LASSA FEVER : IMMUNO-EPIDEMIOLOGICAL APPROACH TO THE STUDY OF AN ENDEMIC VIRAL HAEMORRHAGIC FEVER

J. TER MEULEN

• *Institute for Virology, Philipps University, Marburg, Germany and Projet de Recherche sur les Fièvres Hémorragiques en Guinée (PFHG), Conakry, République de Guinée • Fax : 00 49 40 31182 378 •termeulen@bni.uni-hamburg.de •*

Med. Trop. • 2000 • 60 • 20S-22S

The Lassa virus belongs to the family of *Arenaviridae,* the members of which persist in chronically infected rodents without causing overt pathology in their natural hosts. Rodents shed virus in high concentrations (106/ml) in their urine. Reservoir and vector of the Lassa virus is a small rodent, *Mastomys natalensis,* which is prevalent all over Africa. Since this rodent preferentially lives in a semi-domestic fashion in or near human dwellings, rodent-to-human transmission of the virus occurs via food stuff contaminated with the animal's excrements. Human-to-human transmission is not normally of major importance. In forest areas hunting of small mammals including *Mastomys* as a source of proteins is common practice and manipulation of infected rodents poses a major risk of infection (1).

Lassa fever, named after its first appearance in 1969 in Lassa, Nigeria, is very common in certain areas of West Africa (Nigeria, Liberia, Sierra Leone, Republic of Guinea). The clinical disease must occur undiagnosed in more West African countries, because a recent case of imported Lassa fever in Germany originated either from Côte d'Ivoire, Ghana or Burkina Faso (2). In some hospitals of north-eastern Sierra Leone, up to 15 p. 100 of all medical inpatients suffer from Lassa fever (3). In a study which we conducted in the Republic of Guinea, 16.4 p. 100 of all inpatients with the diagnose «pyrexia of unknown origin (PUO)» had Lassa fever (4). Serosurveys in Sierra Leone and Liberia revealed up to 50 p. 100 seroprevalence of Lassa antibodies in selected villages, with 6 p. 100 of the seronegative persons seroconverting annually. Based on these data it was calculated that 70 p. 100 of all Lassa virus infections are asymptomatic and that the mortality-to-infection ratio is 1-5 p. 100. However, 10-15 p. 100 of symptomatically infected patients die (3).

CLINICAL PICTURE OF LASSA FEVER

The clinical picture of a Lassa virus infection is quite variable. After an incubation period of 7-18 days unspecific flu-like symptoms develop. The fever rises above 40°C between the third and sixth day and a conjunctivitis is frequently observed. Concomitant with the development of thoracic and abdominal pain, vomiting and diarrhea can occur. Typical, but not pathognomonic for Lassa fever are a dry cough accompanied by retrosternal pain, as well as an exsudative pharyngitis with yellowish patches on the tonsills. 50 p. 100 of patients develop a generalized, painless lymphadenopathy. Interstitial pneumonia is quite common and may be the leading pathological finding in a returned traveller with PUO, as was the case in the recent Lassa fever patient imported from Sierra Leone to London (5). The disease then takes two different courses : in the majority of cases, defervescence is accompanied by diminishing headache, thoracic and pharyngeal pain. More than 30 p. 100 of patients develop an uni- or bilateral auditory deficit, which is irreversible in two of three cases and probably caused by an immunopathogenetic process. However, 15 p. 100 of all patients deteriorate rapidly. They suffer from intractable vomiting, develop a typical edema of the face and neck and may additionally become dyspnoic and cyanotic. In fatal cases, encephalopathy, hemorrhages (20 p. 100) and pleural effusions are observed, with circulatory failure and irreversible shock finally leading to death. Bleeding usually occurs only at mucosal surfaces but not into the skin, so that gastrointestinal, vaginal, urogenital and gingival hemorrhages and epistaxis are observed.

A specific therapy of Lassa fever offers the nucleosid-analogue Ribavirin (1-beta-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide). Clinical trials have shown that early administration of the drug, i.e. within six days after onset of symptoms, decreases mortality from 75 p. 100 to 9 p. 100.

PECULIARITY OF THE IMMUNE RESPONSE TO LASSA VIRUS AND THE PROBLEM OF VIRUS VARIABILITY

The Lassa virus is poorly cytopathogenic, causes a persistent infection in its natural host and induces no or only low-titre neutralizing antibodies. Animal vaccination studies using recombinant vaccinia-Lassa constructs have shown that protection against an otherwise lethal Lassa virus infection is solely T-cell mediated (6). Cytotoxic T-cells presumably play the pivotal role, because adoptive CD8 T-cell transfer from guineapigs vaccinated with avirulent arenaviruses protected naive animals against a fatal Lassa virus infection. However, this protection was restricted to cells

Table I - Proliferative responsa of PBMC from Lassa virus antibody positive and negative individuals.

Sex/age	Lassa antibody Titre	Proliferative response JOS-recNP	NEG	P.I.
M/45	1: 20	8200	92	91*
M/23	1:160	1390	40	35*
F/81	1: 20	4670	150	31*
M/24	1:160	2680	90	30*
F/44	1:160	4060	170	24*
M/30	1: 80	3370	160	21*
M/15	neg.	1340	100	13*
M/16	1:160	2960	300	10*
F/62	1: 80	300	60	5
F/19	1:160	320	120	2,7
M/38	1:160	150	60	2,5
M/76	neg.	290	120	2,4
M/86	1: 40	179	79	2,3

sampled during early convalescence of the animals. Interestingly, highly virulent Lassa virus strains did not induce a CTL-response. It is not known whether an immunological memory exists, which is capable of protecting against challenge with other variants. Vaccination of monkeys with recombinant Vaccinia-Lassa constructs protected from disease but not infection upon challenge with a virulent Lassa virus strain (7). The animals developed viremia and mild disease and Lassa virus RNA was detectable by PCR in several organs throughout the observation period (6 months).

In contrast to the animal models, little is known concerning the immunity to Lassa fever in humans. Neutralizing antibodies develop late in convalescence and to low titres, therapeutic efforts using «immune plasma» have therefore not been very successful in the past. We could recently show that peripheral blood mononuclear cells of healthy, Lassa antibody positive and negative donors show a strong proliferative reaction to recombinant Lassa virus nucleoprotein (Table I). The observed proliferation index of up to 90 (specific proliferation in comparison to a control protein expressed in the same *Escherichia coli* expression system) is unusually high and indicates the presence of very active CD4-memory cells. T-cell clones generated from one donor reacted partly strain specific (with the Josiah strain of Lassa) and partly cross-reactive (Nigeria, Mopeia) with their respective peptide epitopes, mapped on the nucleoprotein (8). During field studies, we have identified healthy, asymptomatic individuals with high anti-Lassa IgG but not IgM antibody titres, who had Lassa virus RNA detectable in their urine. Preliminary data suggest that individuals undergoing asymptomatic infection with excretion of viral RNA in their urine show a absent or reduced virus specific CD4 T-cell proliferation compared with those who do not excrete RNA.

The crucial role of T-cell immunity with respect to both clearance of the acute infection and protection from disease upon reinfection, has important implications for the epidemiology of Lassa fever. The MHC-restriction of the T-cell response could negatively affect a cross-protection against different Lassa virus strains, given a sufficient genetic variablity of the virus. To date, only three Lassa virus strains have been completely sequenced and published, revealing approx. 10 p. 100 divergence on the aminoacid (aa) level. Our studies in Guinea produced PCR based evidence of a new Lassa strain with 10.4 p. 100 variation on the aa-level of the nucleoprotein (4). A systematic correlation of Lassa virus strain, disease severity and genetic background of the patient has so far not been carried out, however, virus strains isolated from patients with severe disease tend to be more virulent for guineapigs than others.

A HYPOTHESIS TO EXPLAIN THE OCCURRENCE OF SEVERE LASSA FEVER AND LASSA FEVER EPIDEMICS

Lassa fever epidemics occur in remote, rural areas and rural hospitals, where they have been associated with poor standards of hygiene. Nosocomial transmission usually involves direct contact with the patient's body fluid, wherefore strict «barrier nursing» (gloves, gowns, masks, goggles) can effectively prevent transmission of the virus (9). In contrast, it seems completely unclear why Lassa fever epidemics occur outside of hospitals amidst a population, which should be immune if judged by the prevalence of Lassa virus antibodies and the normally benign course which Lassa fever tends to take. During the last major outbreak in Sierra Leone in 1996/1997 more than 800 Lassa fever cases with a case fatality rate of 20 p. 100 have been reported to WHO (10). We propose that understanding the interplay of three factors will explain these obvious contradictons. First, the mode of transmission of the virus is important ; second, the genetic variability and different pathogenicity of Lassa virus strains ; and third, the extend of cross-protective immunity against different Lassa virus variants in a given population.

If the most prevalent Lassa variant found in *Mastomys* is not very pathogenic, a high seroprevalence will contrast with a low morbidity and mortality in the population. In times of crisis, e.g. during the civil war in Sierra Leone in 1996/1997, when regular field work is almost impossible, the people will have to rely on increased hunting activities to meet their demand for food. With extension of the hunt to small mammals, the possibility of contact with rare and possibly more virulent Lassa virus variants will increase. If the T-cell based immunity against the major, less virulent variant does not prevent disease caused by the rare, more virulent strains, severe cases of Lassa fever will be seen which may reach epidemic proportions due to the potential of the virus for human-to-human transmission.

PERSPECTIVES

Due to the outbreak of Ebola in former Zaire in 1995 the interest in hemorrhagic fever research has risen continuously during the past years. The European Community supports since 1998 a multinational cooperation project between European and West African partners, with the aim to develop technologies for the rapid diagnosis of viral hemor-

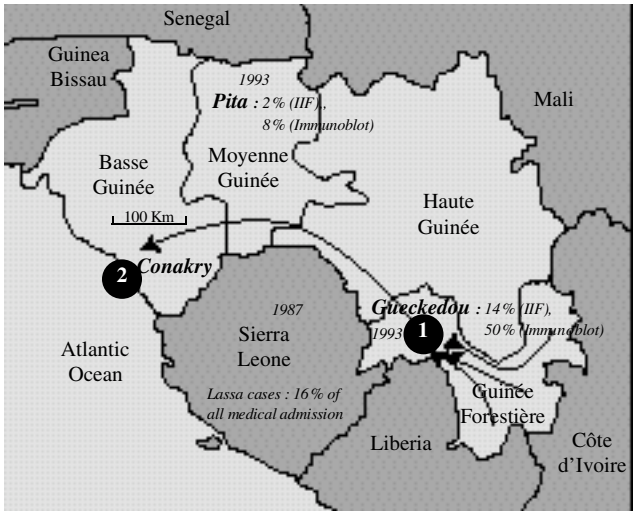

Figure 1 - Projet de Recherche sur les fièvres hémorragiques en Guinée (PFHG). Suspected viral hemorrhagic fevers cases are identified in the South of the Republic of Guinea. Serum is stored in a first-line laboratory in Gueckedou and then transported for futher analysis to Conakry. Seroprevalence of Lassa virus antibodies in Guinea and Sierra Leone is indicated.

rhagic fevers and to deploy them to endemic areas in developing countries. Within this framework the *Projet de Recherche sur les Fièvres Hémorragiques en Guinée* (PFHG) at the Institute for Microbiology, University of Conakry. was founded in 1998. Through prevalence and prospective studies the natural history of Lassa virus and other viral hemorrhagic fever infections (Yellow fever, filovirus infections) shall be elucidated in the Republic of Guinea. Suspected viral hemorrhagic fever cases are presently identified in three regional hospitals in the South of Guinea (Fig. 1). Patient material is transported in a cold chain to Conakry, where a biosafety level 3/4 laboratory was set up for diagnostic and research purposes. Immunofluorescence, ELISA, immunoblots and RT/PCR can presently be performed on the clinical samples. As far as Lassa fever is concerned, the studies focus on the clinical picture of primary infections and reinfections in relation to the infecting viral strain and the patients' T-cell response. During these studies the infrastructure and know-how shall be established to investigate outbreaks of Lassa fever in the Republic of Guinea as well as in neighboring countries to test the hypothesis which was outlined above.

REFERENCES

1 - TER MEULEN J., LUKASHEVICH I., SIDIBE K. et Coll. -Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of guinea. *Am. J. Trop. Med. Hyg.* 1996 ; **55** : 661-666.

2 - GUENTHER S. , EMMERICH P., LAUE T. et Coll. - Imported Lassa fever in Germany: Molecular characterization of the virus by full-length amplification and sequencing of the 3.5kb S-RNA. *Emerg. Infect. Dis.* 2000 ; (in press).

3 - MACCORMICK J.B., WEBB P.A., KREBS J.W. et Coll. - A prospective study of the epidemiology and ecology of Lassa fever. *J. Infect. Dis.* 1987 ; **155** : 437-444.

4 - TER MEULEN J., KOULEMOU K., WITTEKINDT T. et Coll. - Detection of Lassa virus antinucleoprotein immunoglobulin G (IgG) and IgM antibodies with a simple recombinant immunoblot assay for field use. *J. Clin. Microbiol.* 1998 ; **36** : 3143-3148.

5 - PHLS - Communicable Disease Surveillance Center, London. Lassa fever imported to England. Commun. *Dis. Rep. CDR Wkly* 2000 ; **10** : 99.

6 - CLEGG J.C. - Current progress towards vaccines for arenavirus-caused diseases. *Vaccine* 1992 ; **10** : 89-95.

7 - FISCHER-HOCH S.P., MCCORMICK J.B., AUPERIN D. et Coll. - Protection of rhesus monkeys from fatal Lassa fever by vaccination with a recombinant vaccinia virus containing the Lassa virus glycoprotein gene. *Proc. Natl. Acad. Sci.* 1989 ; **86** : 317-321.

8 - TER MEULEN J., BADUSCHE M., KUHNT K. et Coll. - Characterization of human CD4+ T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. *J. Virol.* 2000 ; **74** : 2186-2192

9 - HOLMES G.P., MCCORMICK J.B., TROCK S.C. et Coll. - Lassa fever in the United States : Investigation of a case and new guidelines for management. *N. Engl. J. Med.* 1990 ; **323** : 1120-1123.

10 - WHO - Lassa fever in Sierra Leone. *Weekly Epidemiol. Rec.* 1997 ; **72** : 173-178.