Supporting Information

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SI Text

Materials and Methods ***Antibodies, viruses, and cells.*** The anti-rabbit polyclonal antibodies against human cytomegalovirus (HCMV) assemblin and capsid scaffolding protein (CSP) were kindly provided by Annette Meyer of Warner Lambert Co (Ann Arbor, MI). The monoclonal antibodies that react with HCMV UL44 and gB were purchased from Goodwin Institute for Cancer Research, and the monoclonal antibody against human actin was purchased from Sigma Inc. The propagation of HCMV (AD169) in human fibroblasts was carried out as described previously (1). THP-1 cells (American Type Culture Collection) were differentiated with 100 ng/mL tetradecanoyl phorbol acetate (Sigma) for 24 h before infection (2–4).

In vitro analysis of ribozyme activity. M1GS RNAs and the CSP mRNA substrate were synthesized in vitro by T7 RNA polymerase (Promega Inc.) and further purified on 8% polyacrylamide gels containing 8-M urea. The procedures to measure the equilibrium dissociation constants (K_d) of the M1GS-CSP mRNA substrate complexes were modified from Pyle et al (5) and have been described previously (1, 6). The values of K_d were the average of three experiments. The cleavage reactions were carried out by incubating the ribozyme and [^{32}P]-labeled mRNA substrate at 37 °C in a volume of 10 μL in buffer A (50 mM Tris, pH 7.5, 100 mM NH_4Cl, and 100 mM $MgCl_2$) (1). Cleavage products were separated in denaturing gels and quantitated with a STORM840 phosphorimager (Molecular Dynamics). The overall rate of cleavage [$(k_{cat}/K_m)^s$] was assayed following the procedure described previously (6).

In the cleavage reaction shown in Fig. 1*D*, the substrate (20 nM) was incubated either alone or with 5 nM of M1-C1, M1-C2, or M1-TK. Reactions were carried out for 40 min in buffer A (50 mM Tris pH 7.5, 100 mM NH_4Cl, and 100 mM $MgCl_2$) at 37 °C.

Growth analysis of Salmonella. Growth analysis of *Salmonella* in LB broth was carried out by first inoculating a single colony in 2 mL of LB broth and culturing at 37 °C with shaking at 250 rpm overnight (about 16 h) (7). Thirty microliters of the overnight culture were then inoculated into 3 mL of fresh LB broth and cultured at 37 °C and 250 rpm. At time points of 0, 2, 4, 6, 8, 10, 12, 14, 16, and 24 h after inoculation, 100 μL of bacterial culture were collected and used for analysis by limiting dilution in sterile 96-well plates and then plated on LB agar plates to determine their cfu/mL. Each sample was analyzed in triplicates, and the analysis was repeated at least three times. The average value of cfu/mL was used to generate the growth curve (7).

Analysis of the expression of ribozymes. For Northern analyses of the expression of the ribozymes, both nuclear and cytoplasmic RNA fractions from *Salmonella*-treated cells were isolated (8), separated in gels that contained formaldehyde, transferred to nitrocellulose membranes, hybridized with the [^{32}P]-radiolabeled DNA probes that contained the DNA sequence coding for M1 RNA and H1 RNA, and finally analyzed with a STORM840 phosphorimager. The radiolabeled DNA probe used to detect M1GS RNAs was synthesized from plasmid pFL117 (1, 9).

Analysis of viral mRNA and protein expression. Viral mRNAs and proteins were isolated from HCMV infected cells as described previously (1). The multiplicity of infection (MOI) is specified in *Results*. To measure the levels of viral immediate-early (IE) transcripts, some of the cells were also treated with 100 μg/mL cycloheximide prior to and during infection. The RNA fractions were separated in agarose gels that contained formaldehyde, transferred to nitrocellulose membranes, hybridized with the [^{32}P]-radiolabeled DNA probes that contained the HCMV or human β-actin DNA sequences, and analyzed with a STORM840 Phosphorimager. The DNA probes used to detect M1GS RNAs, human β-actin mRNA, HCMV immediate-early 5 kb RNA transcript, IE2 mRNA, US2 mRNA, and CSP mRNA were synthesized from plasmids pFL117, pβ-actin RNA, pCig27, pIE2, pCig38, and pCSP, respectively.

For Western analyses, the polypeptides from cell lysates were separated on either SDS/7.5% polyacrylamide gels or SDS/9% polyacrylamide gels cross-linked with N,N″methylenebisacylamide and transferred electrically to nitrocellulose membranes. We stained the membranes using the antibodies against HCMV proteins and human actin in the presence of a chemiluminescent substrate and analyzed the stained membranes with a STORM840 phosphorimager. Quantitation was performed in the linear range of RNA and protein detection.

Analysis of the level of viral genome replication. Total and encapsidated (DNase I-treated) DNAs were isolated from HCMV infected cells essentially as described (10) and used as the PCR templates. Viral DNA was detected by PCR amplification of the viral IE1 sequence, using human β-actin sequence as the internal control. The 5′ and 3′ primers were CMV3 (5′-CCAAGCGGCCTCTGATAACCAAGCC-3′) and CMV4 (5′-CAGCACCATCCTCCTCTTCCTCTGG-3′), respectively, while those used to amplify the actin sequence were Actin5 (5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′) and Actin3 (5′-CTAGAAGCATTGCGGTGGCAGATGGAGGG-3′), respectively (11). PCR cycles and other conditions were optimized to assure that the amplification was within the linear range.

The PCR reactions were performed in the presence of α-[^{32}P]-dCTP, and the radiolabeled DNA samples were separated on polyacrylamide gels and then scanned with a STORM840 phosphorimager. A standard (dilution) curve was generated by amplifying different dilutions of the template DNA. The plot of counts for both HCMV and β-actin vs. dilutions of DNA did not reach a plateau for the saturation curve under the conditions described above, indicating that quantitation of viral DNA could be accomplished. Moreover, we observed that the ratio of viral DNA to β-actin remained constant with respect to each DNA dilution in the standard curve, suggesting that the assay is adequately accurate and reproducible. The PCR results were derived from three independent experiments.

Analysis of the expression of Toll-like receptor 9 (TLR9) mRNA. Total RNA was isolated as described previously (1). The levels of TLR9 mRNA were determined using a qRT-PCR assay, in which the mRNA was amplified using a One-Step SYBR RT-PCR kit (TaKaRa) in an IQ5 real-time PCR instrument (Bio-Rad). The level of β-actin mRNA was used as the internal control. The 5′ and 3′ primers for amplification of TLR9 mRNA sequence were tlr9-5 (5′-GTGACAGATCCAAGGTGAAGT-3′) and tlr9-3 (5′-CTTCCTCTACAAATGCATCACT-3′), respectively, whereas those used to amplify the actin sequence were actin-5 (5′-CGTGGGCCGCCCTAGGCACCA-3′) and actin-3 (5′-TTGGCCTTAGGGTTCAGGGGGG-3′), respectively (12, 13). Each reaction (25 μL) included 2 μL total RNAs, 12.5-μL One

Step SYBR RT-PCR kit, 0.5-μL TaKaRa Ex Taq HS polymerase, 0.25-μL M-MLV RTase, 0.5-μL RNase inhibitor, 0.5-μL of each of forward and reverse primers (5 pmol/each) and 8.25 μL H_2O.

Analysis of the inhibition of HCMV growth by M1GS ribozymes. To determine the level of inhibition of viral growth, 5×10^5 differentiated THP-1 cells were first treated with *Salmonella* carrying different constructs at a MOI of 20 bacteria/cell. At 16 h post-treatment, the *Salmonella*-containing cells were isolated by FACS analysis based on GFP expression. The isolated cells were incubated for 8 h and then either mock-infected or infected with HCMV at an MOI of 2. The cells and medium were harvested at 1, 2, 3, 4, 5, 6, and 7 days postinfection. Viral stocks were prepared, and their titers were determined by performing plaque assays on human foreskin fibroblasts (1). The values obtained were the average from triplicate experiments.

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