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Reverse ELISA for IgG and IgM antibodies to detect Lassa virus infections in Africa

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Abstract

Background: Anti-Lassa antibodies are detected by indirect immunofluorescence assay (IFA) or by enzyme-immunoassay (ELISA). Both methods have problems to detect low amounts of specific antibodies.
Objectives: We report here highly sensitive and specific reverse ELISAs to detect Lassa virus IgG and IgM antibodies. Due to the reverse techniques, serum samples could be applied at dilutions of 1:10 without increasing non-specific background reactions.
Study design: For IgM antibody detection microtiter plates were coated with anti-IgM antibodies and for IgG antibody detection with rheumatoid factor (RF) (Sachers M, Emmerich P, Mohr H, Schmitz H. Simple detection of antibodies to different viruses using rheumatoid factor and enzyme-labelled antigen (ELA). J Virol Methods 1985;10:99–110). In both assays a tissue culture antigen was used in combination with a labeled anti-Lassa monoclonal antibody (Hufert FT, Ludke W, Schmitz H. Epitope mapping of the Lassa virus nucleoprotein using monoclonal anti-nucleocapsid antibodies. Arch Virol 1989;106(3–4):201–12).
Results: The reverse ELISA turned out to detect virus-specific IgG and IgM antibody in all 20 samples of West African patients collected 2–8 weeks after onset of Lassa fever. Moreover, both IFA and reverse ELISA found IgG antibodies in 53 out of 643 samples of healthy West Africans (sensitivity of 100%). Six of the 643 samples were positive by reverse IgG ELISA only. Thus, the specificity compared to IIF was 99.0%, but it may be even higher, because compared to IFA the IgG ELISA was clearly more sensitive in detecting low antibody titers.
Conclusions: In Ghana 3% seropositives were found by IFA, but 4% by the reverse ELISA. The reverse ELISAs can be performed with high sensitivity and specificity under field conditions in Africa.

Keywords: Reverse ELISA; Lassa IgG; IgM; Guinea; Ghana

1. Introduction

Lassa fever is highly endemic in Sierra Leone, Liberia and Nigeria (Carey et al., 1972; Frame et al., 1970).

During the first days after onset the clinical diagnosis of Lassa fever is difficult (McCormick et al., 1987a; Schmitz et al., 2002). Therefore, a laboratory diagnosis is needed, although this is difficult to achieve in West Africa without high tech laboratories.

Acute Lassa fever is reliably diagnosed by virus isolation (Johnson et al., 1987; Jahrling et al., 1985a), by antigen detection (Bausch et al., 2000; Jahrling et al., 1985a,b) and by RT-PCR (Demby et al., 1994; Drosten et al., 2002; Lunkenheimer et al., 1990). RT-PCR has turned out to be most sensitive. While RT-PCR and virus isolation pose technical problems in West African field hospitals, antigen tests would be more appropriate. But they are less sensitive and cannot detect low amounts of virus (Bausch et al., 2000; Schmitz et al., 2002).

As an alternative, antibodies tests may help to diagnose an acute Lassa virus infection. Thus, the presence of anti-Lassa IgM antibody or a significant rise in titer of IgG antibodies can prove an acute Lassa virus infection (Johnson et al., 1987;

Abbreviations: RF, rheumatoid factor; SA, soluble antigen; IFA, indirect immunofluorecence assay

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McCormick et al., 1987b; Wulff and Lange, 1975). Besides, IgG antibodies to Lassa may be useful to study the epidemiology of Lassa fever infection (Lukashevich et al., 1993a; McCormick et al., 1987b).

The best-studied method for antibody detection is IFA (Knobloch et al., 1980; Wulff and Lange, 1975). Since IFA has been considerably observer dependent, we have improved the reading of slides by counterstaining the antigen inside the cells (Haas et al., 2003).

As an alternative to IFA, indirect ELISA techniques have been published to detect IgG and/or IgM antibodies using tissue culture (Bausch et al., 2000; Jahrling et al., 1985b; Niklasson et al., 1984) or by recombinant antigens (Barber et al., 1990; Gunther et al., 2001; ter Meulen et al., 1998). Anti-Lassa IgG antibodies were detected by indirect ELISAs. In contrast, IgM antibodies were also detected by a reverse system using anti-IgM coated plates (Bausch et al., 2000; Jahrling et al., 1985b; Niklasson et al., 1984).

Unfortunately, indirect ELISAs were not as sensitive as the IFA in detecting IgG antibody (Bausch et al., 2000). This may be explained by the high serum dilutions of about 1:≥100, which have to be applied to avoid non-specific background staining. Moreover, in the case of recombinant proteins (Barber et al., 1990; Gunther et al., 2001; ter Meulen et al., 1998) the loss of virus-specific epitopes may also contribute to a reduced sensitivity.

In contrast to indirect ELISA techniques, using labeled anti-immunoglobulin conjugates we have applied here a reverse ELISA technique not only for IgM but also for IgG antibodies. To detect IgG antibodies, we have used a solid phase coated with rheumatoid factor (RF) (Sachers et al., 1985), where the amount of bound antibody is measured using either a directly labeled antigen (Schmitz et al., 1980) or an antigen that is detected with a labeled monoclonal antibody (Schmitz and Gras, 1986). Coating of the solid phase with anti-IgG antibodies, similar to anti-IgM coating, would be too insensitive. Instead, we selectively bind Lassa antigen–antibody complexes to RF during simultaneous incubation of the serum with Lassa antigen. The bound antigen is finally detected using a biotinylated anti-Lassa mouse monoclonal antibody.

The reverse ELISAs both for anti-Lassa IgG or IgM antibody detection allowed to apply the sera undiluted without increased background staining. The sensitivity and specificity of the reverse IgG and IgM ELISAs were evaluated with samples of 20 acute Lassa fever patients and with more than 600 serum samples of healthy West Africans.

2. Material and methods

2.1. Serum samples

From studies in Nigeria (Omilabu et al., 2005), Liberia, Sierra Leone and Guinea late serum samples of 20 Lassa fever survivors were available. At onset of the disease all patients had had high fever and elevated aminotransferases. In the samples taken 2–30 weeks after onset, IgM and IgG antibodies to Lassa virus had be detected by IFA. In 11 of the 20 patients, of whom early samples were available, Lassa virus RNA was found by RT-PCR (Drosten et al., 2002).

Moreover, upon ethical clearance, 643 samples were collected from adult, healthy subjects in West Africa (age 18–53 years, m/f 1.2:1). Four hundred and thirty samples were from Ghana and 213 from Guinea. Serum samples of 200 healthy German blood donors were also tested.

2.2. IFA

IgG antibodies to Lassa virus were detected using acetone-fixed Vero cells infected with Lassa virus strain Josiah (Wulff and Lange, 1975). Cultivation of the viruses was carried out in a BSL 4 laboratory. Our own data have consistently shown that from acetone-fixed Lassa virus infected cells no virus can be cultivated upon inoculation of Vero E6 cells. Serum samples were tested in two-fold steps starting at a dilution of 1:20. Cell smears were routinely counterstained with an anti-Lassa nucleocapsid monoclonal antibody 2F1 (Hufert et al., 1989) using Rhodamine-anti-mouse as secondary antibody (Haas et al., 2003). Also cell smears of Vero E6 cells infected with Lymphocytic Choriomenigitis virus (strain Amstrong M20869) and with Mopeia virus (strain 800150, M33879) were prepared.

2.3. Preparation of soluble antigen (SA)

SA was obtained by sonification of Lassa virus (Josiah strain) infected Vero E6 cells of one bottle (50 ml) in 10 ml PBS containing 0.3% detergent (NP 40, Sigma–Aldrich, Munich, Germany). Cell debris was removed by low speed centrifugation (5000 × g, 20 min). The SA could be stored lyophilized at room temperature and was additionally inactivated by gamma irradiation (24 kGray) (Bausch et al., 2000).

2.4. Monoclonal antibody

Monoclonal antibody 2F1 (Hufert et al., 1989) reacts with the nucleoprotein of several Lassa viruses (Josiah isolate of Sierra Leone, AV of Ivory Coast, CSF of Nigeria; Gunther and Lenz, 2004) by IFA. It does not cross react with Mopeia virus or LCMV. It was purified to 1 mg/ml and labeled with NHS biotin (Sigma–Aldrich) according to standard procedures.

2.5. Antigen titration

The Sandwich ELISA has been described in more detail earlier (Schmitz and Wolff, 1986). The antigen was incubated on the antibody (2F1) coated plates over night at 4 °C and was detected using the biotinylated antibody 2F1 at a dilution of 1:4000 followed by strepavidine-peroxidase (see reverse ELISA).