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Specific neutralizing response in plasma from convalescent patients of Ebola Virus Disease against the West Africa Makona variant of Ebola virus

Joanna Luczkowiak[a], José R. Arribas[b], Sara Gómez[a], Víctor Jiménez-Yuste[c], Fernando de la Calle[d], Aurora Viejo[c], Rafael Delgado[a,*]

[a] Department of Microbiology, Instituto de Investigación Hospital 12 de Octubre (imas12), CAA, Avenida de Córdoba sn, 28041 Madrid, Spain
[b] Infectious Diseases Unit, Department of Internal Medicine, Instituto de Investigación Hospital La Paz (IdiPAZ), Paseo de la Castellana, 261, 28046 Madrid, Spain
[c] Department of Hematology, Instituto de Investigación Hospital La Paz (IdiPAZ), Paseo de la Castellana, 261, 28046 Madrid, Spain
[d] Tropical Diseases Unit, Department of Internal Medicine, Instituto de Investigación Hospital La Paz (IdiPAZ), Paseo de la Castellana, 261, 28046 Madrid, Spain

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ABSTRACT

Background: The current outbreak of Ebola Virus Disease in West Africa is caused by a new variant of Ebola virus (EBOV) named Makona 2014, whose sequence differs 3% from isolates from Central Africa such as Mayinga 1976 EBOV. The specificity and kinetics of the neutralizing antibody response induced by the circulating Makona EBOV has not been thoroughly studied.
Methods: We have used a lentiviral EBOV-glycoprotein (GP)-pseudotyped infection assay to measure Makona-GP and Mayinga-GP specific neutralizing activity of plasma from three convalescent Ebola Virus Disease patients from the current EBOV outbreak at 2, 3, 4 and 9 months post-infection. Total anti-EBOV GP IgG was measured by a commercial ELISA assay.
Findings: In convalescent Ebola Virus Disease patients, Makona-GP-specific neutralizing titers increased from 2 months (mean IC_{50} 1/59), 3 months (IC_{50} 1/212), 4 months (IC_{50} 1/239) and up to 9 months (IC_{50} 1/268) post-infection. Neutralizing activity of plasma from the three convalescent Ebola Virus Disease patients was more vigorous against the current Makona-GP pseudotyped EBOV variant than against Mayinga-GP pseudotyped EBOV and this difference was observed at each time point tested: Mayinga vs Makona mean IC_{50} fold = 4.92 at 2 months post-infection, 2.89 fold at 3 months post-infection, 2.23 at 4 months post-infection and 2.98 at 9 months post-infection (all differences $p < 0.01$). Total level of IgG against EBOV-GP did not evolve significantly during the follow up.
Discussion: In convalescent Ebola Virus Disease patients, EBOV-GP specific neutralizing activity increases over time, at least up to 9 months post-infection, which suggests that active affinity maturation of antibodies takes place long after clinical recovery. EBOV-GP specific neutralizing response is significantly higher against Makona EBOV circulating in West Africa than against the variants included in the currently approved vaccines. Correlates of protection for EBOV vaccines have not been completely established and the relevance of a lower neutralizing activity in convalescent plasma from the current outbreak against one of the EBOV-GPs contained in the vaccines in terms of its potential efficacy does not necessarily preclude its efficacy. However, this observation highlights the concern regarding the natural diversity of EBOV and its subsequent challenge for diagnosis, therapy and vaccine design. EBOV-GP neutralizing activity varies considerably over time in convalescent Ebola Virus Disease patients. Titering of convalescent blood products would be desirable to standardize and evaluate their potential therapeutic value.
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* Corresponding author at: Department of Microbiology, Instituto de Investigación Hospital 12 de Octubre (imas12), CAA, planta 4ª, Avenida de Córdoba sn, 28041 Madrid, Spain.
E-mail addresses: joanna.luczkowiak@gmail.com (J. Luczkowiak), joser.arribas@salud.madrid.org (J.R. Arribas), alkalimme@hotmail.com (S. Gómez), vjyuste@gmail.com (V. Jiménez-Yuste), fercalleprieto@gmail.com (F. de la Calle), aurora.viejo@salud.madrid.org (A. Viejo), rafael.delgado@madrid.salud.org, correoderafaeldelgado@gmail.com (R. Delgado).

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1. Introduction

The current outbreak of Ebola Virus Disease in West Africa is caused by the Makona variant of Ebola virus (EBOV) whose sequence appears to be 3% different from EBOV isolated in previously reported outbreaks in Central Africa (Baize et al., 2014; Gire et al., 2014; Kuhn et al., 2014) (Fig. 1). The unprecedented scale of the outbreak, along with the genomic differences in the Makona variant has been the matter of discussion. However, there is no evidence of a different behavior of this virus in terms of transmission route or efficiency, pathogenesis and mortality as compared with other EBOV outbreaks (WHO, 2014). There is no specific antiviral treatment clinically proven for EBOV and blood or plasma from convalescent patients have been anecdotally used in a number of infected patients, however, the potency of the specific neutralizing response and its kinetics have not been thoroughly investigated in convalescent Ebola Virus Disease patients in this outbreak (Kreil, 2015). Furthermore, the vaccines strategies that have been evaluated in experimental animal models and are now being used in the affected areas, have been designed to express GP from reference variants of EBOV isolated in previous outbreaks in Central Africa (Ledgerwood et al., 2014; Marzi et al., 2011). To investigate the specificity of the antibody response, we have measured EBOV-GP specific antibody-neutralizing activity against Makona-GP and Mayinga-GP pseudotyped viruses in three convalescent Ebola Virus Disease patients from the current West Africa outbreak after 2–9 months post-infection.

2. Materials and methods

2.1. Patients

Three convalescent Ebola Virus Disease patients (CP) were studied: CP #1 was a 44 year-old female, nurse assistant infected in Madrid, Spain in October 2014 while providing health care to Ebola Virus Disease patients evacuated from Sierra Leone. She had a severe form of disease and was discharged from the isolation unit after 34 days. CP #2 and CP #3 were, respectively, 45 and 36 year-old black female missionaries involved in health care activity in Liberia and diagnosed with Ebola Virus Disease in August 2014. Both were admitted in a local Ebola Treatment Unit and discharged after 18 and 15 days respectively upon clinical recovery. CP #1 received supportive care, high-dose Favipiravir for 10 days and 5 units of convalescent plasma obtained from CP #2 (3 units, 200 ml each) and CP #3 (2 units, 200 ml each) at 2 months post-infection. The others, CP #2 and CP #3, received standard supportive treatment in local Ebola Treatment Units in West Africa.

2.2. Samples

Blood samples were obtained in EDTA tubes upon informed consent and approval by Hospital Universitario La Paz Internal Review Board (IRB-071015) at the following time points: 2 months (CP #2 and CP #3), 3 months (CP #1, CP #2 and CP #3), 4 months (CP #3) and 9 months (CP #3) post-infection.

2.3. ELISA

For total anti-EBOV-GP IgG detection the Recombivirus™ Human Anti-Zaire Ebola Virus Glycoprotein IgG ELISA Kit (Alpha Diagnostic International, San Antonio, TX) was used according to manufacturer instructions using a 1:500 dilution to get results within the linear range of the assay.

2.4. EBOV-GP neutralizing assays

An EBOV-GP-pseudotyped lentiviral system was used to test neutralizing activity. Briefly, human embryonic kidney 293 T cells were plated at a density of 3×10^6 per 10-cm diameter tissue culture dish and after overnight incubation transfected with pNL4-3.Luc.R$^-$.E$^-$ (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: pNL4-3.Luc.R$^-$.E$^-$ from Dr. Nathaniel Landau) (Connor et al., 1995) and EBOV-GP expression vectors using a standard calcium chloride transfection protocol (Life Technologies, Carlsbad, CA, USA). Expression plasmids for the GP of EBOV variant Mayinga (GeneBank: U23187.1) and Reston virus (RESTV) (GeneBank: U23152.1) were kindly provided by Anthony Sanchez, Centers for Disease Control and Prevention, Atlanta, USA. EBOV variant Makona GP (GeneBank: KM233102.1) (Gire et al., 2014; Kuhn et al., 2014) was synthesized and cloned into pcDNA3.1 by GeneArt® AG technology (Life Technologies, Regensburg, Germany). Supernatants containing GP-pseudotyped viruses were harvested 48 h later, centrifuged to remove cell debris and stored in aliquots at −80 °C. Infectious titers were estimated as tissue culture infectious dose per ml by limiting dilution (1:5 serial dilutions in triplicate) of the lentivirus-containing supernatants on HeLa cells. Luciferase activity was determined by luciferase assay (Luciferase Assay System, Promega, Madison, WI) in a GloMax®-Multi+ Detection System (Promega, Madison, WI, USA).

Plasma samples from convalescent and healthy blood donors ($n = 3$) were heat-inactivated at 56 °C for 30 min and tested in triplicate in three independent assays at dilutions 1:50, 100, 250, 500 and 1000. As a positive control of neutralization, the human monoclonal anti-EBOV-GP antibody KZ52 that recognizes a conserved epitope in both Mayinga and Makona GP (Maruyama et al., 1999) was used at concentrations from 0.0125 to 1.6 μg/ml and tested also in triplicate in three independent experiments.

For neutralization experiments, virus-containing transfection supernatants were normalized for infectivity to an MOI of 0.05 and incubated with the dilutions of plasma samples at 37 °C for 1 h in 96-well plates. After the incubation time, 10^4 HeLa cells were seeded onto the virus-plasma mixture and incubated in a total volume of 100 μl per well. At 48 h after infection, cells were lysed and assayed for luciferase expression as described above.

2.5. Statistical analysis

Statistical analysis was performed by using the software Graphpad Prism v6. Inhibitory concentration 50 (IC_{50}) with 95% confidence interval (95% CI) of Makona-GP and Mayinga-GP EBOV neutralization were estimated by model of nonlinear regression fit with settings for log (inhibitor) vs normalize response curves. The p values neutralizing comparisons were calculated by non-linear fits obtained for Mayinga-GP and Makona-GP pseudotyped viruses using the Extra sum-of-squares F test.

2.6. Sequence alignment

The alignment of the sequences was performed by using Geneious v6.1 bioinformatic software.

3. Results

An assay based on GP-pseudotyped lentiviral particles was applied to measure neutralizing activity against Mayinga EBOV-GP, Makona EBOV-GP and RESTV-GP. The EBOV-GP-specific neutralizing monoclonal antibody KZ52 was used as a positive control throughout our experiments to confirm the validity of our test. The conformational epitope for KZ52 is conserved in both, Makona and

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