

Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses

P. B. Jahrling, J. Geisbert, J. R. Swearingen, G. P. Jaax, T. Lewis, J. W. Huggins, J. J. Schmidt, J. W. LeDuc*, and C. J. Peters**

United States Army Research Institute of Infectious Diseases Fort Detrick,
Frederick, Maryland, U.S.A.

Summary. A commercially available immunoglobulin G (IgG) from horses, hyperimmunized to Ebola virus, was evaluated for its ability to protect cynomolgus monkeys against disease following i.m. inoculation with 1 000 PFU Ebola virus (Zaire '95 strain). Six monkeys were treated immediately after infection by i.m. injection of 6.0 ml IgG; these animals developed passive ELISA titers of 1:160 to 1:320 to Ebola, two days after inoculation. However, the beneficial effects of IgG treatment were limited to a delay in onset of viremia and clinical signs, in comparison with untreated controls. The six IgG recipients had no detectable viremia day 5, in contrast with three virus infected controls whose viremias exceeded $7.0 \log_{10}$ PFU/ml that day. The controls died on days 6, 6, and 7, while two IgG recipients died day 7 and the remaining 4 died day 8, all with high viremias. These results document that passively acquired antibody can have a beneficial effect in reducing the viral burden in Ebola-infected primates; however, effective treatment of human patients may require antibodies with higher specific activities and more favorable pharmacokinetic properties than the presently available equine IgG.

Introduction

The recent outbreak of Ebola fever in Zaire claimed 244 human lives (MMWR 95) among 344 cases and attracted world-wide attention to the highly virulent nature of this viral infection [2, 3, 13]. Unlike some other viral haemorrhagic fevers (VHF) for which effective antiviral drugs have been identified, Ebola virus infections can not be effectively treated [11]. Immune plasma has been used successfully with other VHF infections [8, 9, 11] and there are anecdotal case reports in the literature suggesting the potential benefits of passive immunization

Present addresses: * World Health Organization, Geneva, Switzerland; ** Special Pathogens Branch, Centers for Disease Control, Atlanta, Georgia, U.S.A.

against Ebola infection [1]. In the waning days of the 1995 outbreak, reports began to surface that whole blood transfusions from recently convalescent patients had been used with remarkable success to treat acutely ill Ebola fever patients. Evaluation of those studies must await formal publication and analysis of the virological and immunological aspects of the treated patients in comparison with untreated cohorts. Even if these whole blood transfusion studies withstand scientific scrutiny and prove the principle of antibody therapy for Ebola fever, the problems of acquiring, processing, and storing this material would remain [6].

In June 1995, the World Health Organization (W.H.O.) was made aware of the existence of hyperimmune immunoglobulin G (IgG) against Ebola, prepared by Russian investigators in horses [6]. This product had been reported to protect baboons experimentally infected with Ebola virus [10] and was offered by the Russian Association "Epidbiomed" for sale to the W.H.O. to be used in Zairian patients. The W.H.O. obtained several hundred doses of this product, and requested that the United States Army Research Institute of Infectious Diseases (USAMRIID), in its capacity as a W.H.O. collaborating Center-Designee with BL-4 primate capability evaluate this product in cell culture and in animal models. Purified equine IgG would circumvent many of the problems associated with human plasma acquisition, and would probably contain higher concentrations of neutralizing antibodies. This report documents the initial evaluation of the "Epidbiomed" hyperimmune IgG product.

Materials and methods

The IgG was received in 3 ml glass vials from Epidbiomed, labeled, "Immunoglobulin against Ebola fever, from horse antiserum, liquid, (basic preparation), Lot N34". These vials contained colorless, weakly opalescent liquid. Total IgG was determined by nephthometry, based on antigen-antibody binding between the sample and anti-horse IgG. Purified horse IgG was the standard. Reagents were from Cappel Laboratories, Inc. The sample was devoid of hemoglobin; total protein was 140 mg/ml, and total IgG was 120 mg/ml. By size exclusion chromatography using Pharmacia Superose-12 and Superose-6 columns and a Pharmacia FPLC instrument, chromatographic profiles and elution profiles of the sample were compared with reference (Cappel) equine IgG. On both columns, the sample gave a single symmetrical peak with an elution volume identical to the IgG standard. No albumin or higher molecular weight materials (aggregates) were found in the sample. Sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the IgG sample gave the expected peaks for heavy and light chains; it was free of albumin, and exhibited a high degree of purity.

The anti-Ebola titers of the equine IgG were measured in several standard serological tests. The indirect immunofluorescent antibody titer (IFAT) was measured using Ebola-Zaire infected vero cells [4] and amounted to 1:20 480. By ELISA, using Ebola-Zaire infected vero cell lysate as antigen [7], the IgG titered 1:256 000. The neutralizing antibody titers were determined by plaque reduction in both the serum dilution test (PRNT) against 100 plaque forming units (PFU) of Ebola-Zaire (Mayinga strain) and in the virus dilution test using a constant serum dilution (1:100) which yields a Log_{10} neutralization index (LNI) as described [4]. The 80% PRNT titer was 1:2 560, and the 50% PRNT was 1:10 240. The LNI was 4.2 against both Ebola-Mayinga and against a 1995 isolate of the virus (#807224) obtained from the Centers for Disease Control, Atlanta, GA. These neutralizing antibody