Genome Sequence of Human Cytomegalovirus Ig-KG-H2, a Variant of Strain KG Propagated in the Presence of Neutralizing Antibodies

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ABSTRACT
Human cytomegalovirus (HCMV) replicates poorly when first isolated from clinical material. However, upon serial passage in fibroblasts, improved replication and increased release of cell-free virus are conferred by disruptive mutations in the RL13 gene and one or more of the contiguous genes UL128, UL130, and UL131A (1). The latter mutations disrupt assembly of a pentameric complex on the virion surface that is important for entry into epithelial and endothelial cells but not fibroblasts (2–6).

In our recent work, replicate fibroblast cultures were infected with HCMV in urine from a symptomatic congenitally infected infant (7). One lineage (Ig-KG) was passaged with HCMV-hyperimmune globulin (HIG) (CytoGam) in the culture medium, whereas the other (ϕ-KG) was passaged in the absence of HIG. ϕ-KG lost epithelial tropism and acquired frameshift mutations disrupting RL13 and UL131A, whereas Ig-KG retained epithelial tropism and was intact in these genes after 22 passages. Long-term genetic stability of these lineages (and their mutations) was confirmed by isolating representative viruses by limiting dilution, i.e., Ig-KG-H2 from the Ig-KG passage 22 stock and ϕ-KG-B5 from the ϕ-KG passage 13 stock.

Preliminary Ion Torrent and targeted PCR/Sanger sequence analyses that were focused on protein-coding sequences identified mutations affecting five genes (RL13, UL100, UL102, UL122, and UL131A) in the parental Ig-KG and ϕ-KG stocks that were also present in Ig-KG-H2 and ϕ-KG-B5 (7). Here, we report the complete genome sequence of Ig-KG-H2 and compare it with that of ϕ-KG-B5.

Ig-KG-H2 or ϕ-KG-B5 virions were pelleted from culture supernatants by ultracentrifugation and treated with DNase I prior to DNA purification by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation (7). DNA samples (~100 ng) were sheared acoustically to an approximate size of 450 bp, and sequencing libraries were processed through seven PCR cycles using the LTP library preparation kit (KAPA Biosystems, Wilmington, MA, USA), employing NEBNext multiplex oligos for Illumina (New England Biolabs, Ipswich, MA, USA). Sequencing on an Illumina NextSeq mid-output 300-cycle cartridge generated 9,614,942 and 10,011,260 paired-end reads of 150 bp.
nucleotides for Ig-KG-H2 and φ-KG-B5, respectively. A pipeline included in the GRACy tool (https://github.com/salvocamiolo/GRACy) was used to perform de novo assembly of the Ig-KG-H2 reads. Briefly, reads that aligned with the Hg38 human reference sequence (GenBank GCA_000001405.15) using Bowtie 2 v. 2.3.1 (8) (with the end-to-end flag set) were removed, and sequencing adapters and low-quality reads were removed using Trim Galore v. 0.4.0 (https://github.com/FelixKrueger/TrimGalore) and PRINSEQ v. 0.20.4 (9), respectively. The remaining reads were normalized and assembled using SPAdes v. 3.12 (10), and the resulting contigs were ordered in relation to the HCMV reference strain Merlin genome sequence (GenBank accession number AY446894.2). Gaps were closed using an overlap-layout-consensus algorithm implemented in GRACy, and the assembly was further refined by visualization in Tablet v. 1.19.09.03 (11) of a read alignment that had been generated using Bowtie 2. All tools were used with default parameters unless otherwise specified. The Ig-KG-H2 genome sequence consisted of 236,244 bp (G/H11001C content, 57.4%) and was determined at an average coverage of 4,886 reads/nucleotide. The φ-KG-B5 reads were aligned to the resulting Ig-KG-H2 genome sequence using Bowtie 2, and differences present in the entire population were identified manually using Tablet.

As reported previously (7), φ-KG-B5 had disruptive mutations in RL13 and UL131A, as well as a single amino acid substitution in UL122 (Table 1). In contrast, Ig-KG-H2 lacked disruptive mutations in RL13 and UL128, UL130, or UL131A but contained mutations resulting in four amino acid substitutions in UL102, two amino acid substitutions in UL100, and a distinct single amino acid substitution in UL122. Also, Ig-KG-H2 had two silent mutations in UL57 and UL98. The availability of the genome sequences of Ig-KG-H2 and φ-KG-B5 will facilitate studies of the relative importance of these mutations in the adaptation of Ig-KG-H2 to growth in the presence of HIG.

**Data availability.** The genome sequence of Ig-KG-H2 has been deposited in GenBank under accession number MN274568. Raw reads are available from the European Nucleotide Archive with accession numbers ERR3988552 (Ig-KG-H2) and ERR3988553 (φ-KG-B5).

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### Table 1 Mutations identified in the Ig-KG-H2 and φ-KG-B5 genomes

| Gene       | Protein                        | Mutanta | Mutation(s)b | Consequence                  |
|------------|--------------------------------|---------|--------------|------------------------------|
| None       | None                           | φ-KG-B5 | 1-bp deletion (C6372) | None                         |
| RL13       | Membrane protein RL13          | φ-KG-B5 | 10-bp deletion (CATATTATT at positions 11661–11670) | Frameshift after residue 164 |
| UL57       | Single-stranded DNA-binding protein | Ig-KG-H2 | C89864T substitution | Silent                       |
| UL98       | DNase                          | Ig-KG-H2 | C145699T substitution | Silent                       |
| UL100      | Envelope glycoprotein M        | Ig-KG-H2 | C146566G substitution | E361D                        |
|            |                                 |         | C146750A and T146751G substitutions | S300L                        |
| UL102      | Helicase-primase subunit       | Ig-KG-H2 | C147608A substitution | S15I                          |
|            |                                 |         | C147895G substitution | L23V                          |
| UL122      | Regulatory protein IE2         | φ-KG-B5 | G171290C substitution | F384L                        |
| UL131A     | Envelope protein UL131A        | φ-KG-B5 | G171315T substitution | S376Y                        |
|            |                                 |         | 1-bp insertion (T178079) | Frameshift after residue 27 |

a The virus in which each mutation occurred was identified by comparison with strain Merlin as a representative HCMV strain.
b Coordinates refer to the Ig-KG-H2 genome sequence.

c This is only nominally a mutant, as the mutation represents a difference in the number of nucleotides in a C tract that varies in length among HCMV strains.
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