SYNTHESIS OF HBeAg-SPECIFIC EPITOPE-CLONED HBV BIO-NANOPARTICLES

Authors:
Droungas Y[1,2], Netter H[1], Walsh R[1], Sozzi V[1], Mason H[1], Revill P[1,2]

[1] Victorian Infectious Diseases Reference Laboratory, Melbourne Health, The Peter Doherty Institute for Infection and Immunity, [2] Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity

Introduction:

The Hepatitis B e Antigen (HBeAg) is a secreted, accessory protein of particular interest, responsible for initial establishment of Chronic Hepatitis B (CHB) disease, and acting as a tolerogen against the host's immune responses. Its seroconversion in CHB patients is a treatment end-point and a recognized precursor of Hepatitis B surface Antigen (HBsAg) seroclearance, currently accepted as functional cure. The aim of this study was to generate chimeric Hepatitis B Virus (HBV) Bio-Nanoparticles (BNPs), carrying specific HBeAg epitopes and to test whether they are immunogenic against HBeAg and HBsAg-specific antibodies (Abs).

Methods:

Three variants of the 100% conserved N-terminal HBeAg epitope (a 15-mer (eAgM), a 15-mer dimer (eAgD), and a mutant 15-mer (eAgC4S)) were ligated into the 1st antigenic loop of the HBsAg, and subsequently ligated into the pCAGGs vector. HuH7 and HEK293 cells were transfected with the DNA plasmids. Harvested lysates and supernatants were examined via western blotting and ImmunoFluorescent Assays (IFA) to detect the proteins of interest using a panel of characterized HBeAg and HBsAg-specific antibodies. The chimeric BNPs were visualized via electron microscopy (EM).

Results:

The eAgM and eAgD HBsAg chimeras were successfully detected via western blotting, IFA and EM. However, the HBsAg/ eAgC4S chimeras were not detected, possibly due to conformational changes of the original sequence that prevented their assembly into BNPs.

Conclusion:

With HBV being one of the most important, medically-relevant pathogens, there is an urgent need to address the current lack of therapeutic approaches targeting the HBeAg and HBsAg. This study is the first to describe the expression of chimeric HBeAg epitope-expressing BNPs in cell cultures, whilst maintaining HBeAg and HBsAg antigenicity. Future studies will assess the ability of our BNPs to 'break' immune tolerance in CHB infected mice, ultimately leading to functional cure in patients that currently do not respond to therapy.