Bovine Herpesvirus 1: Differentiation of IBR- and IPV-Viruses and Identification and Functional Role of Their Major Immunogenic Components

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Summary

Infectious Bovine Rhinotracheitis (IBR) and Infectious Pustular Vulvovaginitis (IPV) virus strains of Bovine Herpesvirus 1 (BHV-1) can be differentiated by restriction endonuclease digestion of their DNAs. Antigens and polypeptide patterns of isolates of these different clinical entities are almost identical. Page analysis of immunoprecipitates revealed three major immunogenic components in BHV-1 infected cells. These are glycoproteins with apparent molecular weights of 93,000 (GP93), 74,000 (GP74) and 69,000 daltons (GP69), respectively. Bovine convalescent sera and antisera, which are directed against individual precipitates derived from crossed immunoelectrophoresis, contain antibodies reacting with one or more of these glycoproteins. The experiments with these antisera demonstrate that GP74 and possibly GP93, both structural components of the BHV-1 virion, induce neutralizing antibodies, whereas GP69, a non-structural protein, does not.

Introduction

Bovine Herpesvirus 1 (BHV-1) is one of the major pathogens of cattle and causes several diseases. The main clinical entities are infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV), but other manifestation sites can exist (13). Characterization of the viral genome (Skare, personal communication; 18) and biological properties of BHV-1 allow assignment of this virus to the alphaherpesvirinae (33). Investigations of the viral DNA using restriction enzyme analysis showed strain differences (7) and led to a separation into “IBR-like” and “IPV-like” strains (29).
Investigations of viral polypeptides have revealed a number of 18 to 21 structural components (27, 35) or 25 to 33 polypeptides in the purified virion (4, 22). From these eight (35), possibly up to eleven (22), were considered to be glycoproteins. MISRA et al. (23) described a glycoprotein (GPV 11) which appeared to be involved in antibody- and complement-mediated immunocytolysis of BHV-1-infected cells. Until now, however, no information has been available regarding the nature of the antigenic determinants involved in the humoral immune response.

The identification and characterization of the major immunogenic components of IBR- and IPV-viruses and the differentiation of these two subtypes of BHV-1 are the subject of this report.

**Materials and Methods**

**Cells and Media**

Georgia bovine kidney (GBK) cells, a fibroblastoid rabbit kidney cell line (RR cells, PAULI, unpublished) and primary bovine fetal lung (BFL) cells were all grown in Eagles essential medium, Dulbecco's modification (EDM, Gibco). During cultivation GBK and BFL cells were supplied with 3 per cent FCS, RR cells with 2 per cent rabbit serum.

**Viruses**

The history of the 20 strains of BHV-1 analysed in this study are summarized in Table 1. Stock virus was grown after inoculation of cells with a multiplicity of infection (MOI) of 10^-3 to avoid the appearance of defective interfering particles (6, 38). Cell free virus was harvested 24—36 hours post infection (p. i.), depending upon the MOI.

**Antisera**

Hyperimmune sera were prepared against three strains (B1, B4, MO6) with IPV-like restriction enzyme pattern of the DNA and one strain (MO2) with an IBR-like genome pattern. Infected RR cells, grown in the presence of rabbit serum in order to avoid problems with calf serum proteins which might act as immunogens (30) were harvested 30 hours p.i. For one dose a pellet of approximately 2×10^7 infected cells were suspended in 1 ml PBS and mixed with an equal volume of Freund's adjuvant. A homogenous mixture was produced by pressing this emulsion several times through a hypodermic needle with a conus on both ends for connecting two syringes. Cells with complete (first immunization) or incomplete (booster injections) Freund's adjuvant were administered intradermally into rabbits. Ten days after each immunization the rabbits were bled.

Preparation of antisera against individual precipitation bands obtained after two-dimensional immunoelectrophoresis was performed by immunization of rabbits with such immunoprecipitates (39). Hyperimmune sera from experimentally infected cattle were kindly supplied by Dr. Steinhagen, Neumünster, Federal Republic of Germany. A convalescent serum from a naturally infected cow was taken three weeks after abortion.

**Neutralization Tests**

Neutralization assays were performed using microtitre plates and an overlay medium containing 0.8 per cent carboxymethyl cellulose in EDM medium. The plaques were counted two days p.i. and the serum dilution effecting 80 per cent plaque reduction was calculated (28).