Antiviral Research 140 (2017) 95–105

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Implementation of a non-human primate model of Ebola disease: Infection of Mauritian cynomolgus macaques and analysis of virus populations

Géraldine Piorkowski [a,*], Frédéric Jacquot [b], Gilles Quérat [a], Caroline Carbonnelle [b], Delphine Pannetier [b], France Mentré [c], Hervé Raoul [b], Xavier de Lamballerie [a]

[a] UMR "Emergence des Pathologies Virales" (EPV: Aix-Marseille University - IRD 190 - Inserm 1207 - EHESP - IHU Méditerranée Infection), Marseille, France
[b] Inserm, Laboratoire P4 Jean Mérieux, Lyon, France
[c] Inserm, IAME, UMR 1137, Université Paris Diderot, Paris, France

ARTICLE INFO

Article history:
Received 26 September 2016
Accepted 23 January 2017
Available online 26 January 2017

Keywords:
Ebola virus disease
Non-human primate model
Genome sequencing
Minor variants

ABSTRACT

Ebola virus (EBOV) haemorrhagic fever remains a threat to global public health with an urgent need for an effective treatment. In order to achieve these goals, access to non-human primate (NHP) laboratory models is an essential requirement. Here, we present the first NHP-EBOV laboratory model readily available to the European scientific community, based on infection of Mauritian cynomolgus macaques using a Central-African EBOV strain and increasing virus challenge dose (10, 100, or 1000 focus forming units per animal). The outcome of these experiments was assessed using clinical, hematological, and biochemical criteria. All challenge doses resulted in fatal infections within 8–11 days. Symptoms appeared from day 5 after infection onwards and disease progression was slower than in previous reports based on Asian cynomolgus macaques. Thus, our model resembled human disease more closely than previous models (onset of symptoms estimated 2–21 days after infection) extending the period of time available for therapeutic intervention. To establish the dynamics of virus genome variation, the study included the first detailed analysis of major and minor genomic EBOV variants during the course of the disease. Major variants were scarce and the population of minor variants was shaped by selective pressure similar to genomic mutations observed in Nature. This primate model provides a robust baseline for future genomic studies in the context of therapeutic methods for treating Ebola virus-infected patients.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Non-human primate (NHP) models are an essential requirement for the study of viral haemorrhagic fevers such as those caused by Ebola virus. Indeed, without such *in vivo* methods and appropriate maximum containment BSL4 laboratory facilities, vaccine candidates and potential antiviral treatments cannot be developed safely or validated for use in humans. Previous studies on Ebola virus (EBOV) have used either rhesus macaques (*Macaca mulatta*) (Ebihara et al., 2011; Fisher-Hoch et al., 1985) or cynomolgus macaques (*Macaca fascicularis,* also named "Long-tailed" or "Crab-eating" macaques) from Asia (Geisbert et al., 2003; Marzi et al., 2015; Qiu et al., 2012).

Macaca fascicularis is one of the most commonly used NHPs in academic research, but population genetic research has revealed significant substructure throughout the species distribution that may lead to distinct phenotypic traits which could confound scientific studies (Ogawa and Vallender, 2014). Accordingly, it was important to characterize as completely as possible our EBOV infection model using a known homogeneous sub-population of macaques, potentially overcoming variability as seen in previous experiments performed with macaques presumed to be from a variety of Asian sub-populations.

Accordingly, we present here the first experiments using an NHP model of Ebola virus disease in a European maximum containment (BSL4) laboratory facility (Laboratoire P4 Jean Mérieux, Lyon, France). We used the Zaire EBOV virus strain, as described in a number of previously reported experiments (Ebihara et al., 2011; Geisbert et al., 2003; Marzi et al., 2015; Qiu et al., 2013), as the

* Corresponding author.

http://dx.doi.org/10.1016/j.antiviral.2017.01.017
0166-3542/© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

challenge virus and cynomolgus macaques known to have been resident in Mauritius for approximately 400 years (Lawler et al., 1995), following their introduction presumably from a Sumatran sub-population (Tosi and Coke, 2007).

Intramuscular injections of 10 focus-forming units (ffu), 100ffu or 1000ffu of EBOV were administered, with follow-up of clinical, hematological, biochemical and viral load analyses.

In addition, we present the first analysis of virus population variation during the course of Ebola virus infection. This was achieved by systematic quantified next-generation sequencing and characterization of variants at different stages of infection. EBOV genomic variability was characterized and the information gained will support future experimental studies aimed at developing antiviral therapeutic intervention.

The results of this study will bring to the attention of the European research community the availability of the necessary facilities and protocols with which to extend our capability of developing methods for controlling Ebola haemorrhagic fever.

2. Materials and methods

2.1. Study design

The NHP experiments were performed using cynomolgus macaques (*Macaca fascicularis*) obtained from a Mauritian colony known to be free of herpes B-virus, tuberculosis, simian T-cell leukemia virus and simian type D retrovirus. Prior to the study the animals were quarantined by Silabe ADUEIS (Strasbourg, France). All experiments were performed in the Inserm-Jean Mérieux laboratory BSL4 facilities in Lyon.

The study was conducted using twelve female NHPs (3 years old, weight range 3.5–5.0 kg), housed and monitored in accordance with the guidelines of the European directive 2010/63 and procedures established for use of animals in BSL4 facilities. The primates were anaesthetized via intramuscular injection using Zoletil® (Tiletamine/Zolazepam w/w, 3 mg/kg) and infected by intramuscular injection in the right leg quadriceps of a titrated supernatant fluid containing the Ebola virus Gabon 2001 strain (a Central African strain of EBOV). The primates were divided into 3 groups of 4 animals which were infected with either 10 focus-forming units (ffu), 100ffu or 1000ffu of EBOV, respectively. The experimental protocol received ethical authorization number P4-2014-008 (18th of November 2014, CECCAPP C2EA15 ethical committee, registered with the French Ministry of Research).

2.2. Clinical follow-up, sampling and euthanasia score

(i) Body temperature and weight were measured at days 0, 2, 5 post-infection (pi) and every day when clinical signs indicated progression of disease. All results were expressed as change from day zero.

(ii) Blood collection in the femoral vein under anaesthesia was collected for most of the monkeys at days 0, (before infection the same day), 2, 5 and 7 pi. Additional samples were collected between days 8 and 11 pi on surviving animals. *(iii)* Scoring for disease progression was performed daily from day 7 pi, using the following parameters: temperature, increase or decrease of food and water intake, weight loss, dehydration, hemorrhage, and rash. A score $\geq$15 (Table S1) was the criterion for euthanasia (performed by intra-cardiac administration of 5 mL of pentobarbital under anaesthesia). Necropsy was performed for all animals.

2.3. Biochemical and hematological follow-up

Serum levels of enzymes (ALP, ALT), creatinine, urea, and C Reactive Protein (CRP) were estimated using a Pentra C200 Analyzer (Horiba, Kyoto, Japan) at days 0, 2, 5 and 7 pi. Total leukocyte, lymphocyte, platelet and erythrocyte counts, haemoglobin and hematocrit values were determined from EDTA-treated blood samples using the MS9-5s Hematology Analyzer (Melet Schloesing, Osny, France) at days 0, 2, 5, 7, 9 pi (except for monkey CB821 which died on day 8 pi) at day 10 and day 11 pi in surviving monkeys. All results were expressed as change from day 0.

2.4. Virological follow-up

2.4.1. Molecular viral load

A synthetic RNA template, including the envelope gene region targeted by the Gibb system (Gibb et al., 2001) was produced using the MEGA shortscript™ T7 Transcription Kit (Thermo Fisher Scientific) and quantified by spectrophotometry. EBOV genomic RNA was detected in NHP plasma samples by real time RT-PCR using the Gibb system and the GoTaq Probe one step qRT-PCR kit (Promega) following manufacturer's instructions. Quantification was performed with reference to the standard curve obtained from serial dilutions of the standardized synthetic RNA template. Molecular viral load was assessed at days 0, 2, 5, 7 and 9* pi (*day 8 pi for monkey CB821), occasionally at day 10 pi and day 11 pi in surviving monkeys.

2.4.2. Infectious virus titer

Virus titre in blood was determined using 12-well microplates of Vero E6 cells. Cells were incubated with serial dilutions of plasma (1 h, 37 °C), then grown in the presence of carboxy-methyl-cellulose (37 °C, 7 days). Infectious foci were detected by incubation with a GP EBOV specific monoclonal antibody (generously provided by Laurent Bellanger and Fabrice Gallais, LI2D Laboratory, CEA, Marcoule, France), followed by phosphatase-conjugated polyclonal anti-mouse IgG and 1-step NBT/BCIP plus Suppressor (Thermo Fisher Scientific). Virus titre was expressed as focus-forming units (ffu) per millilitre of plasma. Infectious viral loads were measured in serum samples at days 0, 2, 5, 7 and 9* pi for all monkeys (*day 8 pi for monkey CB821), occasionally at day 10 and day 11 pi in surviving monkeys.

2.5. Next-generation sequencing

Virus sequencing from clinical samples was performed directly without virus isolation in cell culture. Viral RNA was extracted from serum using the QiaCube HT device and Cador Pathogen kit (Qiagen). Eight overlapping amplicons spanning the complete genome sequence were produced from the extracted RNA using the SuperScript® III One-Step RT-PCR System with Platinum® *Taq* High Fidelity kit (Thermo Fisher Scientific) and specific primers. PCR products were pooled in equimolar proportions for library building. Sequencing was performed using the PGM Ion torrent technology (Thermo Fisher Scientific) following manufacturer's instructions. Automated read datasets provided by Torrent software suite 5.0.2 were trimmed according to quality score (99%) and length (reads shorter than 30bp were removed) using CLC genomics workbench software (CLC bio-Qiagen). Primers used for RT-PCR were removed using an in-house software package. Reads were mapped on reference (inoculum strain) using CLC. A *de novo* contig was also produced to ensure that the consensus sequence was not affected by the reference sequence. Substitutions with a frequency higher than 1% (minor variants: variants frequency >1% and <50%) were considered for further analysis. Detailed protocols

Download English Version:

https://daneshyari.com/en/article/5551828

Download Persian Version:

https://daneshyari.com/article/5551828

Daneshyari.com