Expression Studies of VP2 and VP5 Outer Capsid Proteins of Bluetongue Virus

F. Mohd Jaafar[1*] H. Attoui[1] X.W. Li[2]
O. Alpar[2] P.P.C. Mertens[1]

Since 1998, nine bluetongue virus (BTV) strains from serotypes 1, 2, 4, 6, 8, 9 11, 16 and 25 have invaded Europe, killing more than two million animals (mainly sheep). Live attenuated vaccines of BTV-2, 4, 9 and 16 have also been used in the region, in some cases causing further outbreaks of disease. Recent sequence analysis have shown that there have been cases of reassortment between field strains and live attenuated vaccine strains, hence the need for safer vaccines such as killed vaccines or recombinant protein-based subunit vaccines. The outer capsid protein VP2 of *Culicoides*-borne orbiviruses is the cell-attachment protein, which carries sero-neutralization epitopes. We have cloned the open reading frame (ORF) (of segment 2) encoding VP2 and expressed the protein from BTV-4 in a bacterial expression system. The entire protein was found to be insoluble. Bioinformatic and evolutionary analysis showed that VP2 was composed of two 'domains', which were expressed separately using the same system. Optimization of expression conditions generated soluble proteins, indicating a correct fold of the expression products. These protein domains were used as antigens in mice experiments to raise antibodies against conformational epitopes. VP2 of BTV-4 was also expressed in a baculovirus system and was found to be soluble. The ORF encoding VP2 was cloned into a mammalian expression vector (pcDNA3.1) and is currently used (as a DNA vaccine) in animal experiments (using Chitosan as an adjuvant) to assess the antibody raising capacity of this formulation. One of the intended uses of this protein or its domains is to generate crystals for determination of the VP2 atomic structure using X-ray crystallography. VP5 of orbiviruses is a fusion protein that is involved in membrane penetration during initiation of infection. The ORF (of genome segment 6) encoding VP5 of BTV-4 was cloned and expressed in the same bacterial system. Bioinformatic analysis also defined two domains of VP5. Only about 10% of the full length protein was soluble, while the two separated domains were over 90% soluble. Currently, VP5 and the two separate domains are being used in mice experiments to determine whether these can influence the immune response when concomitantly injected with the VP2 domains. VP5 was also cloned in pcDNA and used in mice experiments, formulated using Chitosan as an adjuvant. Antibodies collected from the mice reacted with the recombinant expressed VP5 showing high titres of antibodies. VP5 and its domains will also be used in X-ray crystallography. A plaque reduction assay was developed to determine whether the antibodies generated against VP2 domains or the VP2/VP5 mixture can neutralize virus infectivity in cell culture assays, opening the way for challenge assays in animals.

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1. Arbovirus Molecular Research Group, Vector-Borne Disease Programme, Institute for Animal Health, Pirbright Laboratory, Woking, Surrey, GU24 0NF, United Kingdom.
2. London School of Pharmacy, University of London, United Kingdom.

* Corresponding author
Tel.: +44 14 83 23 11 94; Fax: +44 14 83 23 24 48
E-mail: fauziah.mohd-jaafar@bbsrc.ac.uk

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