B-ARG25.C |
|         | GLU35.B-LYS80.C |        | GLU35.B-LYS80.C |
|         | GLU52.B-Glu78.C |        | GLU52.B-Glu78.C |
|         | GLU52.B-ARG66.C |        | GLU52.B-ARG66.C |
|         | HIS143.A-ASP60.C |       | HIS143.A-ASP60.C |
|         | LYS111.A-HIS103.C |    | LYS111.A-HIS103.C |
| EP2     | ASP43.B-ARG9.C | GEP2    | ARG140.A-PHE44.C |
|         | GLU52.B-ARG66.C |        | GLU52.B-ARG66.C |
|         | HIS143.A-ASP60.C |       | HIS143.A-ASP60.C |
|         | LYS111.A-HIS103.C |    | LYS111.A-HIS103.C |
| EP3     | ASP43.B-ARG89.C | GEP3    | ASP142.A-ARG52.C |
|         | ARG25.B-GLU64.C |        | ARG25.B-GLU64.C |
|         | ARG39.B-GLU64.C |        | ARG39.B-GLU64.C |
|         | ARG48.B-ARG9.C  |        | ARG48.B-ARG9.C  |
|         | ARG140.A-PHE44.C |      | ARG140.A-PHE44.C |
|         | GLU35.B-ARG66.C |        | GLU35.B-ARG66.C |

*Same hydrogen bonds.
interaction (Figure 4f). Considering the epitope hMOG_{92-106}, the presence of glycan increases the interaction of hMOG with HLA (Figure 4i). When we analyzed the hydrogen bond interacting residues in EP3 and GEP3 (Table 3), none of the hMOG interacting residues are from the region of epitope 3. In Table 3, for the system GEP3, the hMOG residues (chain C) are mainly from epitope 2 (35–55) or near epitope 2. Thus it was concluded that the HLA prefers to interact with the epitope 2 of hMOG (hMOG_{35-55}) irrespective of glycan presence.

It was apparent from Table 2, that the average number of water molecules in the hydration shell II of hMOG was greater than that in shell I in all the six systems. This is because hMOG interaction with water molecules was affected by HLA molecule and N-glycan in the hydration shell I. Considering the role of glycan in the hydration shell, it was noted that the average number of water molecules in hydration shell I and II of hMOG was increased in the system GEP2 than in EP2. The important observation here is that the glycan increased the flexibility of hMOG in GEP2 and thereby increased its interaction with HLA and water molecules. On the other hand, the average number of water molecules in the hydration shell I and II of hMOG were reduced in the presence of glycan in the system GEP3. Interestingly, the glycan decreased the flexibility of hMOG in GEP3 and increased the intramolecular interaction of hMOG. The hMOG interaction with water molecules was reduced in the presence of glycan.

The radial distribution function (RDF) of hydration water oxygen around hMOG in all the six systems was calculated with a bin spacing of 0.1. The distance was taken along the X-axis and g(r) the probability of water oxygen presence at a distance r from hMOG was taken along Y-axis. Figure S10 represents the RDF of water oxygen in the hydration shell around hMOG. It was apparent from Figure 7 that the distribution of hydration water molecules around hMOG in both EP1 and GEP1 remains the same, the distribution increases in the presence of glycan in GEP2 and decreases in the presence of glycan in GEP3 systems, respectively.

Here it depicts that as the glycan increased the flexibility of hMOG in both GEP1 and GEP2, hMOG interacts more strongly with the water molecules and HLA molecule whereas when glycan decreased the hMOG flexibility (as in GEP3) its interaction with surrounding water molecules and HLA decreased. Thus in GEP3, we expected the hMOG-HLA interaction to reduce as hMOG flexibility decreased, but the residues of hMOG interacting with HLA were from other epitope 2 or its adjacent residues and not from epitope 3. Thus these hydration water molecules are so important that if the interactions between these water molecules and hMOG get increased the interaction of hMOG with HLA will be reduced. This happens when the glycan increases hMOG flexibility. Hence it becomes more interesting to ponder the transmembrane region of MOG as we observed that the glycan decreased MOG flexibility when HLA interacts with the epitope 3 similar to the study of MOG-Fab (Ramya, 2020).

When we consider the conformation of HLA in all the systems, the average backbone atoms RMSD was increased in the presence of glycan in both GEP2 and GEP3. Thus, the flexibility of HLA was reduced only in GEP1. This again proves that the glycan role was essential when HLA interacts with the hMOG_{1-22} site. The intramolecular interaction of HLA was reduced in the presence of glycan in all GEP1, GEP2, and GEP3 systems.

4. Conclusion
To explore the hMOG epitope interaction sites with HLA, we docked the HLA near all the three epitope sites of hMOG and MD simulation performed with and without glycan presence. The analysis revealed that the glycan plays a crucial role when HLA interacts with the epitope hMOG_{1-22} site. In GEP1, glycan reduced the interaction between HMOG and HLA whereas its presence had no significant role when HLA interacted with epitope hMOG_{35-55} in GEP2. Interestingly, in GEP3, glycan enhanced the interaction of HLA with hMOG but the interacting hydrogen bond residues were not from the epitope 3 site. This emphasizes that in the presence of glycan, HLA preferred to interact with hMOG_{35-55} or with its adjacent residues rather than hMOG_{1-22} and hMOG_{92-106} sites. The hMOG residues Arg9, Arg46, and Arg66 were found to play an important role in interaction with HLA even in the presence of glycan. The mutational studies will be carried out in near future to understand the significance of these Arg residues. Thus the glycopeptide can be designed with more Arg residues which can play a significant role in inhibiting the interaction of MOG and HLA and enhance HLA-peptide interaction. Moreover, it was found that when the glycan increases the hMOG flexibility, the interaction of hMOG with water molecules and HLA increases. If the hydration shell water molecules interact more with hMOG, then the interaction of hMOG-HLA will be reduced.

Acknowledgements
The authors thank the funding agency for the financial support and also acknowledge the support from SASTRA Deemed to be University for providing the necessary computational facilities.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This work was supported by the Science and Engineering Research Board (SERB), Government of India (ECR/2017/000192).

Author’s contribution
LR conceived and designed the study. PJ, RM, and LR performed docking, simulations, and collected data. LR analyzed the data. All authors discussed the results. LR wrote the manuscript and revised it.

ORCID
L. Ramya http://orcid.org/0000-0001-8264-9476
