Marseillevirus-Like Virus Recovered From Blood Donated by Asymptomatic Humans

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(See the editorial commentary by Goodman on pages 1039–41.)

The study of the human virome is still in its infancy, especially with regard to the viral content of the blood of people who are apparently disease free. In this study, the genome of a new giant virus that is related to the amoeba-infecting pathogen Marseillevirus was recovered from donated blood, using high-throughput sequenc-
ing. Viral antigens were identified by an immunoconversion assay. The virus was visualized with transmission electron microscopy and fluorescence in situ hybridization and was grown in human T lymphocytes. Specific antibody reactions were used to identify viral proteins in blood specimens from polymerase chain reactive–positive donors. Finally, we tested 20 blood specimens from additional donors. Three had antibodies directed against this virus, and 2 had circulating viral DNA. This study shows that giant viruses, which are missed by the use of ultrafilters, are part of the human blood virome. The putative pathogenic role of giant viruses in humans remains undefined.

Keywords. viral metagenomics; giant viruses; Marseillevirus; blood virome.

Viruses are the most abundant and diverse entities in the human body [1]. Viral communities in various anatomical sites, including the skin, oropharynx, gut, and blood, have been described [2–4]. Human blood harbors heterogeneous viral flora, and the majority of these communities are commensal and rarely cause disease in healthy people [5, 6]. Determining when a given virus is a component of the typical floral community, rather than an invasive pathogen, has direct clinical applications but remains a challenging process. The detection of new or highly divergent viruses is difficult because of limitations in isolation and cultivation methods and the lack of conserved genetic elements among genomes [7, 8]. During the last decade, the use of sequence- and culture-independent techniques to characterize genetic material from viral populations (a process known as viral metagenomics) has allowed for the discovery of previously uncharacterized viruses [9–12]. However, despite the increase in knowledge gained during the metagenomics era, viral diversity has been mainly assessed on the ultrafilterable fraction (ie, on particles recovered after 0.22-µm filtration), which potentially filters out larger viruses and creates a gap in the complete description of the human virome.

The order Megavirales, also known as nucleocyto-plasmic large DNA viruses, contains 7 viral families: Poxviridae, Iridoviridae, Ascoviridae, Mimiviridae, Phycodnaviridae, Asfarviridae, and Marseilleviridae [13, 14]. Marseillevirus, the first representative of the Marseilleviridae family, was isolated because of its ability to prey on amoebae [15]. Marseillevirus possesses a 368-kb double-stranded DNA genome, the sixth largest known viral genome, enclosed in a 250-nm icosahedral capsid. In this study, we describe a new giant virus, termed “giant blood Marseillevirus” (GBM), recovered from blood donated by asymptomatic humans.
MATERIALS AND METHODS

Blood Sampling
Viral metagenomics studies were performed on blood samples from 10 healthy donors collected at the Etablissement Français du Sang (Marseille, France). Epidemiological analysis was performed on 20 additional blood samples collected at the Etablissement Français du Sang (Montpellier, France). Blood pockets were stored at 4°C before further processing.

Viral Purification and Immunoprecipitation
Blood samples (40 mL) were centrifuged at 3000 × g at 4°C for 10 minutes to obtain pellets of blood cells and cellular debris. Ten milliliters of each cell-free plasma sample were aliquoted and filtered through 0.45-µm-pore Whatman filters. The filtrate was loaded onto a CsCl step gradient consisting of 1 mL each of 1.7, 1.5, and 1.2 g/mL CsCl in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, and 1.4 mM KH2PO4 [pH 7.3]) and then centrifuged at 55 000 × g at 4°C for 2 hours. The 1.2–1.5-g/mL fraction was collected and centrifuged at 55 000 × g at 4°C for 2 hours to obtain pellets of viral particles, which were then resuspended in 200 µL of PBS filtered by 0.02-µm-pore Whatman filters. The purified viral particles were then incubated with 0.2 volumes of chloroform for 10 minutes at room temperature. A pellet of the chloroform solution was obtained, and the supernatant was removed and treated with 2.5 U of DNase I (Sigma-Aldrich) per microliter of sample for 2 hours at 37°C to remove residual host DNA. For GBM immunoprecipitation, serum from blood donor 27 725 was treated with 3.5 × 107 magnetic beads (Dynabeads, Invitrogen) coated with sheep anti-mouse immunoglobulin G (IgG; 10 µL) were incubated with a mixture of 3 types of mouse monoclonal anti-Marseille-virus antibodies (2 µg). On the next day, the solution was incubated with 3.5 × 107 magnetic beads (Dynabeads, Invitrogen) coated with sheep anti-mouse IgG. Beads containing immunoprecipitated viral particles were magnetically pulled down and washed 3 times with PBS and then resuspended in 0.2 mL of PBS. Viral DNA was then extracted using the High Pure Viral Nucleic Acid Kit (Roche) according to the manufacturer’s protocol.

High-Throughput Sequencing
Purified viral DNA was amplified with Genomiphi (GE Healthcare) to generate sufficient material for shotgun 454 pyrosequencing library preparations. The resulting amplified DNA was purified with silica columns (Qiagen) to remove the enzyme, deoxyribonucleotide triphosphates, and primers. DNA was pyrosequenced on a Roche 454 Life Sciences GS FLX Titanium platform (1/16th of the plate was used), generating raw data (11.9 Mb) with an average length of 389 bp. The same protocol was used for the serum from blood donor 27 725, and the immunoprecipitated fraction was then further sequenced on an AB SOLiD 4 System platform, using 1/96th of the plate.

Read Processing, Assembly, and Contig Analysis
The sequences obtained from the 454 pyrosequencing were screened to remove exact and nearly identical duplicates, which are a common artifact of the pyrosequencing technology. Duplicate removal was performed using the CD-HIT-454 program available under the CAMERA 2.0 web portal. This process resulted in 8.2 Mb of nonredundant sequences with an average length of 406 bp, which were then subsequently taxonomically classified by a BLASTn search against the GenBank nucleotide database with an E value of <10⁻⁵.

Read assembly was performed by Newbler software (Roche). We chose a minimum overlap length of 35 bp and a minimum overlap identity of 98%. Only contigs >400 bp were kept for further analyses. Large contigs were classified as those spanning >1500 bp. Contigs were annotated by performing a BLASTx search against the BLAST nonredundant database nr with an E value of <10⁻⁵. SOLID sequencing generated 15 568 200 paired sequences. Marseillevirus was used as a reference for genome mapping (CLC software). The generated consensus genomic sequence was further analyzed for open reading frames (ORFs), using Prodigal. Predicted ORFs were then compared to GenBank nr, using BLASTp with an E value of <10⁻⁵.

Polymerase Chain Reaction (PCR) and Primer Design for Targeting Metagenome and Marseillevirus Homologue Genes
To evaluate the presence of human and mitochondrial DNA contamination, the sample was checked by PCR, using the following specific primers: H18S F5′-TCAAGAAGAAAGTT CGGAGG-3′, H18S R5′-CACGTTTTCACATCTCC-3′, Mit3130 F5′-AGGCAAGAGAAAT AACGCCC-3′, and Mit3301 R5′-AGGCAAAGAAGAATAGGCC-3′. The presence of GBM in human sera was evaluated using primers amplifying orf 152 (orf 152F 5′-AGGCCAAACCTGCAATC-3′ and orf 152R 5′-CCGGAAGATTCCAGGTTCA-3′) and orf 268 (orf 268F 5′-ACAACCTCTATATCC-3′ and orf 268R 5′-AATCT CCTCAGGTCT-3′). Amplification using Phusion DNA Polymerase (New England Biolabs) started with an initial denaturation step at 98°C for 30 seconds, followed by 35 cycles at 98°C for 10 seconds, at 53°C for 30 seconds, and at 72°C for 30 seconds. Sequencing reactions were performed with the agents of the ABI Prism dye terminator cycle sequencing ready reaction kit (Perkin Elmer Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions.
Enzyme-Linked Immunosorbent Assay (ELISA)
The presence of IgG antibodies specific to Marseillevirus-like particles were evaluated using an ELISA with Marseillevirus as the antigen. ELISA-negative controls were obtained using sera from 10 specific-pathogen-free balb/c mice aged 3 months (Charles River, Lyon), which were supposed to be unaffected with GBM. The ELISA was performed as follows. Purified Marseillevirus (20 ng) was coated with carbonate buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, and 0.2 g/L NaN₃ [pH 9.6]) overnight at 4°C in a flat-bottomed 96-well ELISA microplate. The plate was then washed 2 times with PBS, saturated with 5% bovine serum album (Sigma-Aldrich) for 2 hours, and incubated with sera at a 1:100 dilution. IgG titers in blood from donors 27725 and 20363 were estimated using serial dilutions ranging from 1:10 to 1:5000. For a positive control, mouse anti-Marseillevirus antibody was used at a 1:1000 dilution. Following sera incubation, the plate was washed 3 times with PBS 0.1% Tween 20 and incubated for 1 hour with secondary horse-radish peroxidase–conjugated anti-human IgG at a 1:5000 dilution (Jackson ImmunoResearch). Detection was performed at 490 nm using o-phenylenediamine dihydrochloride substrate (Sigma-Aldrich). Average results for IgG levels were obtained from 2 independent experiments. Negative controls (OD₄₉₀ 0.057) and positive controls (OD₄₉₀ 0.474) were included for each plate. The ELISA threshold (OD₄₉₀ 0.260) was calculated using the relative percentage of positivity (RPP) formula: \[ \text{RPP} = \frac{\text{OD}_{\text{threshold}} - \text{OD}_{\text{negative control}}}{\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}} \] An RPP of 50% was used, for which a relative specificity of 99.9% and a relative sensibility of 90.4% for ELISA results were estimated [18].

Cell Culture and Viral Infection
Human cells were maintained under standard culture conditions (37°C and 5% CO₂). THP1 cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 1% penicillin/streptomycin. HeLa cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Jurkat cells were cultured in Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Human primary macrophages were obtained from fresh peripheral blood mononuclear cells (PCR negative for possible GBM infection) incubated with anti-CD14 magnetic beads, in accordance with the manufacturer’s recommendations (Dynabeads CD14, Life Technologies). Purified cells were incubated for 2 days to allow attachment and differentiation. Infections with GBM-containing serum were performed by inoculating cells at 100% confluence with a purified viral suspension diluted 1:10 in cell media for 48 hours. Seven and 14 days after inoculation, 200 µL of cell suspension was harvested and centrifuged at 300 g for 5 minutes. DNA from the cell supernatant and cell pellet was extracted using a High Pure Viral Nucleic Acid Kit and then amplified by PCR, using the orf 152 and H185 primers. For DNase treatment, 35 mL of Jurkat T-cell supernatant 14 days after inoculation was centrifuged at 90000 g for 2 hours to obtain a pellet of viral particles. The pellet was resuspended in 0.5 mL of sterile PBS filtered through 0.2-µm pores. Viral suspension (0.2 mL) was treated with 4 U of TURBO DNase (Life Technologies) for 1 hour at 37°C, following by DNase inactivation by adding 0.1 volume of DNase inactivation buffer. Nucleic acids were extracted using the High Pure Viral Nucleic Acid Kit (Roche) according to the manufacturer’s protocol.

Fluorescence In Situ Hybridization and Microscopy
Observations
For in situ hybridization, concentrated serum (50 µL) was fixed in 4% formol for 1 hour at room temperature, 56000 × g for 2 hours to obtain a pellet, and embedded in sterile agarose. The agarose plug was dehydrated in 100% ethanol for 2 hours, embedded in paraffin, and cut into thin sections. Agarose sections were deposited onto glass slides and deparaffinized. The sections were incubated with 50 ng of DIG-labeled DNA probe (orf152-orf153) for 5 minutes at 95°C and then for 20 hours at 37°C. The sections were washed 3 times in 1:1 (vol:vol) formamide–standard sodium citrate 2× followed by 3 washes with standard sodium citrate 2× for 3 minutes, and they were then washed for 30 minutes in TNB buffer (100 mM Tris-HCl [pH 7.5], 150 mM NaCl, and blocking buffer 1X [Roche]). Anti-DIG fluorescein isothiocyanate mouse antibodies (1:200) were incubated for 30 minutes, followed by 3 washes for 3 minutes in TNT buffer (100 mM Tris-HCl [pH 7.5], 150 mM NaCl, and 0.1% Tween 20), counterstained with DAPI, and stored at 4°C for further observation using a Leica SP5 confocal microscope. For epifluorescence observations, the purified viral suspension (20 µL) was diluted in PBS containing 2% paraformaldehyde and filtered through a 0.02-µm-pore Whatman filter. The filter containing viral particles was stained with 2.5× SYBR Gold (Molecular Probes) for 10 minutes, washed 2 times with PBS 1X, and then observed under an epifluorescence inverted microscope (Leica DMi6000). The average number of virus-like particles was estimated using ImageJ software and was calculated from 3 representative fields. For electron microscopy, Formvar-coated grids were deposited in a 40-µL drop of viral suspension and incubated for 30 minutes at 37°C. The grids were then incubated for 10 seconds on 1% ammonium molybdate, dried on absorbing paper, and observed with a transmission electron microscope (Morgagni 268D, Philips).

2-Dimensional Protein Analysis
A 2-dimensional protein analysis was performed as previously described [15]. Following protein migration, gels were silver stained. Selected excised spots were further identified using MALDI-TOF mass spectrometry. For Western blot analyses,
the samples were transferred to a 0.45-μm nitrocellulose membrane and immunoblotted overnight at 4°C, using a mouse anti-Marseillevirus polyclonal antibody (1:2500) or serum from donor 27 725 (1:1000) in PBS plus 0.3% Tween 20 with 5% nonfat dried milk. Secondary horseradish peroxidase-conjugated anti-mouse IgG goat antibody was used at a 1:5000 dilution.

RESULTS

Pyrosequencing DNA extracted from the viral-enriched fraction of the blood of asymptomatic blood donors generated 20 238 reads (NCBI accession number: PRJNA183996), which were classified by their taxonomic distribution (Figure 1). A best BLAST hits comparison against the GenBank database (BLASTn; E value, <10^{-5}) indicated that 17 257 metagenomic reads (85.3%) had significant hits and that 67.7% of these reads were related to viruses. The majority of these sequences aligned with viruses from the Arenelloviridae family, including Torque Teno virus (TTV), TTV-like virus, SEN virus, TTV midi virus, and TTV-like mini virus (Table 1), which has been previously reported [5, 12, 16]. However, we unexpectedly found that 2.5% of the viral metagenome matched that of Marseillevirus. We were initially unconvinced that these data were correct, as this virus has only been found in our laboratory.

Genomic assembly of the Marseillevirus-related reads identified 2 large contigs that mapped on 2 separate regions localized between positions 111 593 and 125 241 (13 649 bp) and positions 210 367 and 220 539 (10 173 bp) on the Marseillevirus genome (Supplementary Figure 1). Coding-DNA sequence (CDS) predictions on these contigs showed nucleotide differences between the Marseillevirus and metagenome genetic maps. Some of these differences corresponded to stop codons that modify CDS lengths, which strongly suggested the identification of a new virus related to Marseilleviridae (Supplementary Table 1). We thus performed 2 sets of PCRs on the sera pool, using orf 268 primers that are specific to the metagenomic sequences and orf 152 primers, which target both the Marseillevirus genome and the metagenome contigs. PCR with orf 152 amplified a 198-bp amplicon in both metagenome and Marseillevirus genomic DNA, whereas PCR with orf 268 resulted in the amplification of a 608-bp fragment specifically in the metagenomic DNA (Supplementary Figure 1C), thus confirming the identification of new Marseillevirus-like DNA sequences. We next performed PCR with orf 152 on each serum sample (n = 10) separately. We detected Marseillevirus-like DNA in the serum from 1 individual, blood donor 27 725 (Supplementary Figure 1D). The viral fraction from this blood donor serum was concentrated using 3 Marseillevirus-specific mouse monoclonal antibodies to allow for additional SOLiD sequencing (NCBI accession number: PRJNA185405). Genomic mapping was performed with CLC software, using the Marseillevirus genome (NC_013756.1) as a matrix, which allowed for the isolation of 114 278 mapped reads. These reads were organized into a 357 433-bp consensus sequence (GC frequency, 44.73%; fractional coverage, 0.95; average coverage, 7.68) that showed a close resemblance between Marseillevirus and this virus. This virus was named “giant blood Marseillevirus.” When CDS predictions for these regions were performed, a total of 617 CDSs were identified, of which 436 were homologous to that of Marseillevirus, 33 were homologous to that of Lausannevirus, and 148 demonstrated no significant BLAST hit (E value, <10^{-5}; Figure 2A and Supplementary Table 1). A neighbor-joining phylogeny analysis based on concatenated alignments of the D5-helicase primase and the A32 ATPase confirmed the clustering of GBM in the Marseilleviridae family (Figure 2B).

Table 1. Viral Reads Classification

<table>
<thead>
<tr>
<th>Viral Class, Species</th>
<th>Reads, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssDNA virus</td>
<td>11 080 (81.40)</td>
</tr>
<tr>
<td>TTV</td>
<td>1575 (11.57)</td>
</tr>
<tr>
<td>TTV-like virus</td>
<td>576 (4.23)</td>
</tr>
<tr>
<td>SEN virus</td>
<td>12 (0.09)</td>
</tr>
<tr>
<td>TTV midi virus</td>
<td>11 (0.08)</td>
</tr>
<tr>
<td>TTV-like mini virus</td>
<td>6 (0.04)</td>
</tr>
<tr>
<td>RSM1 virus</td>
<td>11 (0.08)</td>
</tr>
<tr>
<td>RSM3 virus</td>
<td>333 (2.45)</td>
</tr>
<tr>
<td>dsDNA virus</td>
<td>7 (0.05)</td>
</tr>
<tr>
<td>Marseillevirus</td>
<td></td>
</tr>
<tr>
<td>Enterobacteria phage λ virus</td>
<td></td>
</tr>
</tbody>
</table>

Viral reads classification was based on BLASTn significant hits (E<10^{-5}) from searches against the GenBank nonredundant database. Abbreviations: dsDNA, double-stranded DNA; RSM, Ralstonia solanacearum phage; ssDNA, single-stranded DNA; TTV, Torque Teno virus.
Figure 2. Genome mapping and phylogenetic analysis of the giant blood Marsilellavirus (GBM) genome. A, Genomic map of the predicted coding-DNA sequence (CDS) of the GBM chromosome, using 454 pyrosequencing and SOLiD data. Forward and reverse CDS data are represented with blue and red.
Figure 2 continued. Lines, respectively. The 2 contigs obtained using 454 pyrosequencing are represented by light green boxes. The BLASTp results of the predicted CDS matching Marseillevirus open reading frames are presented as orange boxes, and those matching Lausannevirus are presented as purple boxes. GC deviation and GC skew from the average are represented in blue and red graphs, respectively.

Figure 3. Detection of giant blood Marseillevirus (GBM) infection and immune response in blood donor 27 725. A, Bidimensional protein analysis by silver staining and Western blot (WB) analysis of serum from blood donor 27 725. The presence of viral proteins in serum 27 725 was evaluated using a mouse polyclonal α-Marseillevirus antibody. Selected spots (black arrows) were cut and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Positive spots were reported on a 2-dimensional gel. B, Negative staining of virus-like particles present in the virus-purified fraction from serum of blood donor 27 725; scale bar, 200 nm. C, Epifluorescence microscopy images obtained from fluorescence in situ hybridization on GBM experiments involving serum from donors 27 725 and 20 363. DNA probes were synthesized using the Marseillevirus genomic region of 152-orf 153 (upper panels). Sections were counterstained with DAPI (middle panels). The merged green and blue channels are presented on the bottom; scale bar, 2 µm. ORF, open reading frame; SDS-PAGE, sodium dodecyl sulfate pulsed-field gel electrophoresis.

Figure 2 continued. Lines, respectively. The 2 contigs obtained using 454 pyrosequencing are represented by light green boxes. The BLASTp results of the predicted CDS matching Marseillevirus open reading frames are presented as orange boxes, and those matching Lausannevirus are presented as purple boxes. GC deviation and GC skew from the average are represented in blue and red graphs, respectively. B, A neighbor-joining tree (1000 bootstrap replications) based on concatenated alignments of 2 nucleocytoplasmic large DNV virus (NCLDV) core proteins: D5-helicase primase and A32 ATPase. The GBM branch is indicated with a red arrow. The NCLDV family is represented as follows: ○, Iridoviridae; ●, Ascoviridae; □, Marseilleviridae; Δ, Phycodnaviridae; ◊, Asfarviridae; and ♦, Poxviridae. Abbreviations: ASFV, African swine fever virus; AV, Ambystoma tigrinum virus; EhV86, Emiliana huxleyi virus 86; EsV1, Ectocarpus siliculosus virus 1; FsV, Feldmannia species virus; HvA-3e, Heliothis virescens ascovirus 3e; IV3, Invertebrate iridescent virus 3; IV6, Invertebrate iridescent virus 6; ISKNV, infectious spleen and kidney necrosis virus; LDV-1, Lymphocystis disease virus 1; LDV-isolate China, Lymphocystis disease virus isolate China; MCV, subtype 1, Molluscum contagiosum virus subtype 1; OsV5, Ostreococcus virus 5; PBCV-FR483, Paramecium bursaria Chlorella virus FR483; SfAV-1a, Spodoptera frugiperda ascovirus 1a; TnA-2c, Trichoplusia ni ascovirus 2c.
A 2-dimensional Western blot coupled with mass spectrometry of a concentrated viral fraction of the serum was performed using an α-Marseillevirus polyclonal antibody to identify potential GBM antigens (Figure 3A). Fifteen spots were selected for identification, 3 of which were further identified as containing viral peptides associated with this virus. These peptides corresponded to 2 hypothetical GBM proteins (ORF 137 and 543) and 1 flavin-containing amine oxidoreductase (GBM ORF 123). To observe the viral particle, we used transmission electron microscopy, and observations of the same sample allowed for detection of virus-like particles with a mean size (± SD) of 216.7 ± 4.7 nm; n = 3), which is compatible with Marseilleviridae family members (Figure 3B). Additionally, fluorescence in situ hybridization using DNA-probe hybridization in the orf 152-orf 153 genomic region identified particles of the same size in serum that had been purified and concentrated (Figure 3C).

We next performed a systematic screen of human cell lines and primary cells to evaluate the ability of GBM to infect human cells. These screens included blood cell lines such as monocytes (THP1), T cells, Jurkat cells, and anti-CD14 purified primary human macrophages. A serum inoculation of 14 days did not result in GBM detection in the supernatant of HeLa cells, THP1, or macrophages. The PCR results were positive for the Jurkat supernatant, which suggested that GBM was able to infect T cells (Figure 4A). In addition, Jurkat supernatant ultracentrifugation combined with DNAse treatment still showed the presence of encapsidated viral DNA, which strongly suggested the production and release of encapsidated viral particles from these cells (Figure 4A). Additional fluorescence in situ hybridization and transmission electron microscopy of Jurkat cell inclusions confirmed these results. GBM DNA and viral particles were detected inside the Jurkat cytosol 21 days after infection (Figure 4B).
ELISA was used to locate specific antibodies with Marseille-virus as an antigen in the GBM-infected blood, compared with a PCR-negative blood control. A high level of IgG antibodies (1:1000) was detected in the infected sample, compared with none in the control (Figure 5A). This serum was subsequently submitted to a 2-dimensional Western blot of the Marseille-virus proteome. We were able to identify 26 protein spots in the genome of GBM, with the most reactive being the major viral capsid protein (MAR_ORF342; Figure 5B).

Finally, we evaluated the prevalence of GBM infection in 20 additional blood donors by performing combined IgG detection and PCR amplification/sequencing on 20 sera sampled from asymptomatic blood donors; all were from Marseille, 65% were male, and the median was 46.5 years. We found 3 blood donors with IgG levels above the calculated ELISA threshold (OD₄₉₀ nm > 0.260). Moreover, 2 were PCR positive for GBM, and 1 had elevated levels of IgG (Table 2 and Supplementary Table 2).

**Table 2. Serological and Molecular Surveys of 20 Blood Donors for Giant Blood Marseille-virus Infection**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood Donors</th>
<th>IgG Positive</th>
<th>PCR Positive</th>
<th>IgG and PCR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample number</td>
<td>20/20 (100)</td>
<td>3/20 (15)</td>
<td>2/20 (10)</td>
<td>1/30 (5)</td>
</tr>
<tr>
<td>Male sex</td>
<td>13/20 (65)</td>
<td>2/3 (66.7)</td>
<td>2/2 (100)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Age, y, median</td>
<td>46.5 (22–65)</td>
<td>53 (44–59)</td>
<td>49 (45–53)</td>
<td>53</td>
</tr>
</tbody>
</table>

Data are no. of blood donors with the characteristic/no. tested (%). The IgG-positive threshold was fixed at 0.26 OD. Results of PCR-positive sera were systematically verified by DNA sequencing. See Materials and Methods for further information.

Abbreviations: IgG, immunoglobulin G; PCR, polymerase chain reaction.
DISCUSSION

In this study, we definitively established the presence of a novel giant virus in the blood of an asymptomatic blood donor by complete genome sequencing, antigen detection, morphological visualization (transmission electron microscopy and fluorescence in situ hybridization), and cell culture. Although these findings should be further confirmed, contamination is unlikely because of the multiple procedures used to identify the virus, because this is the first virus from the Marseilleviridae family detected in human cells, and because of its original genomic sequence.

Recently, another virus of the Marseilleviridae family, Sene- galivirus, was isolated in a human stool sample from an asymptomatic patient [17]. The present study confirms that some giant viruses of the Marseilleviridae family, such as GBM, are associated with humans. Because giant viruses escape current screening techniques, the prevalence of human infection with giant viruses related to the Mimi- and Marseilleviridae families may be underestimated. The fact that GBM infection was found in apparently healthy blood donors suggests that this infection may present asymptptomatically or with only mild symptoms. Additionally, the presence in some cases of both IgG antibodies and viruses in the blood suggests possible chronic carriage. Together, these results place Marseilleviridae in the human virome and raise questions about the long-term consequences of this viral carriage.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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