REFOLDING THE HCV E2 GLYCOPROTEIN TO ENHANCE IMMUNOGENICITY

Phu L[1,2], Center RJ[1,2], Poumbourios P[1,3] and Drummer HE[1,2,3]

[1]Centre for Biomedical Research, Burnet Institute, Melbourne 3004, VIC.
[2]Department of Microbiology and Immunology at the Peter Doherty Institute, University of Melbourne, Melbourne 3000, VIC.
[3]Department of Microbiology, Monash University, Clayton 3800, VIC.

Background: An effective vaccine against HCV could act in concert with antiviral therapies to quell the epidemic. Most successful vaccines are powered by neutralizing antibodies, however the major neutralization target of HCV, the E2 glycoprotein, has very high sequence diversity, making it difficult to achieve E2-mediated pan genotypic protection. An E2 immunogen with the three hypervariable regions deleted (Delta3) induces antibodies able to neutralize a broader range of HCV strains, and this is particularly true of a disulfide-linked high molecular weight (HMW) form of this glycoprotein. The HMW form is expressed as a minor species, whereas monomer is the predominant species. We aimed to convert Delta3 monomer to HMW through limited reduction and refolding.

Methods: Delta3 protein was expressed by stable transfection of 293F cells and purified using metal affinity and size exclusion chromatography. Monomeric Delta3 was subjected to sequential exposure to and removal of reducing agent to achieve refolding followed by assessment of HMW formation by size exclusion. ELISA was used to determine the antigenic reactivity of refolded HMW Delta3. Disulfide bonds of Delta3 were determined by nanoLC ESI MS/MS using deglycosylated and protease digested protein.

Results: Optimization of refolding protocols allowed greater than 50% of monomers to be refolded into oligomeric structures. A glutathione shuffling strategy resulted in the production of dimers, whereas refolding with DTT produced HMW forms. Analysis using ELISA suggested that the refolded HMW Delta3 recapitulated many of the antigenic properties of the native HMW.

Conclusion: Conversion of Delta3 monomer to HMW by refolding is a viable strategy to enhance vaccine production and purification. Refolded HMW Delta3 will be assessed in immunogenicity trials.

Disclosure of Interest Statement: We and the conference collaborators gratefully acknowledge the contribution to professional and research activities made by industry partners. Furthermore, we recognize the importance of transparency and therefore, will disclose potential conflicts of interest in both publications and presentations.