

October 06-08, 2014 Hilton San Antonio Airport, TX, USA

## Search for protective Epstein Barr virus (EBV) epitopes in rabbit model

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s recently reported, EBV infection in New Zealand white rabbits does not cause infectious mononucleosis (Rajčáni J. et.al., Intervirology 2014). The administration of high virus dose (108-109 EBV DNA copy per animal inoculated by naso-oral route) elicited a potent immune response when establishing latency dissimilar to that in humans. For the reliable assessment of IgG antibody response, we applied EA-D (early antigen p54) ELISA and immunoblot (IB) tests. The EBV DNA in peripheral white blood cells (WBC) and spleen was detected by classical and/or nested polymerase chain reactions (PCR) using LMP1 gene primers. In addition, the WBC smears were stained for LMP1 antigen by immunofluorescence (IF). The average positive infection rate in 21 rabbits as detected by IB at 2 different intervals post-infection (p.i.) has reached 69.2% if the antibodies were determined against Zta/BZLF1 antigen (transactivator protein), but was slightly lower (53.4%) when evaluated against the early p54 polypeptide antigen (DNA polymerase cofactor). We strongly believe that coincidence of p54 antibodies in the infected animals as detected by both, IB and EA-D ELISA, might reflect the replication of EBV in lymphatic and/or epithelium cells. No EBNA1 antibody was found in our EBV infected rabbits at any of the intervals tested (over 98 days p.i.); this possibly reflects the development of rare latency 0 in the rabbit model. In contrast to the frequency of serological response, viral DNA could be detected in WBCs and/or spleen of 7 out 21 infected rabbits only (30%). The PCR results were in good agreement with the LMP1 antigen expression. Immunogenic EBV peptides estimated suitable for a protective vaccine were selected according to rational strategy, which combined the sequence based antigenicity prediction of B cell and T cell epitopes (by considering the protective/neutralizing immune responses and pathogenicity mechanisms) with the supertype affinity along with the specificity of prevalent HLA alleles as well as HLA ligand conservation (Sollner J. et al. Immunome Res. 2008; Immunome Res. 2010). Altogether 15 epitopes were selected out of the 52 immunogenic peptides considered. The epitopes (oligopeptides) were coupled to biocompatible microparticles (larger than 100 µm) in precise relative amounts; always 3 peptide combinations created together 19 putative immunogenic mixes in different variations. To each of microcarrier beads one pattern receptor recognizing (PRR) agonist (such as poly I/poly C, CpG and/or R848/resimiquod) was ligated. The 19 putative peptide vaccine mixes in question were used to immunize 3 rabbits in 19 immunization groups, which were challenged with the high EBV dose as above described. Taken together, 2 control groups had been created: those from the preliminary experiment (control A, 21 rabbits) and the group of 3 animals (control B) included into the protection test. The results reflecting the four most reliable infection markers (EA-D ELISA, immunoblot, EBV DNA and LMP1 antigen detections) as determined in control A experiment, were included into efficacy calculations. In these, the positive rates obtained in each immunized group were compared with the frequency of data rates in either of the 2 control groups. At least 5 oligopeptide combinations (immunization groups no. 1, 6, 12, 15 and 18), which contained 7 of the 15 predicted epitopes, comprised the statistical category revealing a considerable or (at least) good protective effect (the total frequency of positive data rates ranged here from 0 to 5% while no EBV DNA was detected). Furthermore, a category of slight protection has emerged (revealing positive data rates up to 20%), which contained 7 immunization mixes (numbers 9, 10, 13, 14, 16, 17 and 19). No effect (positive data rates over 21%) was seen after immunization with the rest of 7 mixes (immunization groups numbers 2, 3, 4, 5, 7, 8 and 11), in which the data positive rates showed no statistically significant difference as compared to both controls. Three of the protective epitope carrying polypeptides were structural (envelope) glycoproteins another 3 were non-structural and/or latency associated proteins. In addition to the 6 protective EBV-coded proteins which had already been declared for immunogenic by others (reviewed by Rajcani et.al., in Recent Patents on Anti-Infective Drug Discovery, submitted), we found one non-structural polypeptide not reported yet in such context.

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