Supporting Information

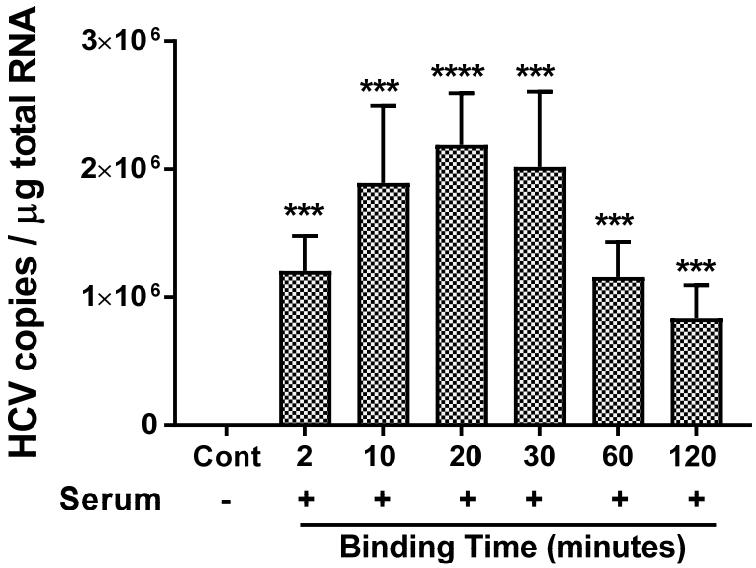
Binding of Free and Immune Complex-Associated Hepatitis C Virus to Erythrocytes Is Mediated by the Complement System

Kazi Abdus Salam, Richard Y. Wang, Teresa Grandinetti, Valeria De Giorgi, Harvey J. Alter, and Robert D. Allison

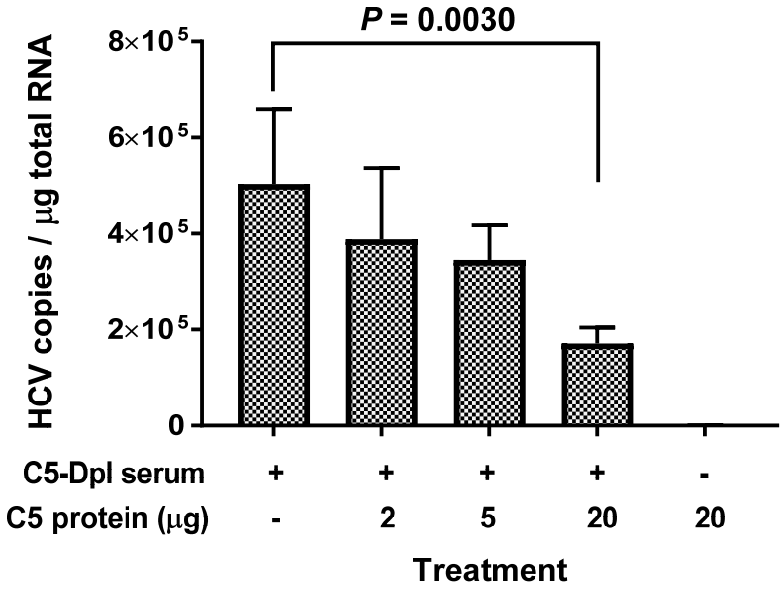
Supporting Methods:

In vitro HCV binding assay with complement-depleted sera

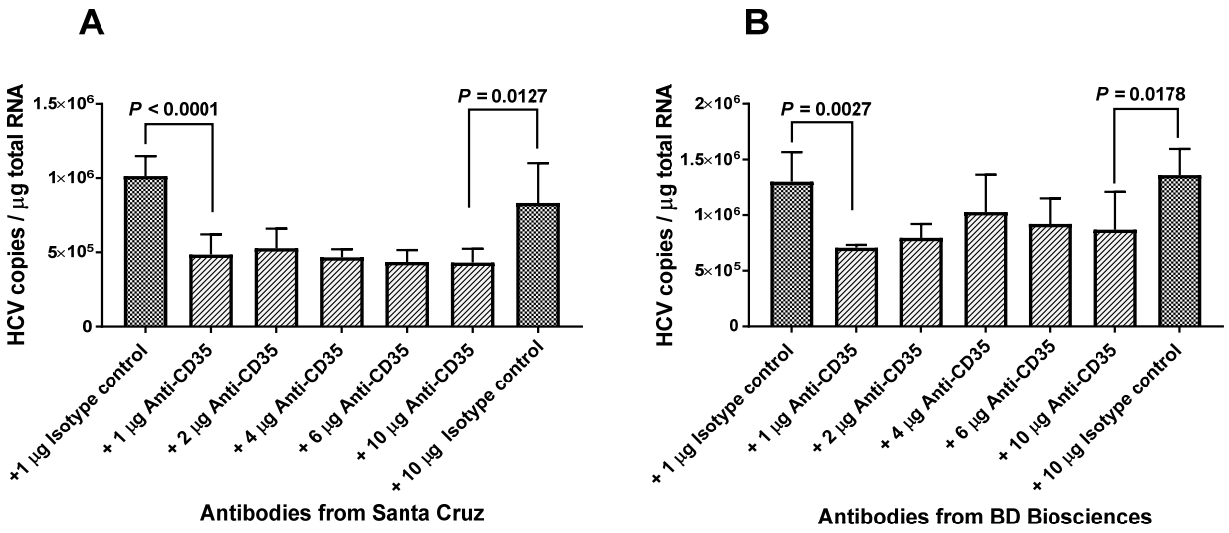
For conducting HCV binding assays using complement depleted sera reconstituted with purified complement proteins, 100 µL of the indicated complement-depleted serum was used for each reaction. All the complement-depleted sera and the purified complement proteins used in this study were obtained from Complement Technology (Tyler, TX). To measure the effect of antibodies against various cell surface receptors on HCV binding to erythrocytes, 2 mL of erythrocytes (5 X 10^8 cells total) were incubated with 6 µg of mouse monoclonal antibodies against the indicated receptors at 25^{o}C for 30 minutes, followed by adding 3 mL of complement activated virus and incubating for 15 minutes. All the antibodies for blocking cell surface receptors used in this study were obtained from BD Biosciences (San Diego, CA) except that a few anti-CD35 (To5) and normal mouse IgG antibodies from Santa Cruz Biotechnology (Dallas, TX) were also tested. The clone numbers for antibodies from BD Biosciences are as follows: CD11b, ICRF44; CD21, 1048; CD32, 3D3; CD35, E11; CD47, B6H12. The epitope mapping for anti-CD21 and anti-CD35 has been previously reported (1, 2).



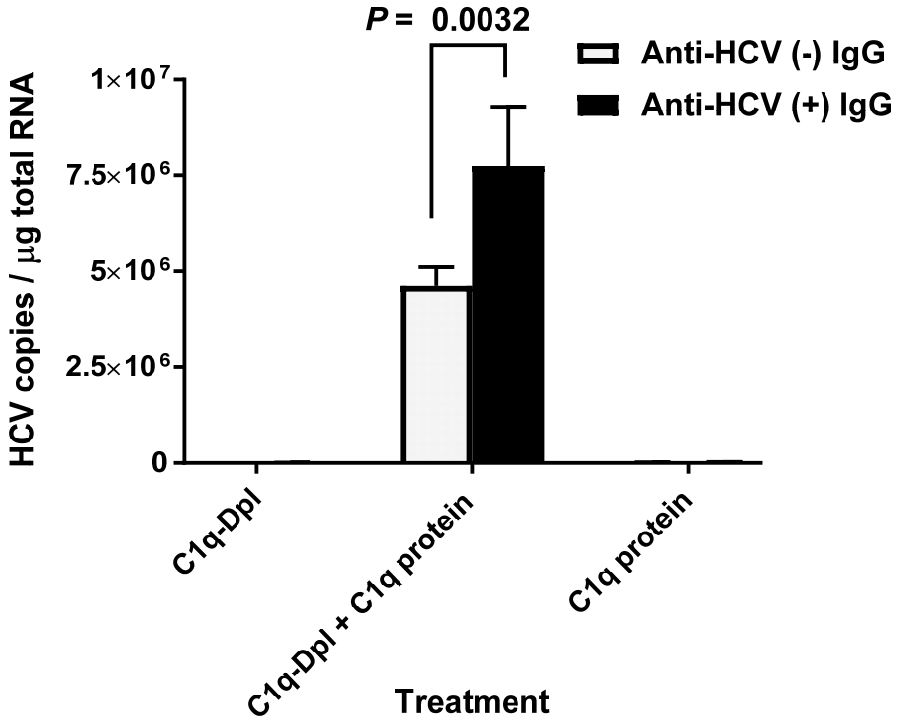
Supporting Fig. S1. Kinetic profiles for the binding of HCV genotype 2a virus to erythrocytes. Three milliliters HCV2a (JFH1) virus (1.9 X 10^8 copies total) was treated in the presence of serum (100 μL) at 25°C for 30 minutes. The erythrocytes (5 X 10^8 cells total) in 2 mL complete RPMI medium were then added and incubated at 25°C with variable times as stated, followed by adding EDTA to a final concentration of 20 mM. The erythrocytes were collected by centrifugation and washed three times with 1X PBS, pH7.4. A control (Cont) experiment without serum (100 μL medium only) was performed for each assay, and incubated at 25°C for the longest time point as indicated. Total RNA isolation from erythrocytes and quantification of HCV RNA were performed as described in Methods section. **** $P < 0.0001$ was expressed when compared to without serum treatment. Each value represents the mean ± standard deviation of six determinations. The data are representative of two independent experiments using erythrocytes from at least two different healthy donors. Cont, control (serum absent)



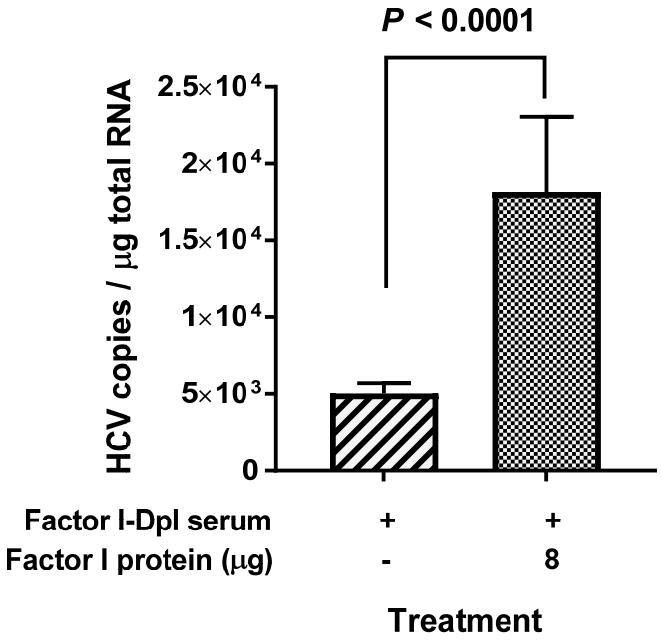
Supporting Fig. S2. Purified complement protein C5 added to C5-depleted serum did not restore HCV binding to erythrocytes. Three milliliters of HCV1a virus (1 X 10^7 genomic copies total) were treated with 100 µL of the C5-depleted serum sample, C5-depleted serum sample plus the indicated amounts of purified C5 protein, or purified C5 protein only. After 30 minutes incubation at 25°C, 2 mL of erythrocytes (5 X 10^8 cells total) in complete RPMI medium were added to the reaction mixture and incubated further at 25°C for 15 minutes, followed by adding EDTA to a final concentration of 20 mM. The erythrocytes were collected by centrifugation and washed three times with 1X PBS, pH7.4. Total RNA isolation from erythrocytes and quantification of HCV RNA were performed as described in Methods section. Each value represents the mean ± standard deviation of six determinations. The data are representative of two independent experiments using erythrocytes from at least two different healthy donors. Abbreviation: Dpl, depleted.



Supporting Fig. S3. Titration of antibody concentration of anti-CD35 (complement receptor 1) from Santa Cruz Biotechnology (SC) and BD Biosciences (BD) in complement-mediated HCV binding to erythrocytes. Two mL of erythrocytes (2.5 X 10^8 cells/mL) were incubated with the indicated amount of anti-human CD35 antibody (clone To5) and isotype control antibody from SC (A), or anti-human CD35 antibody and isotype control antibody from BD (B) at 25°C for 0.5 h; followed by mixing with 3 mL of activated virus (pre-incubation with serum for 0.5 h at 25°C, 1X10^7 HCV1a genomic copies total), and incubating at 25°C for 15 minutes, followed by adding EDTA to a final concentration of 20 mM. The erythrocytes were collected by centrifugation and washed three times with 1X PBS, pH7.4. Total RNA isolation from erythrocytes and quantification of HCV RNA were performed as described in Methods section. Each value represents the mean ± SD of six determinations.



Supporting Fig. S4. C1q protein promoted HCV binding to erythrocytes through both antibody-independent and HCV specific antibody-dependent complement activation. Three milliliters of HCV1a (1 X 10^7 genomic copies total) were incubated with heat aggregated IgG (100 μg/mL) as indicated at 25°C for 30 minutes, followed by mixing with 100 μL of the indicated C1q-depleted serum sample, C1q-depleted serum sample plus purified C1q protein (25 μg), or purified C1q protein (25 μg) only. After 30 minutes incubation at 25°C, 2 mL of erythrocytes (5 X 10^8 cells total) in complete RPMI medium were added to the reaction mixture and incubated further at 25°C for 15 minutes, followed by adding EDTA to a final concentration of 20 mM. The erythrocytes were collected by centrifugation and washed three times with 1X PBS, pH7.4. Total RNA isolation from erythrocytes and quantification of HCV RNA were performed as described in Methods section. Each value represents the mean ± standard deviation of six determinations.



Supporting Fig. S5. Factor I-mediated release HCV2a virus from erythrocytes can infect Huh 7.5.1 cells. Six milliliters of HCV2a virus (6 X 10^8 genomic copies total) incubated with 100 µL Factor I-depleted serum (titrated previously) for 30 minutes at 25°C, followed by adding 4 mL of erythrocytes (1 X 10^9 cells total), incubating at 25°C for 2 hours. After several washing steps, the reaction was carried out in the presence of Factor I (8 μg) for 2 hours at 25°C. The supernatants were collected by centrifugation. One milliliter supernatant was added into each well containing pre-cultured Huh 7.5.1 cells (2.0 X10^5 cells/well) in 6-well plate and incubate the plate in a CO_2 incubator at 37°C. After 2 hours incubation, wash cells and continue to incubate for 20-24 hours. The cells were lysed in 650 µL QIAGEN buffer RLT supplemented with 1% 2-mercaptoethanol. RNA isolation was carried out using the RNeasy Plus Mini kit (Qiagen) according to the manufacturer's instructions. The Huh 7.5.1 cells bound HCV were measured as described in Methods section. Each value represents the mean ± standard deviation of nine determinations.

References

1. Guthridge JM, Young K, Gipson MG, Sarrias MR, Szakonyi G, Chen XS, Malaspina A, et al. Epitope mapping using the X-ray crystallographic structure of complement receptor type 2 (CR2)/CD21: identification of a highly inhibitory monoclonal antibody that directly recognizes the CR2-C3d interface. J Immunol 2001;167:5758-5766.
2. Nickells M, Hauhart R, Krych M, Subramanian VB, Geoghegan-Barek K, Marsh HC, Jr., Atkinson JP. Mapping epitopes for 20 monoclonal antibodies to CR1. Clin Exp Immunol 1998;112:27-33.