

GB Virus C Coinfections in West African Ebola Patients

Michael Lauck,^{a,b} Adam L. Bailey,^{a,b} Kristian G. Andersen,^{c,d} Tony L. Goldberg,^{b,e} Pardis C. Sabeti,^{c,d} David H. O'Connor^{a,b}

Department of Pathology and Laboratory Medicine, University of Wisconsin—Madison, Madison, Wisconsin, USA^a; Wisconsin National Primate Research Center, Madison, Wisconsin, USA^b; The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA^c; Center for Systems Biology, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA^d; Department of Pathobiological Sciences, University of Wisconsin—Madison, Madison, Wisconsin, USA^e

In 49 patients with known Ebola virus disease outcomes during the ongoing outbreak in Sierra Leone, 13 were coinfecting with the immunomodulatory pegivirus GB virus C (GBV-C). Fifty-three percent of these GBV-C⁺ patients survived; in contrast, only 22% of GBV-C⁻ patients survived. Both survival and GBV-C status were associated with age, with older patients having lower survival rates and intermediate-age patients (21 to 45 years) having the highest rate of GBV-C infection. Understanding the separate and combined effects of GBV-C and age on Ebola virus survival may lead to new treatment and prevention strategies, perhaps through age-related pathways of immune activation.

As of this writing, there have been 14,413 confirmed and probable infections and 5,177 deaths in the ongoing and worsening Ebola virus (EBOV) disease outbreak in West Africa (1). Recently, EBOV sequences from Sierra Leone were obtained by unbiased deep sequencing. These patients represented approximately 70% of patients with Ebola virus disease in Sierra Leone from late May to mid-June of 2014 (2).

In the three countries (Sierra Leone, Liberia, and Guinea) where the Ebola virus outbreak is concentrated, GB virus C (GBV-C, also known as human pegivirus) infects between 10 and 28% of individuals (3–6). Although GBV-C causes a prolonged high-titer viremia, GBV-C infection is largely considered to be benign (7, 8). Intriguingly, several epidemiological studies have associated GBV-C infection with lower mortality in HIV-positive people (9–12; see reference 13 for a meta-analysis). Although potential mechanisms explaining this association are still under investigation, a growing body of evidence suggests that GBV-C prevents aberrant immune activation that is a hallmark of HIV pathogenesis and disease progression (i.e., AIDS) (see reference 14 for a review).

We reasoned that the relatively high prevalence of GBV-C in West Africa would result in a significant number of coinfections with EBOV. To examine GBV-C coinfections with EBOV, deep-sequencing data initially published in reference 2 were downloaded from the NCBI Sequence Read Archive (SRA), sequencing

run (SRR) files were converted into fastq files using the SRA toolkit, and SRR identifiers (IDs) were correlated with patient sample IDs using information from supplemental Table S2 in reference 2. Further analysis was confined to the 49 patients for whom EBOV infection outcome, age, and gender information were available (see Fig. S2 in the supplemental material in reference 2; also unpublished data). Samples for which two independent library preparations were performed or which were collected from the same individual at multiple time points were merged into single fastq files. fastq files were then imported into CLC Genomics Workbench 7 and short (<90-bp) and low-quality (Phred quality score <Q30) reads were removed. Samples labeled as potential dupli-

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Address correspondence to David O'Connor, doconnor@primate.wisc.edu.

M.L. and A.L.B. contributed equally to this work.

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TABLE 1 EBOV patients coinfecting with GBV-C

Patient ID	SRR ID(s) ^a	No. of raw GBV-C reads	No. of unique GBV-C reads	No. of EBOV reads	Patient outcome
G3670.1	1553450	191	159	282,289	Discharged
G3765.2	1553501, 1553502	2,161	1,749	2,490	Discharged
G3789.1	1553525, 1553526	343	325	5,508	Discharged
G3796	1553529, 1553530	79,619	40,538	2,090,829	Discharged
G3819	1553559, 1553559	601	554	33,172	Discharged
G3821	1553563, 1553564	4,529	4,047	99,766	Discharged
G3850	1553595, 1553596	797	673	7,363	Discharged
G3764	1553499, 1553500	64	55	5,402,195	Died
G3795	1553527, 1553528	2,671	774	1,005,042	Died
G3808	1553543, 1553544	6,454	5,515	819,166	Died
G3825	1553569, 1553570, 1553571, 1553572	5,093	4,069	2,177,336	Died
G3826	1553573, 1553574	29,752	27,149	867,174	Died
G3845	1553589, 1553590	2,794	2,137	3,377,501	Died

^a SRR, sequencing run.

TABLE 2 EBOV patients not coinfecting with GBV-C

Patient ID	SRR IDs ^a	No. of raw GBV-C reads	No. of unique GBV-C reads	No. of EBOV reads	Patient outcome
G3769	1553503, 1553504, 1553505, 1553506, 1553507, 1553508, 1553509, 1553510	7	0	4,218,762	Discharged
G3799	1553533, 1553534	29	5	1,160,025	Discharged
G3805	1553537, 1553538, 1553539, 1553540	0	0	6,054	Discharged
G3809	1553545, 1553546	6	0	11,344	Discharged
G3810	1553547, 1553548, 1553549, 1553550	0	0	503,396	Discharged
G3817	1553555, 1553556	1	0	920,637	Discharged
G3857	1553603, 1553604	4	2	9,115	Discharged
NM042	1553605, 1553606, 1553607, 1553608, 1553609, 1553610	0	0	574,137	Discharged
EM112	1553429, 1553430	2	0	4,495,065	Died
EM121	1553439, 1553440	0	0	3,393,644	Died
EM124	1553441, 1553442, 1553443, 1553444, 1553445, 1553446, 1553447, 1553448	4	0	2,370,521	Died
G3676	1553451, 1553452, 1553453, 1553454	0	0	1,753,365	Died
G3677	1553455, 1553456, 1553457, 1553458	2	0	2,195,013	Died
G3707	1553471, 1553472	4	0	1,848,917	Died
G3713	1553473, 1553474, 1553475, 1553476, 1553477, 1553478	4	0	11,650,343	Died
G3724	1553479, 1553480	0	0	5,237,956	Died
G3735	1553485, 1553486, 1553487, 1553488	0	0	11,945,730	Died
G3752	1553495, 1553496	2	1	1,181,649	Died
G3770	1553511, 1553512, 1553513, 1553514	15	4	9,932,933	Died
G3798	1553531, 1553532	2	0	1,124,997	Died
G3800	1553535, 1553536	9	0	890,300	Died
G3807	1553541, 1553542	0	0	822,652	Died
G3814	1553551, 1553552	0	0	13,286	Died
G3816	1553553, 1553554	0	0	10,101	Died
G3818	1553557, 1553558	0	0	2,398,770	Died
G3820	1553561, 1553562	2	0	1,890,862	Died
G3822	1553565, 1553566	0	0	3,417,852	Died
G3823	1553567, 1553568	0	0	4,672,245	Died
G3827	1553575, 1553576	0	0	657,986	Died
G3829	1553577, 1553578	0	0	1,619,423	Died
G3834	1553581, 1553582	0	0	646,694	Died
G3838	1553583, 1553584	4	1	2,234,396	Died
G3840	1553585, 1553586	0	0	2,978,090	Died
G3846	1553591, 1553592	6	0	2,829,761	Died
G3848	1553593, 1553594	0	0	3,013,745	Died
G3851	1553597, 1553598	0	0	393,873	Died

^a SRR, sequencing run.

cates in supplemental Table S2 of reference 2 were excluded from the analysis. Reads from all patients were aligned with moderate stringency (length fraction, 0.8; similarity fraction, 0.8; mismatch cost, 2; insertion and deletion cost, 3) against a full-genome GBV-C genotype 1 reference, the predominant genotype in West Africa (15) (GenBank accession number HGU36380). Up to 79,619 reads mapped to GBV-C per individual (Table 1).

A low level of carryover contamination is common in unbiased deep-sequencing experiments. We were therefore concerned that samples with low numbers of GBV-C reads might represent carryover from other samples with high levels of GBV-C. To more rigorously define samples as GBV-C positive or negative, we determined the GBV-C consensus sequence for each sample. We then remapped the reads from each sample against consensus sequences from all samples with high stringency (length fraction, 0.98; similarity fraction, 0.98; mismatch cost, 2; insertion and deletion cost, 3) and discarded reads that mapped to multiple consensus sequences. Twelve individuals had unambiguous evidence of GBV-C viremia supported by at least 100 uniquely mapped reads covering between 63 and 100% of the genome (Tables 1 and 2). A 13th individual (G3764) was putatively categorized as

GBV-C⁺ on the basis of 55 uniquely mapped reads, resulting in 38% coverage across the genome.

The 2014 EBOV sequences from Sierra Leone were on average 99.98% [99.98% to 100%] identical in pairwise comparisons across the genome (data not shown), which is consistent with the recency of this outbreak. In contrast, GBV-C sequences shared on average 91% (86.96 to 98.46%) nucleotide identity (Table 3), suggesting preexisting GBV-C infections rather than cotransmission with EBOV.

Mortality in this cohort of 49 patients with sequence-confirmed EBOV infection was 69% overall, which is comparable to the 65% mortality reported for definitive infections in Sierra Leone before 18 August 2014 (1). Only 6/13 (46%) GBV-C⁺ individuals died, whereas 28/36 (78%) GBV-C⁻ individuals died. Univariate analyses (Table 4) showed that older age was associated with higher mortality (OR, 1.06; *P* = 0.0124) and that GBV-C⁺ status was associated with lower mortality (OR = 0.25; *P* = 0.0402). However, when these factors were considered together in a multivariate analysis (Table 4), GBV-C status became nonsignificant (OR = 0.25; *P* = 0.0835), likely reflecting a confounding effect of age. Our finding of a relationship between older age and

TABLE 3 Pairwise comparison of percentages of nucleotide identity for GBV-C consensus sequences

Patient ID	% nucleotide identity to GBV-C consensus sequence ^a :												
	G3670.1	G3764	G3765.2	G3789.1	G3795	G3796	G3808	G3819	G3821	G3825	G3826	G3845	G3850
G3670.1	100												
G3764	90.86	100											
G3765.2	92.46	91.36	100										
G3789.1	86.96	87.89	88.28	100									
G3795	91.30	90.92	92.79	88.11	100								
G3796	91.36	90.97	92.79	88.11	98.46	100							
G3808	94.22	91.52	92.68	88.61	92.18	92.13	100						
G3819	91.36	91.41	91.30	87.34	91.19	91.03	91.03	100					
G3821	92.46	92.24	92.63	88.61	92.24	92.35	92.85	91.74	100				
G3825	91.41	90.53	93.40	87.78	91.74	91.85	92.13	91.47	92.07	100			
G3826	92.02	91.80	91.80	88.72	91.41	91.63	91.69	91.63	92.57	92.07	100		
G3845	90.97	91.14	92.63	87.89	95.60	95.82	91.85	90.75	92.18	91.52	91.30	100	
G3850	92.18	92.29	92.29	87.40	91.69	91.96	92.68	91.36	92.24	91.69	92.13	91.85	100

^a Pairwise comparisons of GBV-C consensus sequences were generated by aligning sequences with ClustalW. After manual adjustment, the percent nucleotide identity was calculated from the resulting 1,800-bp alignment.

higher mortality is consistent with a recently published study (16). However, GBV-C infection follows a different pattern, being most common in people aged 21 to 45 years (Fig. 1). Thus, age is associated with both EBOV survival and GBV-C status, but the pattern of association is different in each case.

There were both epidemiological and technical aspects of this study that could not be controlled. For example, potentially confounding variables, such as comorbidities, rapidity of diagnosis, and relationships among patients, were not available. Furthermore, the samples were collected opportunistically, possibly introducing selection bias. It is also possible that we were not able to detect low-titer GBV-C viremia in some patients. Because sequencing reads were generated in an “unbiased” fashion, patients with very high EBOV titers may have “swamped” the sample, effectively reducing the number of GBV-C reads. We believe that this is unlikely because (i) we detected GBV-C in patients with EBOV plasma loads of $>10^8$ (see supplemental Fig. S2 in reference 2) and (ii) in previous studies, we have detected multiple viruses from a single sample using a similar methodology, even in samples where at least one virus was highly concentrated (17–20). Recovery of unique reads targeting the majority of the viral genome provide unequivocal evidence for GBV-C infection in all but one of the samples; however, in the one sample where less than half of the genome is covered, verification of GBV-C status using an independent assay (e.g., reverse transcription-quantitative PCR [RT-qPCR]) would be ideal but is not currently possible.

Nonetheless, these results demonstrate that approximately 27% of EBOV patients in this cohort are coinfecting with GBV-C, an immunomodulatory virus that attenuates the pathogenesis of HIV. The association between GBV-C status and Ebola virus dis-

ease survival is intriguing, although confounded by age. We speculate that GBV-C may interact with the host immune system in ways that modulate the overexuberant immune response characteristic of EBOV-related pathogenesis (21–27). However, our analyses are also consistent with a primary effect of age on both Ebola virus disease-related survival and GBV-C infection. Resolving the direction of causality would require additional data on the time course of infection and coinfection, as well as direct measures of immunity.

EBOV and GBV-C appear to infect different types of immune cells. EBOV infects primarily myeloid-lineage cells (28–31), while GBV-C appears to target lymphoid-lineage cells (32, 33). The interaction of immune cell populations—both locally in lymphoid tissues and systemically via secreted factors—provides a biologically plausible mechanism for an interaction between GBV-C and EBOV. If GBV-C infection attenuates EBOV pathogenesis, it is possible that this occurs through modulation of the host immune response. In the context of HIV infection, GBV-C has been associated with a reduced production of proinflammatory cytokines and a reduction in T-cell activation *in vivo* and *in vitro* (34–44). Conversely, robust production of proinflammatory cytokines and lymphocyte activation followed by massive T-cell death are thought to play a major role in EBOV pathogenesis and have been associated with poor clinical outcome in retrospective studies (21–27).

Although our data are preliminary and potentially influenced by confounding variables, the results that we present here indicate

TABLE 4 Factors associated with mortality in EBOV⁺ patients^a

Variable	Univariate model		Multivariate model	
	Odds ratio (95% CI)	<i>P</i> value	Odds ratio (95% CI)	<i>P</i> value
Age (yr)	1.06 (1.01–1.11)	0.0124	1.09 (1.02–1.16)	0.0076
Sex (male)	0.56 (0.16–2.00)	0.3735	0.30 (0.05–1.91)	0.2028
GBV-C ⁺	0.25 (0.06–0.94)	0.0402	0.25 (0.05–1.20)	0.0835

^a Analyses are based on 49 patients for whom complete data were available. First-order interaction terms were not statistically significant and are therefore not included. *P* values are 2-tailed and based on chi-square tests from univariate and multivariate logistic regression, performed using the computer program R (46). Statistically significant *P* values are shown in bold. CI, confidence interval.

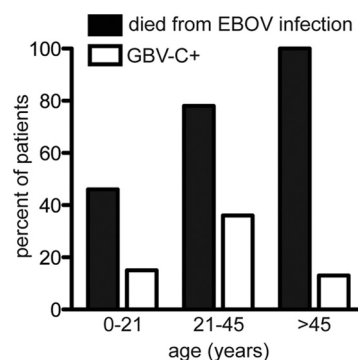


FIG 1 Ebola virus mortality and GBV-C coinfection status by age.

that further study of GBV-C/EBOV coinfection may be warranted. Such investigations should endeavor to follow patients of different ages longitudinally and to collect immunological data, with the goal of establishing the temporal sequence of events that leads to EBOV-related survival and mortality, with and without coinfecting GBV-C.

Nucleotide sequence accession number. We have made consensus GBV-C sequences available in GenBank (accession numbers [KM670096](#) to [KM670110](#)).

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