CATIONIC LIPOSOMES ENABLE BOVINE HERPESVIRUS TYPE 2 DNA TO INFECT CELLS

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DNA transfection of eukaryotic cells with virus DNA has usually included the use of either calcium phosphate precipitation or DEAE-dextran adsorption. Calcium phosphate has been in common use with a variety of herpesviruses to create either intratypic or intertypic recombinant viruses *(1, 2)*.

In an attempt to use bovine herpesvirus type 2 (BHV-2) as a bovine vector virus *(3, 4)* no infectious BHV-2 DNA was ever detected using DEAE-dextran or the calcium phosphate method on any cell line, with or without the use of either glycerol or dimethyl sulphoxide shock treatment of the cells. However use of the cationic lipid N-[1-(2,3-dioleyloxy) propyl]N, N, N-trimethyl amonium chloride (DOTMA; Lipofectin) that forms positively charged liposomes which can interact with DNA to form complexes, enabled infectious BHV-2 DNA from two different strains (BHM-1 or CSIRO 290) to be readily obtained. BHV-2 DNA purified by the method described previously *(3,4)* using sodim iodide gradients without sodium metabisulphete centrifuged at 43,000 rev/min for 72 hr from either infected BHK-21 or MDBK cells was infectious in BHK-21 cells when added with the cationic lipid. Isolation of DNA by another method for extracting herpesvirus DNA described by Pignatti *et al.* *(5, 6)* did not produce infectious DNA even with the use of the cationic lipid. The concentration of the prepared virus DNA was quantitated spectrophotometrically by readings at 260 nm. Further, purity (usually 95 %) of the BHV-2 DNA preparations was checked by restriction endonuclease *Sal*1 digestion and subsequent agarose gel electrophoresis of the cleaved BHV-2 DNA, a method which also distinguishes between the two BHV-2 strains used *(7)*.

Essentially 3-5 x 10^5 BHK-21 cells in 50nm^2 tissue culture plates were transfected with BHV-2 DNA 5 - 24 hr after seeding (in serum - free medium) with 100 μ l (4 - 8 μ g) of BHV-2 DNA plus 35 μ l (35 μ g) of Lipofectin (Bethesda Research Laboratories; Maryland U.S.A.) after first adding these together for 5 min in a polystyrene tube. This combination was found optimal as lesser amounts of Lipofectin dramatically lowered the amount od DNA transfected and higher amounts damaged the BHK-21 cells. Newborn calf serum was added to 10% and 24 - 48 hr later the virus CPE could be noticed. The CPE could be prevented by the adding 50 μg/ml of arabinosyl thymine (AraT; *1, 2, 8*) either at the time of transfection or up to 24 hr later; this amount of AraT did not damage BHK-21 cells. Usually 10 - 15 PFU/μg BHM-2 DNA was achieved on BHK-21 cells resulting in an infectious virus which can be further grown on MDBK cells. Whether transfection using cationic liposomes is general for all bovine herpesvirus DNA, still depends on whether the virus can grow in certain cells.

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