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Analysis of IgG Anti-HEV Antibody Protective Levels During Hepatitis E Virus Reinfection in Experimentally Infected Rhesus Macaques

Youkyung Choi[1], **Xiugen Zhang**[1], **Brianna Skinner**[2]

[1]Laboratory Branch, Division of Viral Hepatitis, National Centers for HIV/AIDS, Viral Hepatitis, STD, and TB prevention

[2]Comparative Medicine Branch, Division of Scientific Resources, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract

Background.—Secondary spread of hepatitis E virus (HEV) infection occurs often in endemic settings in developing countries. The host immune signatures contributing to protection against subsequent HEV reinfection are unknown.

Methods.—Twelve seroconverted rhesus macaques were reinoculated with homologous HEV genotype 1 (gt1, Sar-55) and followed for 115 days. HEV RNA, HEV-specific T-cell responses, IgG anti-HEV antibody, and the IgG anti-HEV avidity index were tested.

Results.—Four animals with baseline IgG anti-HEV levels from 1.5 to 13.4 World Health Organization (WHO) U/mL evidenced reinfection as determined by HEV RNA in stool, and increase in IgG anti-HEV levels between 63- and 285-fold ($P = .003$). Eight animals with baseline IgG anti-HEV levels from 2.8 to 90.7 WHO U/mL did not develop infection or shed virus in feces, and IgG anti-HEV antibody levels were unchanged ($P = .017$). The 4 reinfected animals showed a lower HEV-IgG avidity index (average 35.5%) than the 8 protected animals (average 62.1%). HEV-specific interferon-gamma–producing T cells were 2-fold higher in reinfected animals ($P = .018$).

Correspondence: Y. H. Choi, PhD, Laboratory Branch, Division of Viral Hepatitis, National Centers for HIV/AIDS, Viral Hepatitis, STD, and TB prevention, Centers for Disease Control and Prevention, Atlanta, Georgia 30329, brt5@cdc.gov.

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Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Conclusions.—Preexisting antibody and high IgG avidity index (>50%) are important factors for protection against HEV reinfection. HEV-specific T-cell responses were elevated in reinfected animals after subsequent exposure to HEV.

Keywords

hepatitis E virus; genotype 1; reinfection; IgG anti-HEV antibody; avidity of IgG anti-HEV antibody; hepatic host immune response gene expression; rhesus macaques

Globally, hepatitis E virus (HEV) causes as many as 20 million infections annually, resulting in an estimated 52 100 deaths each year [1]. HEV infection associated with genotypes (gt) 1 and 2 presents a significant public health problem in parts of Asia, Africa, and the Middle East [2]. The highest clinical attack rate is among older children and young adults [3]. HEV seroprevalence varies from 34% to 64% in hyperendemic areas, including Nepal, Bangladesh, southwest France, and Uganda [4, 5]. Among displaced persons in South Sudan, anti-HEV IgG seroprevalence is 71% [6]. IgG anti-HEV antibody prevalence may suggest frequency of HEV exposure in these populations.

HEV gt1 or gt2 are transmitted through the fecal-oral route as a waterborne disease and cause self-limited disease, but can lead to fulminant liver failure, particularly in pregnant women [7]. HEV gt3 or gt4 infections are associated with sporadic cases and small outbreaks linked to exposure to infected animals and consumption of raw or undercooked meat from infected animals [8]. After the infection is cleared, anti-HEV antibodies are produced and provide protection against HEV infection [9]. The presence of IgM anti-HEV in blood is a marker of an acute infection and IgG anti-HEV antibody is a marker of past infection. In addition, the IgG anti-HEV avidity test is used to identify possible HEV reinfection in immunocompetent patients [10, 11].

IgG anti-HEV antibodies provide protection against HEV reinfection in nonhuman primates and humans [12–15]. HEV reinfection can occur in immunocompromised patients with low anti-HEV IgG levels and can lead to chronic hepatitis [16], and a Dutch study noted seroreversion among healthy blood donors [17]. However, the minimum concentration and avidity index of anti-HEV antibodies providing protection against the reinfection have not yet been determined. Furthermore, host immune responses, including cellular immune response profiles involved in protection against subsequent HEV infection, are unknown.

MATERIALS AND METHODS

Rhesus Macaques

Twelve rhesus macaques (*Macaca mulatta*) that were previously experimentally infected with HEV were examined in this study. Primary infection of RH623 was previously reported [18]. The Comparative Medicine Branch at the Centers for Disease Control and Prevention (CDC) provided care and husbandry for the animals in accordance federal laws, regulations, and the Guide for the Care and Use of Animals. All animal protocols and procedures involved in this study were reviewed and approved by the CDC Institutional Animal Care and Use Committee (IACUC protocol number 2643CHOMONC).

Inoculum and Collection of Samples

After clearance of the primary infection (Supplementary Figures 1 and 2), homologous HEV Sar-55 strain (gt1) was reinoculated intravenously to 2 rhesus macaques (RH624 and RH625) with 1000 monkey infectious doses (MID) (6.4 $\log_{10}$ World Health Organization [WHO] U/mL) and 100 MID (4.9 $\log_{10}$ WHO U/mL) for RH637, RH621, RH617, RH636, RH619, RH622, RH631, RH620, RH626, RH627, and monitored for 115 days. RH623, RH633, and RH634 were inoculated with either 100 or 1000 MID and used as primary infection controls (Table 1). A liver biopsy tissue was obtained from each animal (19 days after inoculation [DAI] from RH622, RH624, RH617, RH619, and RH631; 23 DAI from RH625, RH620, RH627, and RH626; and 28 DAI from RH621, RH636, and RH637). Multiple preinoculation and weekly postinoculation blood samples and daily stool samples were collected from all of the animals. The alanine aminotransferase (ALT) activity in sera was measured using a VetScan VS2 (ABAXIS, Union City, CA) according to the manufacturer's instructions. ALT activity was measured as described before [18]. Reinfection was defined as the detection of a new HEV infection evidenced by detection of viral RNA in stool and serum by real-time polymerase chain reaction (PCR) and elevated serum alanine aminotransferase (ALT) activity. Protection was analyzed based on IgG anti-HEV antibody concentration and IgG anti-HEV avidity index in serum samples.

Determination of HEV RNA Levels by Real-Time PCR

Total RNA extraction, cDNA synthesis, and real-time PCR were performed as described previously [18]. The HEV RNA level in liver, stool, and serum specimens was determined as WHO international units (WHO U/mL) by comparison with a standard curve of serial $\log_{10}$ dilutions of the WHO standard (6329/10, Paul-Ehrlich-Institut, Langen, Germany) [19].

Quantitation of IgG anti-HEV Antibody

IgG anti-HEV antibody levels were measured using Wantai HEV IgG enzyme immunoassay (EIA) kits according to the manufacturer's instructions using the recommended cutoff values (WE-7296, Wantai Biologic Pharmacy Enterprise, Beijing, China). Quantitation of IgG anti-HEV antibody was adapted as described previously [10]. Briefly, each sample was serially diluted 10-fold in the range from 1/10 to 1/8000 with dilution buffer from the kit to determine the levels of IgG anti-HEV antibody. Signal-to-cutoff ratios (S/CO) of IgG anti-HEV antibodies were calculated as sample OD_{450}/cutoff OD_{450} according to the manufacturer's instructions. All samples with a S/CO ratio of ≥1 were considered positive. The WHO IgG anti-HEV reference standard (95/584, National Institute for Biological Standards and Control, South Mimms, United Kingdom) was used to determine the concentrations of anti-HEV antibodies. The detection limit of the Wantai anti-HEV IgG EIA kit was 0.25 WHO U/mL and a linear range was obtained from 0.25 to 5 WHO U/mL. All of the samples were diluted to achieve an OD_{450} value within the linear range of the WHO anti-HEV IgG reference.

IgG anti-HEV Antibody Avidity

IgG anti-HEV antibody avidity was determined as described previously with slight modifications [10]. Briefly, duplicate serum samples were incubated for 30 minutes at

37°C in Wantai anti-HEV IgG EIA enzyme-linked immunosorbent assay (ELISA) plates, 1 sample from the pair followed the standard procedure, and the other sample was exposed to a 7 M urea solution in the washing buffer for 10 minutes at room temperature. The serum samples were diluted to have an OD_{450} in the range between 0.8 and 2.1. IgG anti-HEV antibody avidity was calculated after accounting for dilution effects as:

$$\frac{\begin{array}{c}percentage\ of\ measures\\ antibody\ concentration\\ in\ the\ urea-treated\ well\end{array}}{antibody\ concentration\ in\ the\ untreated\ well}\times 100$$

Enzyme-Linked Immunospot Assay

Whole blood collected in BD Vacutainer Mononuclear Cell Preparation Tubes (CPT; BD Bioscience, San Jose, CA) containing sodium heparin as an anticoagulant was processed according to the manufacturer's instructions. The CPT tubes were inverted several times after blood drawing and centrifuged for 20 minutes at 1800*g* at room temperature. The cell interface layer containing peripheral blood mononuclear cells (PBMC) was harvested and isolated PBMCs were resuspended in EmbryoMax 2X freezing medium (Millipore, Billerica, MA). Human interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISPOT) assays were performed using the 96-well ImmunoSpot kit according to manufacturer's instructions (CTL, Shaker Heights, OH). Three time points (baseline [0 DAI], at the peak of antibody responses [40 DAI for RH617, 619, 622, 624, and 631; 52 DAI for RH620, 625, 626, and 627], and at the end of observation [115 DAI]) were used to determine levels of HEV-specific IFN-γ–producing T cells. Briefly, the PBMCs were washed twice with 5 mL CTL-Test Medium, cells were counted with the Countess Automated Cell Counter (Invitrogen, Carlsbad, CA), and cell numbers adjusted to 4×10^6 cells per mL. HEV ORF2 recombinant protein, HEV-236 (20 μg, 452–617 amino acids) (Prospec-Tany TechnoGene, East Brunswick, NJ) was used to stimulate 2×10^5 cells for 24 hours and a final concentration of 1 μg HEV-236 protein was added to each well and incubated at 37°C in 5% CO_2. Anti-human IFN-γ detection solution was added according to manufacturer's instructions. For positive control, cells were stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (Sigma, St Louise, MO) and 500 ng/mL ionomycin (Sigma), and 1% dimethyl sulfoxide (Sigma) was used as negative control. All assays were performed in duplicate. Cytokine-producing spots were scanned and counted with a CTL Analyzer (Shaker Heights, OH).

Enzyme-Linked Immunosorbent Assay

Supernatant from the HEV-236 stimulated PBMCs was used to analyze cytokines/chemokines generated by HEV-specific T-cell responses using the Human Multi-Analyte ELISA Array (QIAGEN, Germantown, MD). Twenty-four hours after stimulation, supernatants were collected from the ELISPOT plates and stored at −80°C until ELISA testing. Thawed supernatants were added to the Human Multi-Analyte ELISA Array containing interleukin (IL)-1B, IL-4, IL-6, IL-10, IL-12, IL-17A, IFN-γ, tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta 1 (TGF-β1), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1α),

and MIP-2α, and followed according to the manufacturer's protocol. Protein expression levels were measured as absorbance at 450 nm using a Biotek ELISA plate reader (Winooski, VT) and relative levels of cytokines and chemokines were compared to baseline samples.

Rhesus Macaque-Specific Immune Response Gene Array

RNA extraction and synthesis of cDNA were carried out as described before [18]. Rhesus macaque specific innate and adaptive immune response (PAQQ-052Z), Th1 and Th2 response (PAQQ-034Z), and type I interferon response (PAQQ-016Z) RT2 profiler PCR arrays (QIAGEN, Carlsbad, CA) were performed according to the manufacturer's protocol. Gene expression was normalized using the expression of 5 genes, ACTB, B2M, GAPDH, HGPRT, and RPL13A in each PCR array as housekeeping control genes. Liver tissues obtained from 2 anti-HEV antibody-negative animals were used as baseline control samples for determination of differential gene expression. Changes in gene expression of >2-fold compared to baseline were considered significant.

Statistical Analysis

Statistical analysis were performed with GraphPad version 7.04 (La Jolla, CA). Statistical significance was tested using a 2-tailed *t* test. *P* values < .05 were considered significant.

RESULTS

HEV Genotype 1 Primary Infection in Rhesus Macaques

As primary infection controls, 3 anti-HEV antibody-negative rhesus macaques were infected with HEV gt1, Sar-55. One of the primary infection controls (RH623) was reported in a previous study [18]. These animals exhibited the typical course of acute HEV infection (Figure 1). HEV RNA was detected in stools between 3 and 7 DAI, in serum from 10 to 42 DAI, and cleared between 20 and 67 DAI in all of the animals. Serum ALT activity was elevated from 31 to 45 DAI in RH623 and RH633. IgM anti-HEV antibody became detectable from 14 to 35 DAI and disappeared from 52 to 104 DAI. IgG anti-HEV antibody response was observed from 14 to 38 DAI, peaked from 28 to 35 DAI, and declined from 63 to 80 DAI.

Outcome of Homologous HEV Genotype 1 Reinfection

Duration between the primary inoculation and the reinoculation varied from 1.2 to 5.2 years (Table 1). Four macaques were reinfected as evinced by HEV RNA in stools, and 8 animals did not develop infection or shed virus in feces after reinoculation with HEV gt1, and were thus protected (Figure 2). In the reinfected animals, HEV RNA levels in stools was lower (average $3.9\log_{10}$ WHO U/mL) than the primary HEV infection (average $8.4\log_{10}$ WHO U/mL). Duration of viral shedding in stools was also shorter (average 17 days) than the primary infection (average 41 days). None of the reinfected macaques showed IgM anti-HEV antibody responses or elevation of ALT activity (Figure 2 and Table 1).

Cellular Immune Responses Following Exposure to Homologous HEV Genotype 1 Reinfection

Homologous HEV reinfection after the primary infection was associated with significantly higher levels of HEV-specific T-cell responses. Sufficient numbers of PBMCs were obtained from 2 of 4 reinfected and 7 of 8 protected animals to determine cellular immune responses. Levels of HEV-specific IFN-γ–producing T-cell responses in reinfected animals (RH617, $707/10^6$ PBMCs and RH624, $547/10^6$ PBMCs) were higher than 7 protected animals (average of $77/10^6$ PBMCs) both at elevated levels of antibody responses and at the end of observation (RH617, $292/10^6$ PBMCs and RH624, $342/10^6$ PBMCs in reinfected animals and average $49/10^6$ PBMCs in protected animals; $P = .0086$) (Figure 3A). High levels of IL1-β, TGF-β, IL-6, MCP-1 (CCL-2), MIP-1α (CCL-3), and MIP-2α (CXCL-2) expression in supernatants from HEV 296 protein-stimulated PBMC cultures were detected in reinfected animals (Figure 3B). Levels of IL-1β (2-way ANOVA, $P = .0098$), TGF-β ($P = .0066$), and IL-6 ($P = .0033$) expression were elevated at the peak of antibody response, and MCP-1 ($P = .0069$), MIP-1α, and MIP-2α expression peaked at the end of observation in the reinfected animals. In the protected animals, TGF-β and IL-6 expression was downregulated at the peak of antibody response and the end of observation.

IgG Anti-HEV Antibody Levels and Avidity in Primary Infection and Reinfection

In the 3 control animals, IgG anti-HEV antibody response became positive at 38 DAI in RH623, 37 DAI in RH633, and 14 DAI in RH634, and peaked at 56 DAI (578 WHO U/mL) in RH623, 52 DAI (3136 WHO U/mL) in RH633, and 28 DAI (868 WHO U/mL) in RH634 (Figure 1 and Figure 4). Baseline IgG anti-HEV antibody concentration in the 4 reinfected animals ranged from 1.5 to 13.4 WHO U/mL (Table 1). IgG anti-HEV concentration rose between 63- and 285-fold in the reinfected animals ($P = .003$) (Figure 2 and Figure 4). Baseline IgG anti-HEV antibody concentration ranged from 2.8 to 90.7 WHO U/mL in 8 protected animals (Table 1). IgG anti-HEV antibody levels were unchanged in the protected animals following reinoculation ($P = .0172$), except a slight increase was observed between 80 and 115 DAI in RH627; 21 to 113 DAI in RH621; and 50 and 115 DAI in RH622 (Figure 2).

IgG avidity in the primary infection controls ranged from 25% to 34% (mean 28%) (Figure 4 and Table 1). Baseline IgG avidity levels in the reinfected animals ranged from 17% to 27% (mean 24%) and 24% to 80% (mean 74%) in the protected animals. During the second infection, IgG avidity ranged from 30% to 42% (mean 34%) in the reinfected animals ($P = .0014$), and 32% to 88% (mean 62%) in the protected animals ($P < .001$) (Figure 4). Baseline IgG avidity levels were correlated with IgG avidity during secondary infection and the correlation was statistically significant ($r = 0.844$, $P < .00075$) (Figure 4C).

Expression of Hepatic Immune Response Genes in HEV gt1 Reinfected and Protected Macaques

A liver biopsy specimen was obtained at either 19, 23, or 28 DAI from all of seropositive animals and host immune response-related genes associated with the outcome of HEV gt1 reinfection were analyzed. Type 1 IFN response-related genes, CAV1, IFNW1, NOS2, SOCS1, and TNFα were upregulated in protected animals. Th1 and Th2 response-related

genes, CCR5, CCR2, CD40LG, FASLG, ICOS, IL1R1, IL1RL1, Il7R, CCL7, and TNFSF4 were upregulated in reinfected animals, and TNFRSF9, TYK2, VEGFA, and IRF7 genes were downregulated in reinfected animals (Figure 5).

DISCUSSION

Neither natural HEV infection nor vaccination provide lifelong immunity against reinfection, where loss of anti-HEV antibody has occurred 5 to 23 years after natural infection and 4.5 years after vaccination [20–22]. Longitudinal analysis of HEV infection has demonstrated that reinfection and seroreversion were common and serologic evidence of HEV reinfection was found to be 9% in adult humans [23, 24]. In this study, the host immune signatures contributing to reinfection and protection against subsequent homologous HEV gt1 reinfection were investigated using seropositive rhesus macaques. We found that HEV reinfected animals had shorter viremia, lower HEV RNA levels, no IgM anti-HEV antibody response, and no ALT elevation. Anti-HEV antibody can wane after 5.2 years of primary infection and animals with a high avidity of IgG anti-HEV antibody (>50%) index were protected against reinfection.

Previous studies of HEV reinfection with homologous or heterologous virus in rhesus macaques have shown that preexisting IgG anti-HEV antibody protects against HEV reinfection [13–15]. Monkeys with preexisting anti-HEV IgG titers 1:1000 were protected against HEV reinfection, whereas evidence of reinfection leading to pronounced disease was documented in a monkey with an IgG anti-HEV titer of 1:100 [13]. An anti-HEV antibody concentration of 12 WHO U/mL in immunocompetent and <7 WHO U/mL in immunocompromised patients were not protective against HEV reinfection [16, 23]. In our study, the animals with lower baseline IgG antibody levels (mean 6 WHO U/mL) were reinfected and those with higher baseline IgG concentration (mean 36 WHO U/mL) were protected from homologous HEV challenge. We also found that 1 animal (RH621) with baseline IgG 2.8 WHO U/mL and 24% baseline avidity was protected, but another animal (RH624) with baseline IgG 13.4 WHO U/mL and 27% baseline IgG avidity was reinfected after HEV reinoculation. The differences between these 2 animals were HEV inoculation dose and IgG avidity (52% in RH621 and 41% in RH624) following reinoculation. It is possible that the higher inoculum dose (1000 MID) for RH624 may have induced reinfection compared to RH621 (100 MID) [25]. In addition, 8 animals protected against reinfection had IgG avidity of 63%, while the 4 reinfected animals had a mean avidity of 36% during the second infection, suggesting that an avidity >50% in RH621 during the second infection may have provided protection against reinfection. Previous studies showed that low IgG anti-HEV antibody avidity (<25%) was associated with acute infection and high avidity (>50%) was reported 6 months after HEV exposure [10, 11]. However, we observed a mean 29% IgG avidity during the primary infection, the seropositive animals with baseline IgG avidity of 22% (2.8 to 5.2 year after the primary infection) resulted in reinfection, and the animals with a baseline avidity of 58% (1.2 to 2.8 year after the primary infection) were protected against reinfection. Our study suggested that IgG avidity decreases with time after the primary infection.

Innate and adaptive T-cell immune responses against HEV infection have been shown to play critical roles in viral clearance [26–28]. Potent and multispecific $CD4^+$ and $CD8^+$ T-cell responses to the ORF2 protein were found in patients with acute hepatitis E, and weaker HEV-specific $CD4^+$ and $CD8^+$ T-cell responses were associated with chronic hepatitis E in immunocompromised patients [26]. In addition, T-cell response can be detected for 12 years after primary infection in humans and its response rapidly declined with time [28]. In our study, PBMCs were stimulated with a truncated HEV ORF2 protein containing 452–617 amino acids. IFN-γ ELISPOT assay using PBMC stimulated with ORF2 protein could identify HEV-specific T-cell responses [29] and amino acids between positions 533 and 552 in ORF2 protein found to contain at least 2 T-cell epitopes and induced a vigorous antigen-specific T-cell responses in immunized mice [30]. This study is the first to show HEV-specific T-cell responses against HEV reinfection. We found that high levels of HEV-specific T-cell responses to ORF2 protein were associated with HEV reinfection and these responses were correlated with HEV RNA level, suggesting that control of a secondary infection is associated with robust T-cell responses. Moreover, IgG anti-HEV concentration increased between 63- and 285-fold in reinfected animals during the second infection, whereas no significant changes were detected in the protected animals.

Hepatic gene expression related to reinfection and protection against second HEV infection was determined in the liver tissues obtained from each animal. We found that hepatic type I IFN-response–related gene expression was upregulated in protected animals, but these genes were either not regulated or downregulated in reinfected animals. Elevated levels of hepatic Th1/Th2-response–related gene expression were found in reinfected animals (Figure 5). High levels of Th1/Th2 gene expression may be associated with elevation of cellular immune responses detected in reinfected animals (Figure 3). In our previous study, we reported that type I IFN-response–related genes were highly expressed in rhesus macaques with primary HEV gt1 infection [18]. In this study, the type I IFN-related genes were not regulated in reinfected animals, but high levels of type I IFN gene expression were detected in protected animals. Our observations suggest that protection against HEV reinfection may involve an activation of the type I IFN signaling pathway induced by a primary HEV gt1 infection. Our study has some limitations as short intervals between primary and secondary infection (from 1.2 to 5.2 years) were investigated and would not allow us to draw conclusion about levels of preexisting IgG antibody and avidity against reinfection after longer exposure of the primary infection.

In conclusion, we have shown that preexisting IgG antibody avidity (>50%), and activation of the type I IFN response pathway can provide protection from homologous HEV reinfection. Further studies on animals challenged with heterologous strains of HEV are warranted for better understanding host immune signatures for HEV reinfection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

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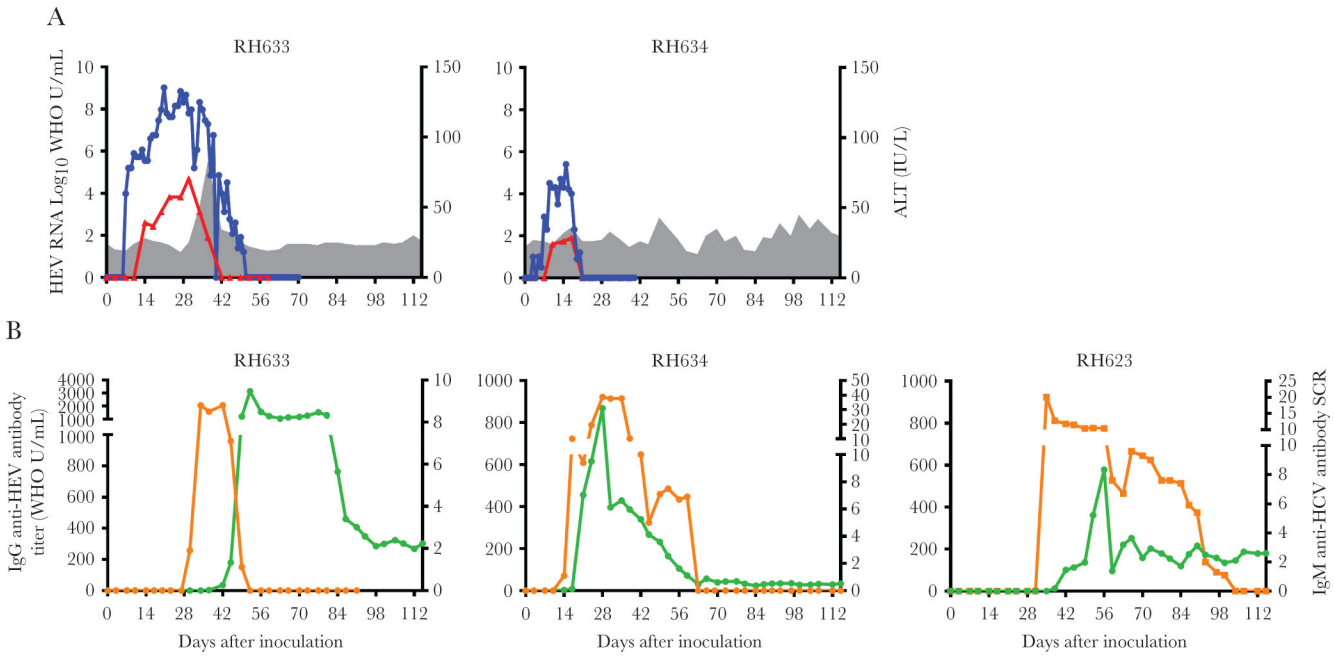

Figure 1.

Primary hepatitis E virus (HEV) genotype 1 (Sar-55) infection in rhesus macaques. *A*, Levels of HEV RNA in stool (blue line), serum (red line), and alanine aminotransferase (ALT) activity (grey shade) in RH633 and RH634. *B*, levels of IgM (brown line) and IgG (green line) anti-HEV antibody response in RH633, RH634, and RH623 during primary HEV infection. Primary infection of RH623 was reported before [18]. Antibody SCR indicates IgM anti-HEV antibody response signal cutoff ratio. Abbreviation: WHO, World Health Organization.

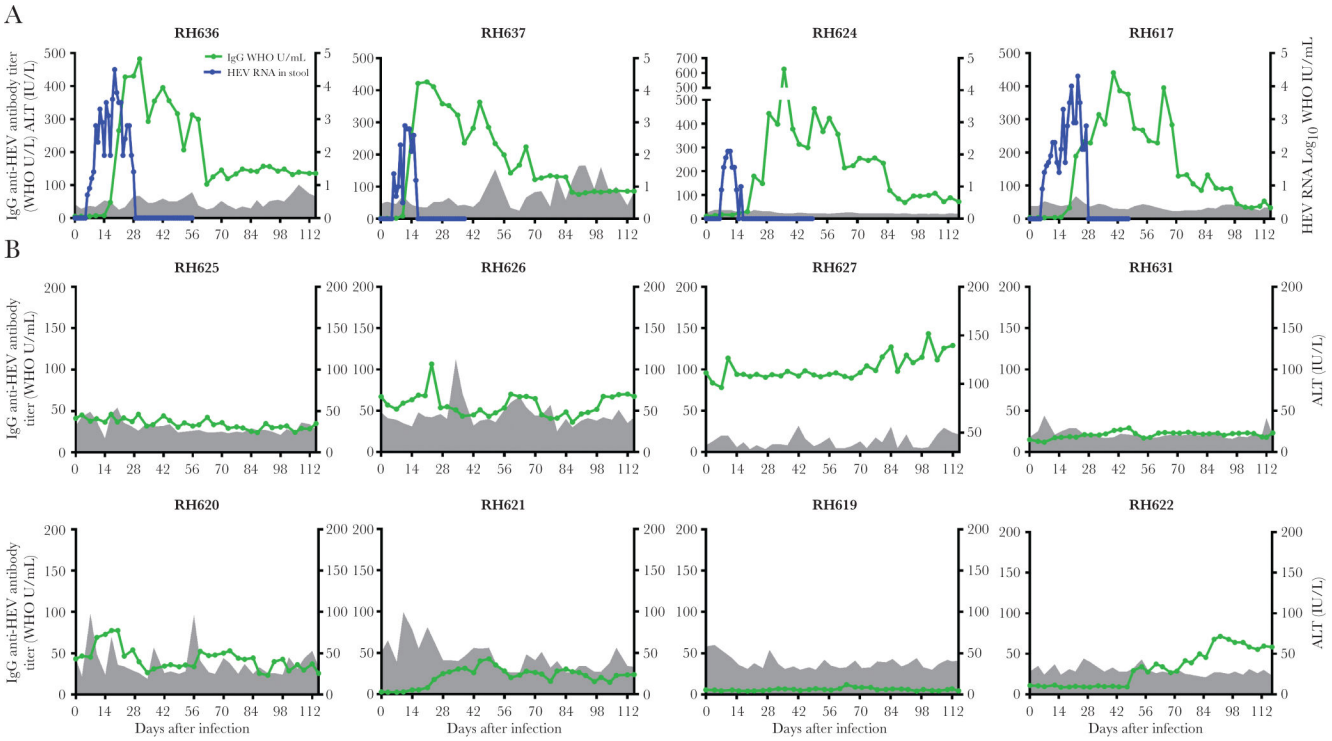

Figure 2.

Levels of IgG anti-hepatitis E virus (HEV) antibody following the HEV reinoculation. *A*, Levels of HEV RNA in stool (blue line) and IgG antibody (green line) in reinfected rhesus macaques. *B*, IgG anti-HEV antibody (green line) and alanine aminotransferase (ALT) activities in protected animals (grey shade). Abbreviation: WHO, World Health Organization.

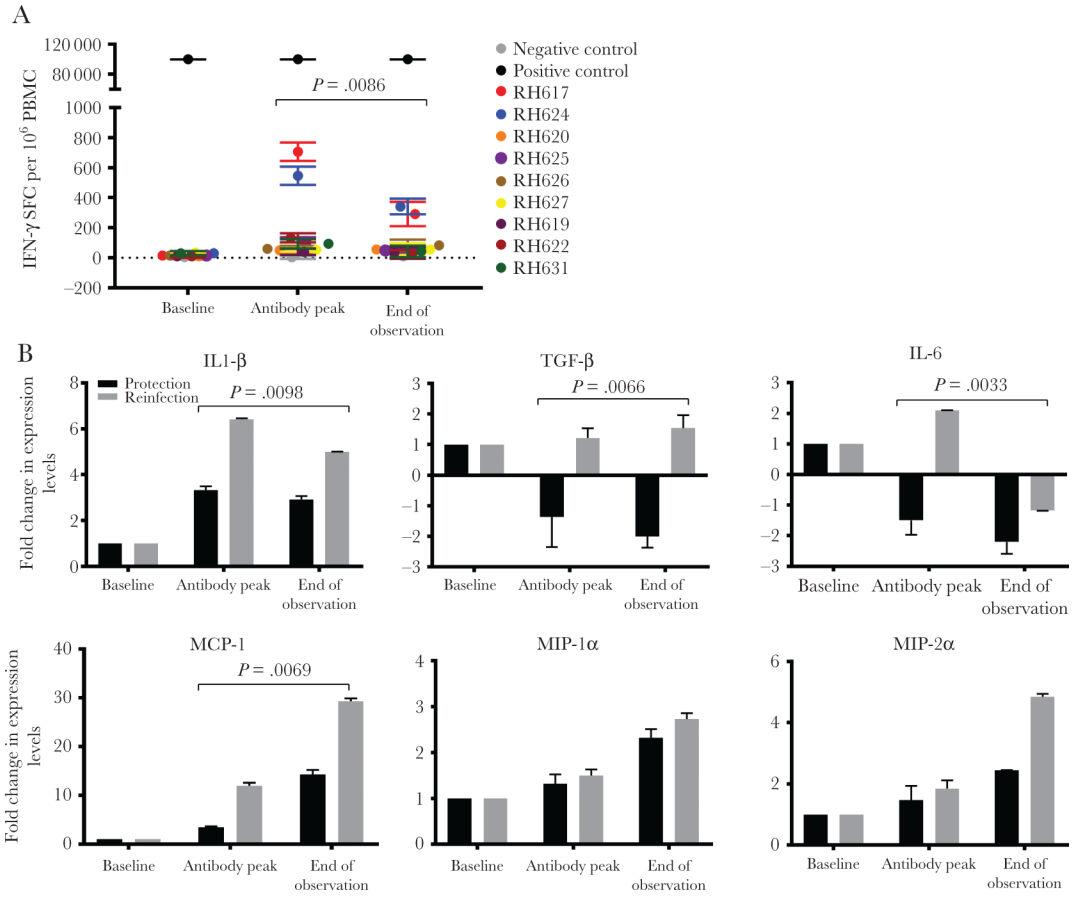

Figure 3.

Hepatitis E virus (HEV)-specific T-cell responses in protected and reinfected animals against homologous HEV reinfection. *A*, Frequencies of HEV-specific cells secreting IFN-γ were determined by ELISPOT. T-cell responses were analyzed using samples obtained from baseline, antibody peak, and end of observation. Data were expressed as SFC per 10^6 PBMCs and represent median levels in 7 of 8 protected and 2 of 4 reinfected animals. *B*, Secreted cytokines and chemokines in HEV-specific T cells induced by reinfection. Levels of IL-1β, IL-6, TGF-β, MCP-1, MIP-1α, and MIP-2α were analyzed by ELISA in supernatants of HEV ORF2 stimulated cultures of PBMCs after reinfection. Relative absorbance at 450 nm of each cytokine was obtained by comparison to baseline samples. Median levels in each experimental group are shown as bar graphs. Abbreviations: ELISA, enzyme-linked immunosorbent assay; ELISPOT; enzyme-linked immunospot; IFN-γ, interferon-gamma; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PBMC, peripheral blood mononuclear cells; SFC, spot-forming cells; TGF-β, transforming growth factor-beta 1.

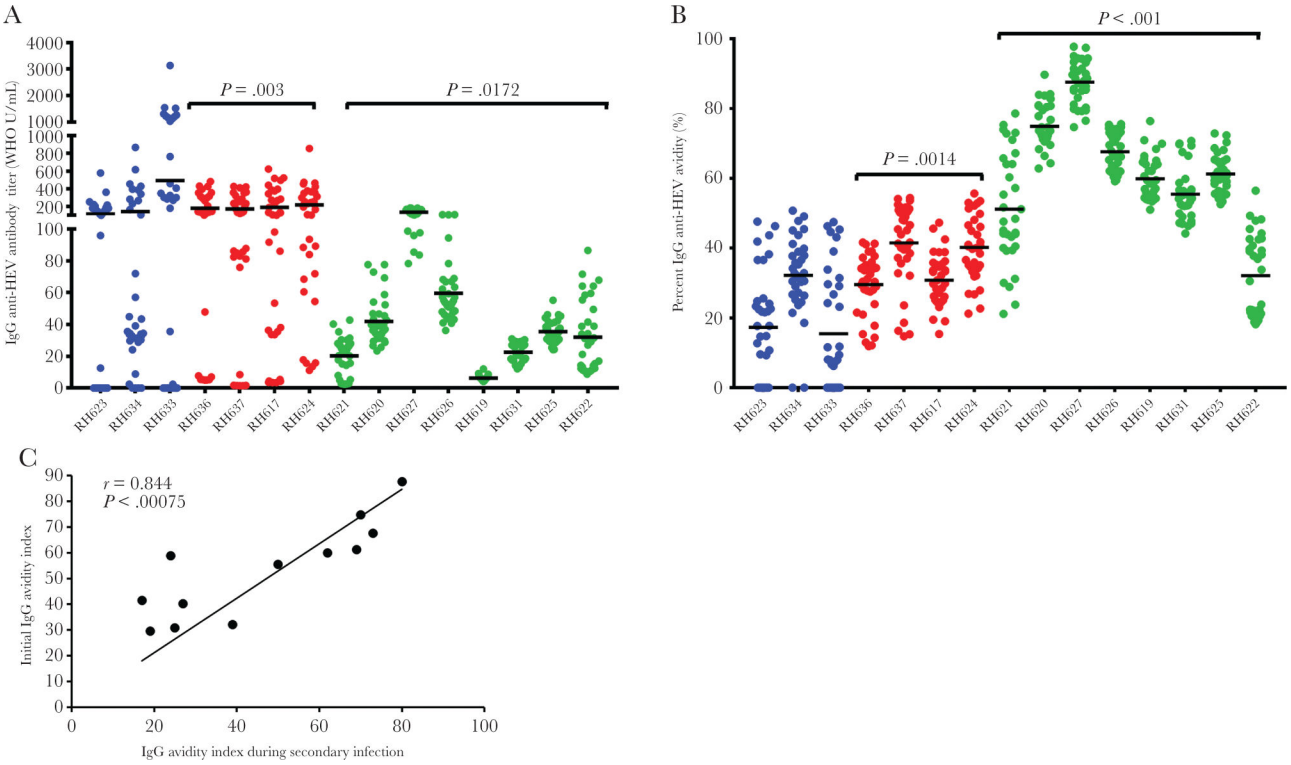

Figure 4.

IgG anti-hepatitis E virus (HEV) antibody concentration and avidity during primary infection and the subsequent HEV reinoculation. *A*, IgG anti-HEV antibody titers (WHO U/mL) during primary infection (blue dot), reinfected (red dot), and protected animals (green dot). *B*, IgG avidity in primary (blue), reinfected (red dot), and protected animals (green dot). *C*, Correlation of IgG avidity index between initial and secondary infection. Pearson correlation test was performed. Abbreviation: WHO, World Health Organization.

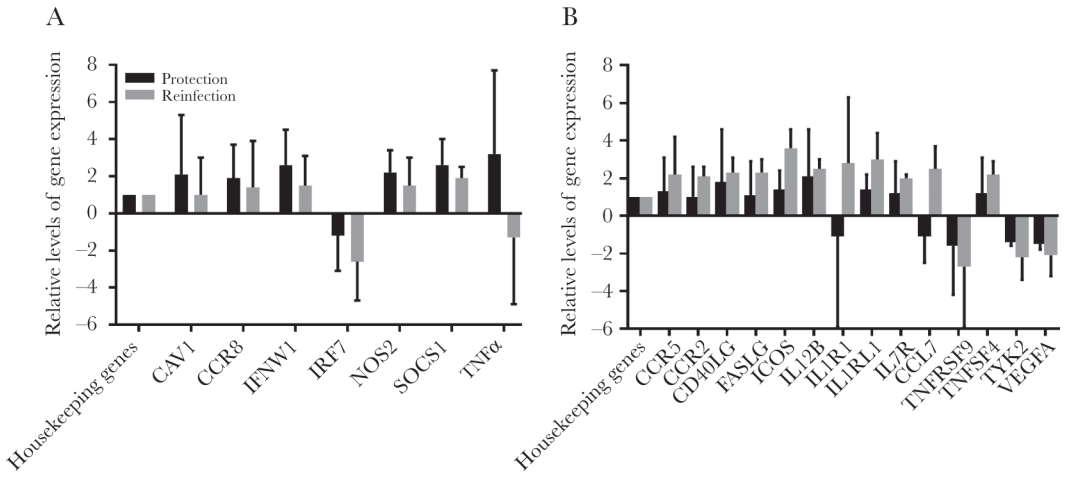

Figure 5.

Expression levels of type 1 interferon (IFN) and Th1/Th2-related gene expression during the hepatitis E virus (HEV) reinoculation. *A*, Relative levels of type I IFN-response–related gene expression. *B*, Th1/Th2-response–related gene expression in protected and reinfected animals against homologous HEV reinfection. Five housekeeping genes (ACTB, B2M, GAPDH, HGPRT, and RPL13A) in each polymerase chain reaction array were used as housekeeping control genes.

Table 1.

Summary of Homologous HEV Genotype 1 (Sar-55) Reinoculation into Seroconverted Rhesus Macaques

Animal ID	Initial IgG Anti-HEV Concentration, WHO U/mL	Initial IgG Avidity, %	Time Between 1st and 2nd Infection, y	MID of Inoculum	DAI of HEV RNA in Stool (Peak Levels of HEV RNA WHO U/mL)	IgM Anti-HEV AB, DAI	Elevation of ALT Activity	Outcome of Reinoculation	Avidity of Anti-HEV IgG, %
RH633	0	0	0	1000	7 to 50 (9.0)	30 to 49	Yes	Infection	24.6
RH634	0	0	0	100	3 to 20 (5.4)	14 to 129	No	Infection	37.4
RH623[a]	0	0	0	100	4 to 67 (10.8)	35 to 100	Yes	Infection	25.3
RH637	1.5	17	5.2	100	6 to 16 (2.9)	NR	No	Reinfection	41.5
RH621	2.8	24	2.6	100	NEG	NR	No	Protection	58.9
RH617	3.9	25	2.8	100	6 to 27 (4.3)	NR	No	Reinfection	30.8
RH636	5.3	19	5.2	100	6 to 28 (4.6)	NR	No	Reinfection	29.5
RH619	6.3	62	2.8	100	NEG	NR	No	Protection	59.9
RH622	13.0	39	2.8	100	NEG	NR	No	Protection	32.1
RH624	13.4	27	2.8	1000	7 to 16 (2.1)	NR	No	Reinfection	40.2
RH631	14.6	50	2.4	100	NEG	NR	No	Protection	55.5
RH625	48.3	69	1.3	1000	NEG	NR	No	Protection	61.2
RH620	51.0	70	1.2	100	NEG	NR	No	Protection	74.7
RH626	60.0	73	1.3	100	NEG	NR	No	Protection	67.6
RH627	90.7	80	2.3	100	NEG	NR	No	Protection	87.6

Abbreviations: AB, antibody; DAI, days after infection; MID, monkey infectious dose; NEG, negative; NR, no response; WHO, World Health Organization.

[a]Primary infection of RH623 was reported previously [18].