Bovine Herpesvirus 1: Molecular and Antigenic Characteristics of Variant Viruses Isolated from Calves with Neurological Disease

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Summary

This report presents data showing that several virus isolates recovered in Argentina, mainly from calves with non-purulent meningo-encephalitis, represent a hitherto unrecognized antigenic variant of BHV-1. The following experimental approaches have been adopted to demonstrate both the unique features among and the relatedness with BHV-1 of these isolates: i) crossed serum neutralization test with rabbit immune sera, ii) analysis by SDS-polyacrylamide gel electrophoresis of radio-labeled virus induced polypeptides and glycoproteins, iii) discriminating reactivity of a panel of monoclonal antibodies which recognize known virus types, and iv) restriction endonuclease analysis of viral DNA. Another strain of BHV-1, which exhibits a specific neuropathogenic potential [HALL et al., Austral. Vet. J. 42, 229—237 (1966)] shares all major features with the viral strains originating from Argentina.

Our results imply that antigenic variants of BHV-1 exist and that they can be accurately and easily identified and differentiated by the available methods.

Introduction

It is generally accepted that all strains of bovine herpesvirus 1 (BHV-1) evaluated to date can be assigned to one out of two major virus types (type 1 and type 2) by analysing viral DNA with restriction endonucleases (for review see 13, 14, 18). More recently (18) it has been shown that the two virus types can also be differentiated from each other by evaluating virus
induced polypeptides in SDS containing polyacrylamide gels or by the discriminating reactivity of certain monoclonal antibodies.

During studies aiming at characterizing various isolates of BHV-1, which had been recovered in Argentina from calves suffering from a neurological disease (4) it became apparent that these isolates represented a hitherto unrecognized antigenic variant, which could not be grouped with established virus types.

The purpose of the present study was to compare the newly recognized virus isolates with known virus types and subtypes (18) as based on genome, glycoprotein, polypeptide, and antigen analyses. Data presented will show that antigenic variants of BHV-1 do exist. The significance of the findings was substantiated by the observation that the Australian BHV-1 strain N569 (6, 7) shares all major features with the isolates obtained from Argentina. Interestingly the N569 strain, also originating from a case of neurological disease (11) has been shown to exhibit a specific neurotropic potential (2, 9).

Materials and Methods

Viruses and Cell Cultures

All virus strains used in this study have received a limited number of in vitro passages, i.e. less than ten. Strains Jura, Spiel and Freiburg were chosen as reference viruses, representing established types and subtypes (type 1, subtype 2a, subtype 2b) of BHV-1 (18). Strain Jura was isolated in Switzerland in 1978 from an animal with acute respiratory tract disease. This virus has been used as challenge virus (12). Origin and characteristics of the isolates Spiel and Freiburg have been described (18). Strain N569 (6, 7) was kindly supplied by Dr. O. C. Straub (BFA, Tübingen, FRG). Strain A 663 was chosen out of several isolates with corresponding characteristics (4). Strain E/CH of caprine herpesvirus 1 (BHV-6) (5) was used for crossed neutralization tests. Virus stocks were grown from plaque purified viruses using MDBK cells (18). For most experiments MDBK cells were used. Embryonic bovine lung cells (eBLC) were used as indicated under results. Similar results were obtained irrespective of the host cell used. Hybridomas producing antibody to the LA strain of BHV-1 were generated and grown as described elsewhere (18).

Infection and Radio-labeling Cells

Preconfluent monolayers were infected with 10 to 20 TCID₅₀ per cell of the various virus strains. Infected monolayers (1 hour, 37°C) were washed with PBS (phosphate buffered saline) and fed with Eagle's MEM containing 1/10 the normal concentration of methionine. At 6 hours post infection the cultures received 5 μCi/ml of [³⁵S]methionine (specific activity >1,000 mCi/mmol). To label glycosylated polypeptides infected cultures were maintained in Eagle's MEM containing half the normal concentration of glucose and 2 μCi/ml [¹⁴C]glucosamine (specific activity 329.6 mCi/mmol). Radiochemicals were purchased from New England Nuclear Corp. At the end of the labeling period, usually at 22 to 24 hours post infection, the cells were washed with cold PBS, dislodged and centrifuged. Infected cell proteins (ICP) were extracted in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (62.5 mM Tris-HCl, pH 6.8, containing 3 per cent SDS, 1 per cent 2-mercaptoethanol, 15 per cent glycerol and 0.01 per cent bromphenolblue) by heating for 3 minutes at 100°C.