Production and Characterization of Monoclonal Antibodies to Peste des Petits Ruminants (PPR) Virus

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ABSTRACT

Peste des petits ruminants (PPR) is an acute, febrile viral disease of small ruminants, caused by a virus of the genus Morbillivirus. PPR and rinderpest viruses are antigenically related and need to be differentiated serologically. In the present study, 23 mouse monoclonal antibodies were produced by polyethylene glycol (PEG)-mediated fusion of sensitized lymphocytes and myeloma cells. Among these, two belong to the IgM class and the remaining 21 to various subclasses of IgG. The MAbs from the IgG class designated 4B6 and 4B11 neutralized PPR virus in vitro. In radioimmunoprecipitation assay, 10 MAbs recognized nucleoprotein, 4 recognized the matrix protein and one each haemagglutinin and phosphoprotein. The remaining 7 MAbs failed to precipitate any defined viral protein. The reactivity pattern of the monoclonal antibodies in indirect ELISA indicated a close antigenic relationship within three Indian PPR (lineage 4) virus isolates and also within two rinderpest vaccine strains. All PPR virus isolates could be distinguished from rinderpest vaccine viruses on the basis of the reactivity pattern of all MAbs and anti-N protein MAbs. A set of six monoclonal antibodies specific to PPR virus could also be identified from the panel. From the panel of MAbs available, two MAbs were selected for diagnostic applications, one each for the detection of antigens and antibodies to PPR virus.

Keywords: antigen, monoclonal antibody characterization, peste des petits ruminants virus, rinderpest virus

Abbreviations: CPE, cytopathic effect; ELISA, enzyme-linked immunosorbent assay; HRPO, horse-radish peroxidase; kDa, kilodalton; MAb, monoclonal antibody; MEM, minimum essential medium; OPD, ortho-phenylenediamine; PEG, polyethylene glycol; PMSF, phenylmethylsulphonyl fluoride; PPR, peste des petits ruminants; RIPA, radioimmunoprecipitation assay; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TCID, tissue culture infective dose

INTRODUCTION

Peste des petits ruminants (PPR) is an acute, febrile viral disease of small ruminants, characterized by necrotizing and erosive stomatitis, enteritis and pneumonia (Ismail et al., 1995). The disease is caused by an RNA virus (PPR virus) belonging to the genus Morbillivirus of the family Paramyxoviridae. The virus is antigenically related to other morbilliviruses (Gibbs et al., 1979), e.g. rinderpest virus (RPV), canine distemper virus (CDV) and measles virus (MV). Among the morbillivirus group, PPR and rinderpest
viruses have been found to cause similar diseases in small ruminants (Diallo et al., 1989). The two viruses show very close antigenic and genetic relationship. The genome of these viruses is divided into six transcriptional units encoding two non-structural proteins (V, C) and six structural proteins. The surface glycoproteins include fusion (F) and haemagglutinin (H) proteins. Internal virion proteins include the matrix (M) protein, the nucleoprotein (N), and the phosphoprotein (P), which forms the polymerase complex in association with the large (L) protein (Crowley et al., 1988; Sidhu et al., 1993; Diallo et al., 1994). Morbilliviruses differ considerably in epitope homology of H proteins, while epitopes in F protein are relatively conserved. The internal virion proteins such as N and M have been shown to have both specific and cross-reacting epitopes (McCullough et al., 1986; Sheshberadaran et al., 1986). Therefore, anti-N protein monoclonal antibodies to rinderpest virus alone were used to establish relationships between different morbilliviruses. The nucleocapsid gene and the protein have been the focus of attention for differentiation of PPR and rinderpest viruses by various workers (McCullough et al., 1986; Diallo et al., 1987, 1989).

The main objective of the investigation of the properties of monoclonal antibodies (MAbs) to PPR virus was to select and identify these for antigenic characterization, and detection of antigen and antibodies to PPR virus using suitable ELISA systems. Recent epidemiology of PPR viruses in India and more broadly in Asia, suggests that all the viruses belong to lineage 4 (Dhar et al., 2002) with the exception of ‘India/TN/92’, a lineage 3 virus (Shaila et al., 1996). In a changing epidemiological scenario, a well-characterized panel of monoclonal antibodies could be an important tool for morbillivirus research. Further, the use of monoclonal antibodies to PPR viruses, though reported scantily, will lead to better understanding of antigenic relatedness between various members of the morbillivirus genus.

In the present study, we report (i) the production of 23 MAbs from a single fusion using a crude antigen derived from an Indian isolate of PPR virus; (ii) the characterization of MAbs for class and subclass, virus neutralization ability, antigenic specificity using radioimmunoprecipitation assay and reactivity with various PPR and rinderpest virus isolates in ELISA; and (iii) the subsequent use of MAbs to determine antigenic relationships between established rinderpest viruses and PPR virus isolates.

MATERIALS AND METHODS

Cell lines

Vero cells, B95a cells and myeloma cells used in the present study were available in the authors’ laboratory. Vero cells between passages 130–160, propagated in Earle's MEM with 10% fetal calf sera, were used to prepare the antigen for immunization of mice. B95a cells are lymphoblastoid cells from the marmoset monkey (Kobune et al., 1991). These cells were propagated in RPMI medium supplemented with 10% fetal calf serum and maintained in the same medium with 2% fetal calf serum. B95a and Vero cells were used for production of PPR virus antigen for screening of primary hybridoma, characterization of monoclonal antibodies and virus neutralization test. Myeloma cells