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Production of monoclonal antibodies against Rift Valley fever virus Application for rapid diagnosis tests (virus detection and ELISA) in human sera

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Abstract

This paper describes the production and characterization of RVFV monoclonal antibodies. The characteristics of 32 out of 55 ELISA and/or IFA positive monoclonal antibodies were determined, including the RVFV components against which they are directed. One monoclonal antibody recognized the nucleoprotein, 15 the Gc and 16 the Gn. Among the latter ones, five monoclonal antibodies possess another specificity and recognized both Gn and either the nucleoprotein (four of them) or the NSs protein (one). To validate the use of these monoclonal antibodies for diagnosis tests, a pool of monoclonal antibodies reacting with the structural proteins was prepared and used successfully to detect RVFV from cell culture as well as viral antigen–antibody complex in ELISA.

Keywords: Plebovirus; Bunyaviridae; Hybridoma; Immunofluorescence; Serological tests

1. Introduction

Rift Valley fever (RVF) is an emerging epidemic disease of humans and livestock which is caused by Rift Valley fever virus (RVFV). This virus belongs to the family Bunyaviridae, and the plebovirus genus (Elliott, 1996). The virus is transmitted to livestock and humans by bites of infected mosquitoes or exposure to tissues or blood of infected animals. Massive epizootics are typically observed in livestock during times of unusually high and sustained rainfall because of the presence of breeding sites and overabundance of adult competent mosquito vectors (Linthicum et al., 1999). Infections caused by RVFV are characterized by severe disease and abortion in livestock, particularly sheep and cattle. In the epidemic region, populations are at high risk for RVFV infection, potentially leading to thousands of human cases. Humans infected with RVFV have self-limited febrile illness, but retinal degeneration (5–10%), hemorrhagic fever (<1%), or encephalitis (<1%) may also develop (Meegan and Bailey, 1989; Laughlin et al., 1979). The virus used to be restricted to sub-Saharan Africa, but in 1977 it emerged for the first time in Egypt causing severe epizootics and epidemics with increased morbidity and mortality (Meegan, 1979; Laughlin et al., 1979). In 2000, the virus spread beyond Africa causing severe epidemics in humans in Saudi Arabia and Yemen (CDC, 2000a, 2000b). RVFV has a trisegmented RNA genome of negative (L and M segments) or ambisense (S segment) polarity. The L and M segments code, respectively, for the RNA-dependent RNA polymerase and for a polypeptide precursor which is cleaved during translation, thus generating the envelope glycoproteins Gn, Gc and the nonstructural proteins 14K and 78K. The S segment utilizes an ambisense strategy: the 5′ halves of the antigenomic and genomic strands code, respectively, for the nucleoprotein N and the nonstructural protein NSs (Giorgi, 1996; Schmaljohn, 1996).

Diagnosis of RVF can be achieved by different methods, namely virus isolation, RT-PCR or detection of viral

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antigens. Virus isolation is considered as the gold standard but it requires biosafety type three laboratory. RT-PCR is highly sensitive and reproducible (Garcia et al., 2001; Sall et al., 1999, 2002; Drosten et al., 2002). However, it is still a research tool which is not available in every laboratory. The viral antigens can also be detected in patient blood during viremia, but the assays may lack sensitivity. In parallel with virological methods which detect the virus or its components, IgM capture and IgG sandwich ELISAs (Niklasson et al., 1984; Martin et al., 2000; Johnson et al., 2000; Paweska et al., 2005), are widely utilized in clinical laboratories. These techniques are rapid, sensitive, specific and useful to reveal infected animal in endemic areas or during an epizootic (Paweska et al., 2003a, 2003b). However, they require RVFV specific antigens and antibodies to monitor the presence of antibody-antigen complexes. RVFV specific polyclonal antibodies are usually produced in mice but the antibody titer varies with each batch. On the other hand, the production of monoclonal antibodies specific for RVFV insures a constant source. These antibodies are revealed with anti-mouse IgG conjugated to peroxidase or are themselves conjugated to this enzyme. Not only, the RVFV monoclonal antibodies are necessary for serology but also they can be helpful for a rapid identification of a RVFV infected cell culture by indirect immunofluorescent assay (IFA). This paper describes the production of monoclonal antibodies that can be used to identify RVFV in cell cultures or to detect RVFV antibodies by IgM or IgG capture ELISA using a monoclonal antibody-based capture ELISA was reported. These monoclonal antibodies were pooled and, for IgM capture ELISA, they were conjugated with peroxidase.

2. Materials and methods

2.1. Cells

C6/36 cells were grown in Eagle minimal essential medium supplemented with 10% foetal calf serum (FCS), 10% tryptose phosphate; BHK-21 in Glasgow minimal essential medium (MEM) supplemented with 5% FCS, 10% tryptose phosphate and 10 mM HEPES; BSR cells (a clone of BHK-21cells) in Glasgow MEM supplemented with 10% FCS; and Vero cells in Dulbecco modified Eagle medium supplemented with 5% FCS. Penicillin (5 U/ml) and streptomycin (5 μg/ml) were added. The cells were incubated at 37 °C in a 5% CO_2 atmosphere except for C6/36 which were cultured at 28 °C in closed flasks.

2.2. Virus

The virus Gizan 02 strain was isolated in Saudi Arabia during the 2000 epidemic and passaged in suckling mice twice. The Kenyan strain (Ken97) was kindly provided by Swanepoel. The ZH548 strain was isolated from a human infection during the 1977 outbreak in Egypt. Virus stocks were prepared by infecting vero cells at low m.o.i.

2.3. RVFV antigen preparation for immunization

Suckling mice were injected with the Gizan 02 strain by intracerebral route. When symptoms appeared, mice were killed and brains were harvested and homogenized as 10% (w/v) suspension in phosphate buffered saline which was formalin inactivated when used for mice immunization.

2.4. Animal immunization

Groups of five BALB/c female mice of 8 weeks age were first inoculated subcutaneously with two doses of formalin-inactivated RVFV infected brain suspension at 15 days interval followed by three doses of infectious virus at 10-day interval. The first two injections were combined with Freund's complete adjuvant, while the rest of injections were combined with Freund's incomplete adjuvant. The mice were killed 4 days after the last injection, their sera analyzed for the presence of antibodies and their spleens were used for production of hybridoma.

2.5. Hybridoma production

The splenocytes from antibody-positive mice were fused with SP2/0-Ag14 myeloma cells by using 50% (w/v) polyethylene glycol (molecular weight, 1300–1600; Sigma Chemical Co.) according to the procedure described (Xu et al., 1997). The fused cells were grown in hybridoma selective medium (Iscove's modified Dulbeco medium; Sigma Chemicals Co.) containing 20% fetal bovine serum (Gibco BRL) and hypoxanthine–aminopterin–thymidine (HAT) selective medium (Sigma Chemical Co.). After 2 weeks, HAT was replaced by hypoxanthine–thymidine (HT) medium (Sigma Chemical Co.). After 5 days of incubation, the supernatants from viable hybridoma clones were screened for antibodies against RVFV by IFA. Positive hybridoma cells were subcloned two to three times by limit dilution. Supernatants from positive hybridoma were further analyzed by ELISA, Western blotting and plaque reduction neutralization tests to determine their specificity.

2.6. Immunofluorescence assay

IFA was used to screen hybridoma clones and determine the specificities of the monoclonal antibodies. RVFV infected Vero cells were mixed with non infected Vero cells and deposited on Teflon coated 10-well slides. The slides were air dried inside a biosafety cabinet and fixed in chilled acetone or acetone/methanol for 20 min. The assay was modified from a previously described procedure (Xu et al., 1997). Briefly, the wells were overlaid with 30 μl of supernatants from hybridoma clones, and the plates were incubated in a moist chamber at 37 °C for 30 min before they were