Cell

Supplemental information

CROSS-REACTIVE AND POTENT NEUTRALIZING ANTIBODY RESPONSES IN HUMAN SURVIVORS OF NATURAL EBOLAVIRUS INFECTION

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Viruses.

The mouse-adapted EBOV strain Mayinga was originally generated by Dr. Mike Bray (U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland) (Bray et al., 1998). The virus was provided originally by the Special Pathogens Branch of CDC, deposited at WRCEVA, and amplified by one passage in Vero-E6 cells. The guinea pig-adapted EBOV strain Mayinga was generated originally by Dr. Brett Cononolly (U.S. Army Medical Research Institute of Infectious Diseases) (Connolly et al., 1999) and was provided by Dr. Alexander Freiberg (UTMB) through Dr. Heinz Feldmann (Special Pathogens Program, National Microbiology Laboratory, Canadian Science Centre for Human and Animal Health, Winnipeg, Canada) and amplified by one passage in Vero-E6 cells. The recombinant EBOV strain Mayinga expressing eGFP was generated by a reverse genetics technique (Lubaki et al., 2013) as previously described (Towner et al., 2005) from plasmids provided by Drs. Jonathan Towner and Stuart Nichol (CDC) and Drs. Yoshihiro Kawaoka (University of Wisconsin) and Heinz Feldmann (NIH), and passaged three times in Vero E6 cells. The EBOV Makona strain from the 2014-2015 West African outbreak, which was provided by Dr. T. Geisbert (UTMB), was isolated originally from serum of a fatally infected patient in early 2014 in Guekedou, Guinea, and was passaged two times in Vero E6 cells.

EBOV and MARV neutralization experiments.

Neutralizing activity of BDBV223 was also tested against the EBOV Makona strain by a classic plaque reduction assay, which was performed as follows. Triplicate samples of 150 PFU of the virus were mixed with serial dilutions of mAbs, with or without 5% guinea pig complement in a

total volume of 100 μL, incubated for 1 hour at 37°C, and placed on Vero E6 cell monolayers. After a 1 hour-long virus adsorption at 37°C, cells were overlaid with 0.8% tragacanth (Spectrum Chemical Mfg. Corp., New Brunswick, NJ) solution in Minimal Essential Medium containing 10% FBS (HyClone, Logan, Utah) and 0.1% gentamicin (Mediatech, Manassas, VA), and incubated for 14 days. Plaques were visualized by staining of monolayers with 0.25% crystal violet (Thermo Fisher Scientific, Waltham, MA) in 10% formalin.

Epitope mapping using an EBOV GP alanine-scan mutation library.

Residues 33-676 of full-length EBOV GP were mutagenized to create a library of clones, each representing an individual point mutant. Residues were changed to alanine (with alanine residues changed to serine). GP residues 1-32, which constitute the GP signal peptide, were not mutagenized. The resulting EBOV GP alanine-scan library covered 99.5% of target residues (641 of 644). Clones were arrayed into 384-well plates, one mutant per well. The EBOV GP mutation library was transfected into HEK-293T cells and allowed to express for 22 hours. Cells were fixed in 4% paraformaldehyde in PBS plus calcium and magnesium, or were left unfixed, and were then incubated with an Ab diluted in 10% normal goat serum (NGS) (Sigma-Aldrich, St. Louis, MO). The cells were incubated with primary antibody for 1 hour at room temperature, followed by a 30 minute incubation with Alexa Fluor 488-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Westgrove, PA) in 10% NGS. Cells were washed twice with PBS without calcium or magnesium and resuspended in Cellstripper (Cellgro, Manassas, VA) plus 0.1% BSA (Sigma-Aldrich, St. Louis, MO). Cellular fluorescence was detected using the Intellicyt high throughput flow cytometer (Intellicyt, Albuquerque, NM). Background fluorescence was determined by fluorescence measurement of vector-transfected control cells.

Ab reactivities against each mutant EBOV GP clone were calculated relative to wild-type EBOV GP reactivity by subtracting the signal from mock-transfected controls and normalizing to the signal from wild-type GP-transfected controls. Before screening, the immunoreactivities of MAbs BDBV270, BDBV289, and BDBV324 were optimized by determining reactivity with fixed or unfixed cells over a range of mAb concentrations to identify optimal signal-to-background ratios (>5:1) and to ensure that signals were within the linear range of detection. MAb BDBV289 also screened as a Fab after conversion by papain digestion. Control mAbs 2G4 and 4G7 were kindly provided by Gary Kobinger, Public Health Agency of Canada. Mutated residues within critical clones were identified as critical to the Ab epitope if they did not support reactivity of the test Ab but did support reactivity of other control EBOV mAbs. This counter-screen strategy facilitates the exclusion of GP mutants that are locally misfolded or that have an expression defect. The detailed algorithms used to interpret shotgun mutagenesis data are described elsewhere (patent application 61/938,894), (Davidson and Doranz, 2014).

Generation and Sequence Analysis of VSV/BDBV GP Escape Mutants.

Briefly, 200 PFUs of VSV/BDBV-GP virus were pre-incubated with 2-fold decreasing concentrations of mAbs before each passage, starting from 200 μg/mL, and serially passaged 3-10 times under selective pressure of the corresponding mAbs. After each passage, virus aliquots were harvested and titrated. A suspension containing 200 PFUs from the virus-positive aliquot with the highest mAb concentration was used for the next passage. Finally, viruses were plaque-purified, and the genes encoding the BDBV GPs were sequenced. Viral samples derived from plaques containing any amino acid substitutions were propagated further in the presence of the corresponding mAb, and tested for neutralization resistance by plaque reduction assay.

Supplemental References

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Table S1. BDBV-specific human neutralizing antibodies: heavy chain gene usage. Related to Figure 2.

Group	Donor	mAb	V_H	D	J_H	CDR3 length	CDR1	CDR2	CDR3
1A	1	231	4-59	3-3	2*01	14	SDSIRSYS	IYYSGNI	ARDWITIFGRYFDV
	3	329	1-69	1-26	6*03	33	GGTFDTYA	IIPVLGIV	ARGLRSLSPRGQEGPTPAPGWRRAQYHYYYMDV
		335	4-61	3-16	6*02	19	GGSINSDSYY	VYTSGST	ARVVWGSYRSYHYSYGMDV
		354	1-18	2-15	3*02	18	GYAFTTYA	ISTYYGTT	VRDRSWLATSRPYDAFDI
		377	3-33	3-10	6*02	21	GFTFNSYG	IWFDGSKK	AKDLLYGSGMVPNYYYYGLDV
		386	4-61	3-10	4*02	17	GGSISSGRFY	IYTSGST	ATELYYYGSGSYDPLWS
		397	4-61	3-22	4*02	15	GGSISSGSYF	IYTSGTT	ATSPYYYDSSHYYDY
		399	4-31	3-10	4*02	12	GGSISNGGYH	IYYSGST	ARDRIRGGPIDY
		415	1-69	3-22	2*01	23	GGTFSSYG	IIPKFATA	AGHFPQRKPITTIVVITYWSLDL
	4	315	4-61	3-3	6*02	19	GDSISSGSYY	IYTSGST	ARDPITIFGGVIFGWGMDV
		343	1-69	3-10	6*02	18	GVTFSRYT	ISPILGTA	ARDAPIILVEGPETGMDV
1B	1	255	3-21	2-21	4*02	14	GFTLSTYS	ISSSSTYK	SRADWDSGKGDLDS
	3	353	1-2	3-10	6*01	18	GYTFSDYY	INPYSGGT	ARLYGAGSHYNHYNGMDV
	4	432	4-61	1-26	4*02	14	GDSSGRYY	ISYTGST	ARGGWNLLVSYFDF
2A	3	392	3-30	4-17	2*01	19	GFTFSSFG	IRYDGSDK	AKRGGHDYGYYDNNRYIDL
		425	1-18	6-19	4*02	18	GYTFTSFG	INTYNGDT	ARDSHLISIAVANTPNDF
	4	342	1-69	2-21	4*02	22	GGTFSSYA	IIPIFGKP	ARGQGEIVVMVGHDDGGDYLGY
		407	4-59	2-8	6*02	21	GGSIRSYF	IYYSGRP	ARDERLLVEVGTDHFYYGLDV
		426	4-30	3-22	4*02	22	GGSISSDDRY	IYYSGST	ATVTAYSPATMIVVGTEHGFDY
3A	2	270	4-61	3-9	6*02	21	GASISRGLYY	IYTSGSI	VRDAPWGDFLTGYFGFYGMDV
	3	324	1-8	2-2	6*02	24	GYTFTSFE	MNPKSGDT	ARGPHVGEVVPGLMAGTYYFPLDV
	7	43	1-69	6-6	5*01	15	DSFSRKYG	IMPIVGLT	ARDEIIGARPHWFDS
		289	1-69	5-24	6*03	21	GATFGSDT	IIPFFGEA	ARQINEMATFGEIHYYTYMDV
3B	1	223	4-34	2-15	6*03	16	GGSFTTTY	VNYSGNA	TSRIRSHIAYSWKGDV
	4	317	3-30	6-13	4*02	11	GLTFSNFG	IRFDGSNK	GRVLYGAAADF
		340	4-61	5-12	4*02	17	GGSISSGSFY	FYTTGST	ARGPVSYYSGNLYYFDY

Table S2. Glycoprotein gene mutations in viral RNA isolated from tissues of representative mAb-treated animals that died. Related to Figure 7.

Animal	Treatment group	Survival curve in Figure 7A	Day of death	Mutations in GP sequence (as compared to the viral inoculate)*	
				Nucleotide change	Amino acid change
9	Single dose BDBV223	Column 2, Red line	8	None	None
10	Single dose BDBV223	Column 2, Purple line	8	T6861G** A7109G** Insertion of extra uridine in the 7 uridine stretch of the transcription editing site***	W275G (glycan cap) R358G (mucin-like domain)
11	Two doses BDBV223	Column 3, Black line,	8	None	None
21	Single dose BDBV289	Column 4, Black line	7	T6909G** (detected in part of the population) G6910C** (detected in part of the population)	W291G, W291S, W291A**** (glycan cap)
25	Single dose BDBV289	Column 4, Purple line	8	None	None

*We amplified the EBOV GP gene by RT-PCR from RNA extracted from the suspension of guinea-pig adapted EBOV that was used to inoculate the animals and from RNA extracted from blood collected on day 6 after inoculation with the guinea-pig adapted EBOV.

** (+) DNA sense indicated.

***This previously described mutation results in the disabling of expression of the secreted form of GP, consistent with previous studies of sequence analysis of the GP gene of EBOV replicating in guinea pigs (Volchkov *et al.,* 2000, *Virology;* 277: 147-155).

****These mutations are located near the expected epitope (see Figure 5); however, an IgG form of mAb BDBV289 bound to a cell-surface expressed GP with the W291A polymorphism at 186% of the level of binding to wild-type GP, and an Fab form of mAb BDBV289 bound at 290% of the level of binding to wild-type GP in the alanine scanning mAb fluorescence binding studies. Thus, these polymorphisms are unlikely to be escape mutations.