MONOCLONAL ANTIBODIES TO TWO BOVINE CORONAVIRUS GLYCOPROTEINS NEUTRALIZE
VIRUS INFECTIVITY

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The Quebec isolate of BCV was found to contain eight proteins with molecular weights (M.W.) of 190,000, 160,000, 124,000, 100,000, 53,000, 26,000, 25,000 and 23,000. Of these, five were glycoproteins and were designated gp190, gp124, gp100, gp26 and gp25. The three unglycosylated proteins were designated p160, p53 and p23. The glycoprotein gp124 was found to be a disulphide-linked dimer, reducible to subunits with a M.W. of 62,000 (gp62).

Three proteins (p160, gp124 and p53) were found to be resistant to bromelain digestion. The glycoproteins gp26 and gp25 were apparently digested by bromelain to a species that migrated in the vicinity of p23.

Based on these results and for reasons given below, the following assignments were made. Of the above proteins, gp190 and gp100 represented the peplomeric proteins analogous to the E2 species (gp180/gp90) of mouse hepatitis virus (1,2). The most abundant protein species, p53, was the nucleocapsid protein and p160 was apparently a trimer of this protein (several hybridoma supernatants reacted with both of these proteins in western immunoblots). The proteins gp26, gp25 and p23 represented proteins analogous to the E1 species of mouse hepatitis virus (2). Finally, gp124/gp62 represented the same protein identified previously as the BCV hemagglutinin (3).

Monoclonal antibodies to BCV proteins have been produced and are presently being used to study the antigenic structure of these proteins. Five monoclonal antibodies which immunoprecipitate gp190, gp100 and a precursor glycoprotein with a M.W. of 170,000 (gp170) from BCV-infected cell lysates were highly effective in neutralizing virus in vitro with plaque reduction (50%) titers (PRD50) as high as 130,000. Immunoprecipitation patterns indicate that monoclonal antibodies reactive to gp124 bind to at least three distinct epitopes. Specifically, these monoclonal antibodies reacted differentially with an apparent degradation product of gp124 [a polypeptide of about 64k (M.W.) that was later found to be reducible to 33k (M.W.) subunits by a reducing agent]. Two of these monoclonal antibodies (reactive to two distinct epitopes) were very efficient at neutralizing virus in vitro with PRD50 titers of 50,000. Thus, both glycoproteins have epitopes that can generate highly neutralizing antibodies.

Western immunoblotting studies indicate that the epitopes defined by six of these neutralizing monoclonal antibodies, are dependent upon disulphide linkages for their antigenic integrity. One epitope, on gp124, was apparently destroyed by boiling alone.

Pulse chase experiments utilizing polyclonal antisera and monoclonal antibodies indicated that processing of the peplomeric proteins of BCV occurs in a similar manner as that suggested for mouse hepatitis virus (2,3). The large precursor glycoprotein, gp170, undergoes further glycosylation to yield gp190. Subsequent proteolytic cleavage of gp190 yields gp100. Pulse chase experiments dealing with the putative hemagglutinin (gp124/gp62), in which samples were subjected to nonreducing or reducing conditions, were consistent with the following interpretation. The primary precursor, a protein with a M.W. of 59,000 (later found to also be a glycoprotein) undergoes rapid dimerization (by disulphide bridging) to gp118. Further glycosylation of gp118 yields gp124. Thus, gp124 is very likely a homodimer.

References

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