Acute hepatitis A virus infection is associated with a limited type I interferon response and persistence of intrahepatic viral RNA

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Hepatitis A virus (HAV) is an hepatotropic human picornavirus that is associated only with acute infection. Its pathogenesis is not well understood because there are few studies in animal models using modern methodologies. We characterized HAV infections in three chimpanzees, quantifying viral RNA by quantitative RT-PCR and examining critical aspects of the innate immune response including intrahepatic IFN-stimulated gene expression. We compared these infection profiles with similar studies of chimpanzees infected with hepatitis C virus (HCV), an hepatotropic flavivirus that frequently causes persistent infection. Surprisingly, HAV-infected animals exhibited very limited induction of type I IFN-stimulated genes in the liver compared with chimpanzees with acute resolving HCV infection, despite similar levels of viremia and 100-fold greater quantities of viral RNA in the liver. Minimal IFN-stimulated gene 15 and IFIT1 responses peaked 1–2 wk after HAV challenge and then subsided despite continuing high hepatic viral RNA. An acute inflammatory response at 3–4 wk correlated with the appearance of virus-specific antibodies and apoptosis and proliferation of hepatocytes. Despite this, HAV RNA persisted in the liver for months, remaining present long after clearance from serum and feces and revealing dramatic differences in the kinetics of clearance in the three compartments. Viral RNA was detected in the liver for significantly longer (35 to >48 wk) than HCV RNA in animals with acute resolving HCV infection (10–20 wk). Collectively, these findings indicate that HAV is far stealthier than HCV early in the course of acute resolving infection. HAV infections represent a distinctly different paradigm in virus–host interactions within the liver.

innate immunity | viral persistence | immune evasion

Hepatitis A virus (HAV) is a small, hepatotropic positive-strand RNA virus. Although it is classified among the Picornaviridae, it differs in several important respects from most other human picornaviral pathogens in having a very slow and nonlytic replication cycle (reviewed in ref. 1). Several primate-derived cell lines are permissive for infection by HAV, and in the absence of extensive adaptation of the virus to cell culture, these infections are typically noncytopathic. Despite this, HAV causes only acute disease in humans and has never been documented to establish long-term persistent infection within the liver (1–3). However, relatively little is known about the pathogenesis of HAV infections and how the virus is cleared from the liver as there has been little impetus for research on hepatitis A since the development of effective inactivated vaccines almost 2 decades ago.

The absence of long-term persistent HAV infection is well documented by disappearance of the virus from isolated human populations over time (4, 5) and differs dramatically from the outcome of hepatitis C virus (HCV) infections, which persist for life in the majority of persons (6). HCV is the cause of considerable human morbidity and mortality. Like HAV, it is a positive-strand RNA virus, albeit classified among the Flaviviridae. Despite significant differences, the replication strategies of these viruses share a number of features, including the production of double-stranded RNA (dsRNA), a potent inducer of innate immune responses during the replication of their genomes by cytoplasmic membrane-bound replicase complexes. Moreover, recent data indicate that both viruses have evolved similar strategies to disrupt early innate immune responses involved in the induction of IFN synthesis (reviewed in ref. 7). These similarities in the biology of these viruses stand in marked contrast to the differences in infection outcome and suggest that studies comparing the early host responses to HAV and HCV might provide unique insight into the role of innate immunity in the control of intrahepatic infections by RNA viruses. Such studies could provide important clues to the mechanisms underlying HCV persistence.

Other than humans, chimpanzees (*Pan troglodytes*) represent the only animal species permissive for both HAV and HCV infection. Chimpanzee studies have provided a wealth of valuable information concerning the pathogenesis of both acute and chronic HCV infection (8). Similarly, chimpanzee studies carried out in the 1970s revealed many features of the natural history of hepatitis A and provided an important scientific platform for vaccine development (9–12). Such studies have not been done in recent years, however, and there are no studies using contemporary molecular and immunological techniques on which a detailed comparison of the early events in HAV and HCV infection can be made. The genetic, physiological, and immunological similarities of chimpanzees to humans make them unique models of human infectious diseases, and the only animal species in which this kind of comparative study can be done.

Here, we describe the early innate immune responses to HAV infection in three chimpanzees. We compare virologic events and intrahepatic host transcriptional responses to historical data from chimpanzees undergoing acute, self-limited HCV infection. We show that viral RNA is much more abundant in the HAV-infected liver, despite similar levels of viremia, and persists for much longer than HCV RNA in animals with acute resolving HCV infection. Surprisingly, we find a paucity of type I IFN

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responses in the HAV-infected liver, which is in marked contrast to the robust intrahepatic type I IFN-stimulated gene (ISG) response that typifies both acute and persistent HCV infection in chimpanzees and humans (13–16).

Results

HAV Persists in the Liver Following Its Clearance from Serum and Feces. Two adult chimpanzees, 4x0293 and 4x0395, were infected by i.v. challenge with the HM175 strain of HAV. A third animal (4x0396) was infected by natural exposure while cohoused with 4x0293. HAV RNA was detected in liver, feces, and serum within 2 d of challenge, reaching peak levels in all three compartments by weeks 2–3 (Fig. 1). Peak viremia ranged from 3 to 10×10^6 genome equivalents (GE)/mL of serum and was similar in magnitude to peak HCV viremia in a cohort of eight chimpanzees with acute resolving infection (Fig. 2*A*). Shedding of HAV into the feces peaked coincidently with viremia, reaching a maximum of 4×10^8 GE/g of feces in 4x0395 (Fig. 1). HCV is not found in feces, possibly because it is not transported vectorially from the hepatocyte into billiary canaliculi like HAV (10, 17) or because its virion contains a lipid envelope and is not stable in bile. HAV RNA also peaked in the liver at weeks 2–3 (Fig. 1), reaching levels up to 9×10^6 GE/μg total liver RNA, ~100-fold higher than HCV RNA in acute resolving infections (Fig. 2*A*). HAV RNA levels were thus much higher in liver than HCV RNA levels, whereas the magnitude of viremia was similar in the two infections.

HAV was cleared rapidly from the blood beginning ~3 wk after infection. Serum HAV RNA declined with a mean half-life ($t_{1/2}$) of 1.6 d and was no longer detectable by weeks 6–8. Although fecal shedding of viral RNA declined by more than a factor of 10^4 during this period, clearance was slower from feces (mean: $t_{1/2}$ 3.8 d). Viral RNA was detected in feces by sensitive RT-PCR assay as late as 20 wk postinfection in 4x0395, 12 wk after clearance of viremia (Fig. 1). Remarkably, the amount of HAV RNA within the liver declined very slowly (mean: $t_{1/2}$ 8.9 d). Whereas fecal viral RNA declined 1,000-fold between weeks 2–4 in 4x0293, HAV RNA in the liver decreased only 2.5-fold. During the first 10 wk, viral RNA in the liver declined by only 10-fold and was still detectable at 48 wk. Two-photon fluorescent microscopy revealed the presence of HAV capsid antigen in ~20% of cells in the liver at 3 wk and in ~5% at week 10; these cells were distributed in a patchy fashion with no particular concentration in periportal or centrilobular regions (Fig. S1*A*). Rare antigen-positive cells were present at week 27. dsRNA could be identified by antibody labeling in ~10% of cells at week 3 and in ~1% of cells at 10 wk (Fig. S1*B*), suggesting that HAV replicase complexes may persist in a small proportion of cells. A single follow-up biopsy at 72 wk was negative by RT-PCR. This pattern of intrahepatic viral RNA persistence was observed in all three animals. Although the $t_{1/2}$ of HAV RNA in serum (1.6 d) is similar to that reported for HCV following the initial elevation of alanine aminotransferase (ALT) activity (1.8 d) (18), the persistence of HAV RNA in the liver contrasts sharply with what is typically observed in chimpanzees with acute resolving HCV infection, where viremia and hepatic viral RNA are cleared coincidently, typically within 10–20 wk of infection (Fig. S1*C*) (13, 19). The mean half-life of viral RNA in the liver of HCV-infected chimpanzees was 4.2 d, compared with 8.9 d for HAV (Fig. S1*C*).

The earliest evidence of host control of the infection, reflected in initial declines of viral RNA in all three compartments, occurred at weeks 3–4, coincident with or slightly before peak elevations in serum ALT and the appearance of anti-HAV antibodies (Fig. 1). Anti-HAV antibodies have potent virus-neutralizing activity and are protective against reinfection (20), but appeared to have little impact on hepatic viral RNA copy numbers in these animals. Although fecal shedding of the virus initially declined in parallel with the drop in viremia, 4x0293 and 4x0395 demonstrated a biphasic pattern of viral clearance from feces, with a transient rebound in fecal RNA shedding at weeks

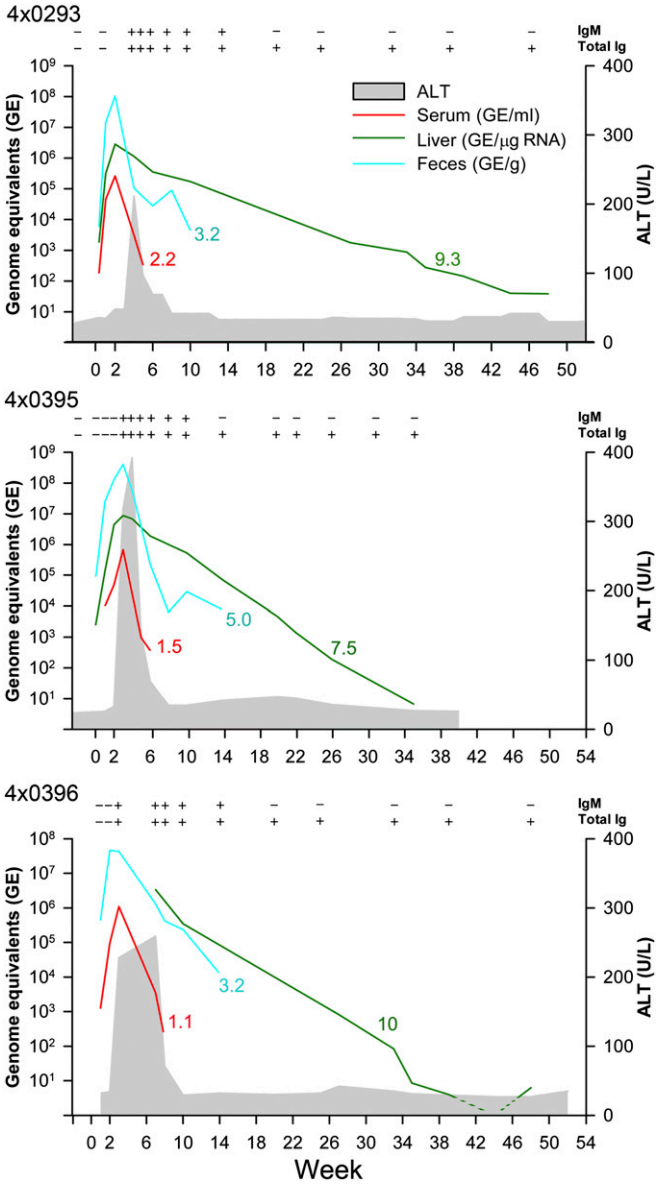

Fig. 1. Persistence of hepatic viral RNA in HAV-infected chimpanzees. Two chimpanzees (4x0293 and 4x0395) were inoculated intravenously using 5,000 chimp infectious doses of HAV HM175 containing 3×10^7 GE (a gift of Robert Purcell, National Institutes of Health, Bethesda, MD). A third animal (4x0396) became infected naturally while housed with infected animals. The exact day of natural infection was not known, but alignment of the three infection profiles by peak viremia, seroconversion for HAV IgM, and peak ALT suggests that infection of 4x0396 occurred 4 wk after inoculation of his cage mate, 4x0293; thus, this week was designated as week 1 for 4x0396. HAV RNA was quantified by TaqMan RT-PCR assays. The limit of detection varied in each compartment due to differences in the samples processed for the assay: serum (red line: linear limit of detection 10^3 GE/mL serum), liver (green line: linear limit of detection 10^1 GE/μg total RNA), and feces (blue line: linear limit of detection 5×10^3 GE/gm feces). Samples from all three compartments were analyzed throughout the study, but only positive samples are shown in the line graphs. The number to the right of each line represents the calculated $t_{1/2}$ for clearance from that compartment. ALT levels are indicated by the gray shaded area. IgM anti-HAV and total anti-HAV was determined by ELISA and are shown as positive or negative (+ or –) at the top of the graphs. The duration of follow-up differs for each animal.

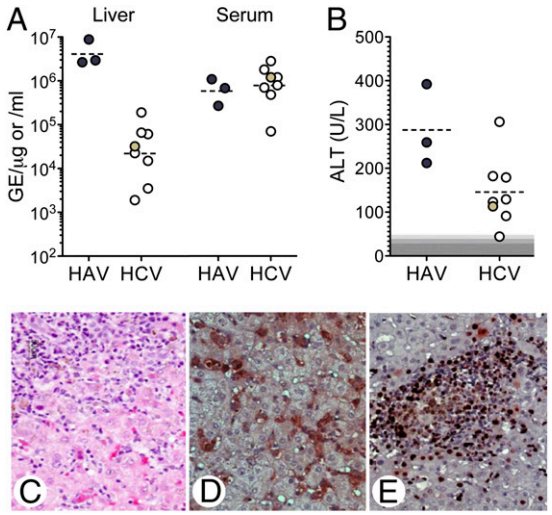

Fig. 2. Comparative features of acute HAV and acute resolving HCV infection in the chimpanzee. (*A*) Maximum viral RNA copy numbers in liver tissue (GE/μg total RNA) and serum (GE/mL). HCV infections were with genotype 1 (open circle) or genotype 3 (solid circle, 4x0296) virus. Dashed lines indicate mean values. (*B*) Maximum serum ALT activities. The shaded areas at the bottom indicate the mean baseline ALT activity plus one and two SDs. (*C–E*) Mononuclear cell infiltration, cell death, and proliferation in acute HAV infection. Formalin-fixed, paraffin-embedded liver tissue obtained 4 wk after HAV challenge (animal 4x0395) was (*C*) stained with hematoxylin and eosin, (*D*) immunostained with antibody to activated caspase 3 as a marker of apoptosis, or (*E*) labeled with antibody to Ki67 as a marker of cell proliferation. Liver tissue samples from preinfection and weeks 3, 4, and 10 after infection are shown in Fig. S2.

8–10 (Fig. 1). A similar pattern may have occurred in 4x0396. This was not matched by quantitative changes in RNA copy numbers in serum or liver of any animal, suggesting the possibility of an independent gastrointestinal replication compartment as proposed previously (21).

Maximum ALT elevations were somewhat greater than in acute resolving HCV infection (Fig. 2*B*) and coincided with a massive periportal mononuclear cell infiltrate that extended into the parenchyma at weeks 3 and 4 (Fig. 2*C* and Fig. S2 *A–D*). The inflammatory infiltrate was associated with severe hepatocyte swelling and necrosis. Numerous cells (up to 40% in selected fields by week 4) stained positively for activated caspase 3, a marker of apoptosis (Fig. 2*D* and Fig. S2 *E–H*). Many of the caspase 3-positive cells were hepatocytes (Fig. S2*M*); however, others appeared to be in sinusoidal spaces and resembled lymphocytes. Ki67, a marker of cell proliferation, was expressed by numerous hepatocytes (Fig. S2*N*) and infiltrating mononuclear cells at weeks 3–4 (Fig. 2*E* and Fig. S2 *I–L*). The number of proliferating cells decreased markedly by weeks 6 and 10.

Collectively, these data indicate that an acute inflammatory host response develops 3–4 wk into the infection, following a clinically silent early infection phase several weeks in length that is marked by robust replication of virus within the liver. Appearance of anti-HAV antibodies has relatively little impact on the intrahepatic viral load. Viral RNA copy numbers decline slowly over a period of months in parallel with decreasing numbers of hepatocytes staining positive for HAV antigen. Viral RNA and HAV antigen indicative of assembled virus capsids (22) persist long after ALT elevations have returned to normal and hepatic inflammation has resolved.

Intrahepatic Transcriptional Responses to HAV Infection. We examined the intrahepatic transcriptional response to HAV infection in serial liver biopsies from 4x0293, 4x0395, and 4x0396 using Affymetrix whole-genome microarrays. This revealed few changes in transcript abundance and no up-regulated gene common to all three animals 2 d after infection (Dataset S1), at which time the HAV RNA copy number exceeded $>10^3$ GE/μg RNA in the liver. Two-dimensional clustering and heat map analysis of the entire data set confirmed that there were relatively few consistent changes in the hepatic transcriptome early in the course of HAV infection (Fig. S3). Importantly, there were more differences between the various preinfection biopsies from the animals than between the preinfection and 2-wk biopsy from 4x0293 (when there were $>10^5$ HAV GE/μg RNA in the liver).

The first evidence of an IFN response occurred at week 1 when low-level increases in ISG15 transcripts appeared in both 4x0293 and 4x0395. These were accompanied by similar small increases in other type I IFN-regulated genes, such as MX1, IFIT1 (ISG56), IFIT3, and IFI6 in 4x0293. However, these and other type I ISGs were not induced in 4x0395 until week 2, even though viral RNA copy numbers exceeded 10^5 in the liver at week 1. This paucity of type I IFN-induced ISG expression early in infection contrasts sharply with prior studies of acute resolving HCV infection, in which these genes are induced by day 2 of infection and at much higher levels (13, 15). Fig. 3 (see also Fig. S4) compares the responses of 16 selected type I IFN-responsive genes in the HAV-infected animals and in an animal with acute resolving HCV infection (4x0296). Most of these ISG transcripts were up-regulated to a minimal degree and for only a few weeks

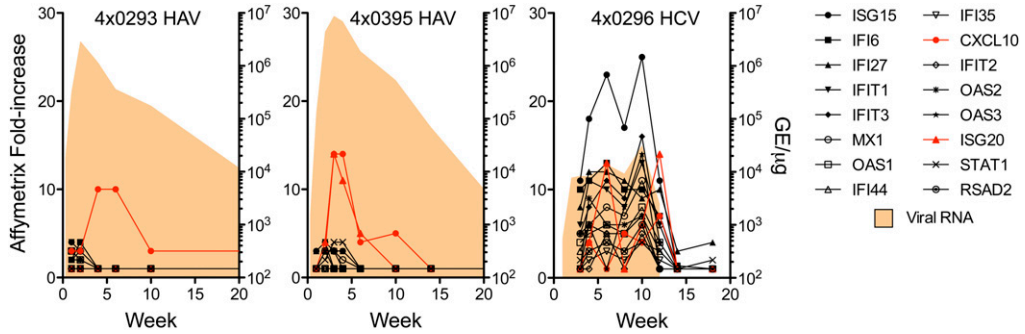

Fig. 3. Intrahepatic ISG responses determined by whole-genome microarray analysis. Total RNA was extracted from liver biopsies collected at the times indicated from animals with acute HAV infection (4x0293 and 4x0395) or acute resolving HCV infection (4x0296, genotype 3) and subjected to Affymetrix GeneChip (U133 Plus 2.0) analysis. Numbers represent the fold-change in the Affymetrix signal from uninfected baseline samples. CXCL10 and ISG20 responses, which are known to be driven by IFN-γ as well as by type I IFN-α/β, are shown in red. Viral RNA copy numbers in liver tissue (GE/μg total RNA) are represented by the shaded area. The results of individual animals are presented in Fig. S4, and results from a second HCV-infected animal are shown in Fig. S5. The entire Affymetrix data set from HAV-infected chimpanzees is shown in Dataset S1.

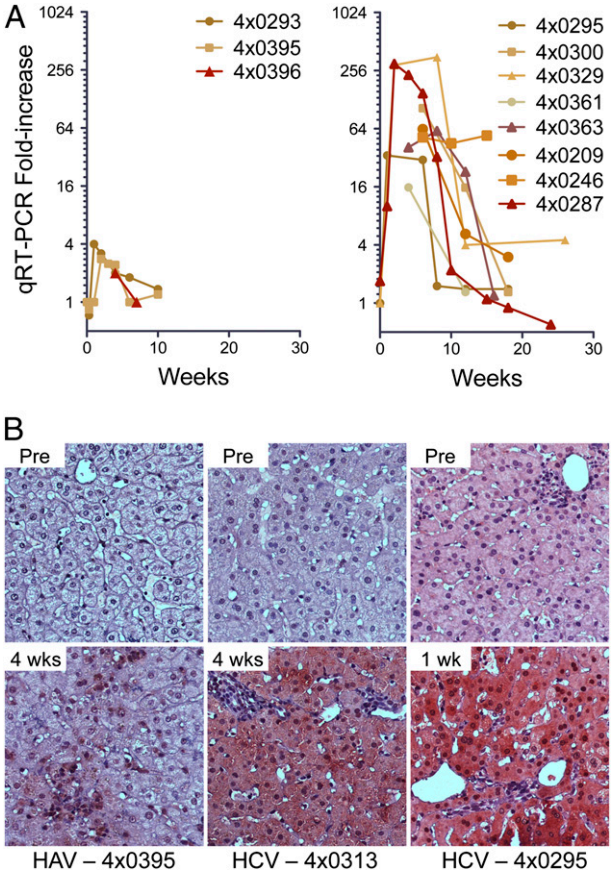

Fig. 4. Comparative analysis of ISG15 expression in HAV- and HCV-infected chimpanzees. (*A*) ISG15 transcript levels, quantified by TaqMan qRT-PCR and normalized to GAPDH transcript abundance, in liver tissue from (*Left*) three chimpanzees with acute HAV infection versus (*Right*) eight animals with acute resolving HCV infection. Results shown represent the fold-change from pre-infection biopsy tissue. (*B*) Immunohistochemical staining for ISG15 expressed in liver tissue before and 1–4 wk after challenge with HAV (*Left* panels: 4x0395) or HCV (Center and *Right* panels: 4x0313 and 4x0295). Chimpanzee 4x0313 was treated with the immunosuppressive agent FK506 and had no evidence of an adaptive immune response at the time of biopsy (4 wk); this animal is not included among the data shown in Figs. 2 *A* and *B* and 4*A*.

in the HAV-infected animals despite 100-fold higher levels and much longer persistence of viral RNA in the liver (Fig. 3). The two ISGs that were up-regulated over 10-fold, CXCL10 and ISG20, are genes that are also responsive to type II IFN (23). In HCV infection, all of the ISGs were highly up-regulated for as long as HCV RNA was present (Fig. 3 and Fig. S5).

Despite the minimal expression of type I IFN-stimulated genes, many other genes became transcriptionally activated in the liver at weeks 3–4 with the onset of inflammation. A total of 3,864 genes were up- or down-regulated twofold ($P = 0.02$) or more (detailed results are presented in Dataset S1). Affymetrix results from biopsies taken during this phase of the infection clustered together and were distinguished by the induction of genes stimulated by IFN-γ or involved in B-cell development (Fig. S3). Ingenuity Pathway Analysis confirmed remarkable activation of genes involved in B-cell development in all three HAV-infected animals (Table S1). These included numerous Ig genes and CXCL13, a chemokine involved in recruitment of B cells to the liver that was highly up-regulated (20- to 100-fold) in all three animals. Ig genes including heavy chains A, G, and M and light chains λ and κ were highly induced at 3–4 wk in all animals. Some remained induced in 4x0395 for up to 26 wk after infection. Many T-cell–associated transcripts were also up-regulated at weeks 3–4, including CD3G, CD3D, granzyme B, and perforin (Table S1). However, transcriptional responses associated with these adaptive immunity genes declined significantly after week 6, well before the elimination of viral RNA from the liver. Animals with acute HCV infection generally exhibit minimal activation of these gene sets, possibly reflecting less lymphocytic infiltration of the liver (13, 15). Lymphocyte-associated transcripts are often undetectable, even though it is well established that T-cell responses resolve HCV infection (24, 25).

We validated the microarray results by comparing them with quantitative RT-PCR (qRT-PCR) measurements of ISG15 and -4 other gene transcripts in the liver, noting a high level of correlation for each (Table S2). We also used qRT-PCR to compare ISG15 transcriptional responses in the three HAV-infected animals and a panel of eight chimpanzees with acute resolving HCV infection. Maximum increases in hepatic ISG15 transcripts were about 4-fold in the HAV-infected chimpanzees, compared with >256-fold in acute HCV infection (Fig. 4*A*). This large difference in the magnitude of ISG15 induction is remarkable given that the viral RNA copy numbers were on average about 100-fold less in the HCV compared with HAV-infected animals (Fig. 2*A*). Immunohistochemical stains demonstrated robust ISG15 expression as early as 1 wk after HCV infection, with virtually every hepatocyte staining positive (Fig. 4*B*). In contrast, much lower levels of ISG15 expression were evident in HAV-infected liver where ISG15 staining occurred in a patchy distribution of mostly mononuclear cells and was not present in most hepatocytes (Fig. 4*B*).

Discussion

Collectively, the data presented here reveal that HAV is a remarkably stealthy virus, with infection eliciting only a very limited type I IFN response within the liver. This minimal ISG response differs dramatically from what is observed in HCV infection (13–16) and comes as a surprise, given that HAV causes only acute disease whereas HCV infection typically persists and frequently causes chronic hepatitis. We have previously concluded that intrahepatic ISG responses are maximally induced in HCV-infected chimpanzees, even at relatively low levels of viremia (13, 14, 26, 27). The level of induction of type I ISGs during acute HCV infection appears to be of similar magnitude in self-limited resolving infections, such as those described here, and in those that become persistent (15, 19). Such responses do not occur in acute hepatitis A, despite the fact that viral RNA abundance is 100-fold greater within the liver than in acute hepatitis C (Fig. 2*A*) and that dsRNA, a potent inducer of IFN responses, can be demonstrated in up to 10% of hepatocytes (Fig. S1*A*). Two of the animals that we studied, 4x0293 and 4x0395, were infected by i.v. inoculation, an unusual route for HAV transmission in humans. However, 4x0396 became infected naturally, presumably by fecal-oral transmission while caged with 4x0293, and demonstrated a similar paucity of type I ISG responses. We conclude that low-level hepatic ISG responses typify HAV infection in the chimpanzee. Given that humans and chimpanzees are closely related phylogenetically, and that HAV infection in the chimpanzee otherwise accurately models human infection (9–12), type I IFN responses are likely to be similarly muted in humans.

Viral RNAs are sensed in hepatocytes by the cytosolic helicases, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), or by Toll-like receptor 3 (TLR3), which is expressed in an endosomal compartment (reviewed in ref. 28). This activates signaling pathways that lead to the phosphorylation of IFN-regulatory factor 3 (IRF-3) and type I IFN synthesis. In HCV-infected cells, this signaling is disrupted by the major viral protease, NS3/4A, which efficiently cleaves mitochondrial antiviral signaling protein (MAVS; also known as IPS-1, VISA, or Cardif), an essential adaptor protein for RIG-I, as well as by the TIR-domain–containing adaptor inducing IFN-β (TRIF;

otherwise known as TICAM-1), an essential TLR3 adaptor protein (29–34). The robust expression of ISGs within the HCV-infected liver, both in humans and in chimpanzees, is thus somewhat paradoxical and not well explained, given the capacity of the virus to disrupt these signaling pathways. One possibility is that infected hepatocytes are sensed by TLR7 expressed in plasmacytoid dendritic cells (pDCs) (35), professional IFN producers that account for most type I IFN in many viral infections.

Because HAV, like HCV, replicates its genome through a dsRNA intermediate and dsRNA is readily detected in the HAV-infected liver (Fig. S1*A*), the data presented here indicate that HAV must also possess potent mechanisms to disrupt IFN synthesis. This is supported by in vitro studies that show that HAV infection blocks the capacity of superinfecting viruses to induce type I IFN through the RIG-I/MDA5 pathway (36, 37). We have shown that this is due to the HAV 3ABC protease, which is directed to the mitochondrial membrane where it cleaves MAVS (36). Recent data also indicate that the 3CD protease-polymerase processing intermediate disrupts TLR3 signaling by directing the cleavage of TRIF (37). HAV and HCV thus have evolved in a remarkably congruent fashion to target these signaling pathways and disrupt their ability to induce type I IFN synthesis. HAV must have an additional advantage over HCV, however, to account for the dramatic difference that we have demonstrated in the IFN response to acute infection. Unlike enveloped viruses, the activation of pDCs by picornaviruses appears to require the presence of anti-viral antibodies (38, 39). Although the pDC response to HAV has not been studied, the weak induction of ISGs by HAV could reflect a failure of pDCs to sense infection. This cannot be explained entirely by an absence of antibody, however, as IgM anti-HAV appeared by week 3 (Fig. 1) without a concomitant increase in ISG expression. Luminex assays for serum IFN-α in the HAV-infected chimpanzees revealed a transient and minor elevation at week 2. This was matched by low-level fluctuating increases (up to approximately threefold) in IFN-α, IFN-β, and IL28 transcripts in the liver of 4x0395 during the first 3 wk of infection. In both cases, positive results were near the limit of detection, but nonetheless suggestive of induction and then active suppression of type I IFN.

A second major surprise was how long HAV RNA persisted in the liver of these animals following its clearance from both feces and serum (Fig. 1). Viral RNA was reported recently to be present in the liver of cynomolgous monkeys as late as 9 wk after experimental HAV infection (40, 41), but its continued presence beyond 46 wk in the chimpanzee (Fig. 1) was unexpected and represents another major difference from acute resolving hepatitis C. In acute resolving HCV infection, viral RNA is typically cleared by 10–20 wk of infection (Fig. S1*C*). It is difficult to know whether the persistence of HAV RNA reflects ongoing viral replication. The antigen that we identified in scattered cells as late as 10 wk after challenge (Fig. S1*A*) is conformation-dependent and indicative of assembled HAV capsids. In early studies, HAV particles were found within resident hepatic macrophages (Kupffer cells) (42), and thus the RNA could possibly represent viral particles trapped in Kupffer cells. However, such long-term persistence of a positive-strand RNA virus without some level of replication would be unprecedented. The detection of dsRNA in a very small number of cells at 10 wk (Fig. S1*A*) suggests the possibility of ongoing replication, but additional studies are needed to confirm this. A continuing low level of viral replication within the liver could be relevant to the pathogenesis of relapsing hepatitis A, which occurs in up to 20% of patients in the months following apparent resolution of acute hepatitis (43). It could also contribute to the persistence of antibody to HAV following infection.

Although viral RNA copy numbers peaked at 3–4 wk in blood, feces, and liver, the kinetics of decline in these compartments were dramatically different. Viremia rapidly declined to undetectable levels following the appearance of antibody, suggesting that neutralizing antibody plays a major role in clearing viremia (20). Transcriptome analyses indicated that B-cell responses are highly induced at this time (Table S1). Fecal shedding of virus was initially reduced in parallel with viremia (Fig. 1), but then followed an unexplained biphasic decline in at least two of three animals. Viral RNA declined much more slowly in the liver and was still highly abundant at the time that it disappeared from feces. Exactly how and when HAV is finally cleared from the liver remains unknown. Neutralizing antibody may halt spread to uninfected cells, whereas the residual infected cells may be slowly cleared by natural turnover or T-cell activity. Similar mechanisms may be involved in the clearance of hepatocytes containing residual hepatitis B virus covalently closed circular DNA after viremia is cleared during acute infection (44).

Blunted production of type I IFN could influence the strength and effectiveness of T-cell immunity that is important for control of HAV infection. Type I IFN acts directly on CD8+ T cells to promote differentiation and/or acquisition of effector functions (45), although the requirement for this cytokine may be pathogen-dependent (46, 47). Our data suggest that the chimpanzee model of human HAV infection will be of general importance in defining the relationship between early innate and adaptive immune responses.

One of the most striking conclusions to come from these studies is that there is much remaining to be learned about hepatitis A infections and HAV, a virus that has been largely neglected by the research community in recent years. Comparative studies with HCV are long overdue and are likely to provide insight into the mechanisms of viral persistence in chronic hepatitis C.

Materials and Methods

Complete methods are presented in *SI Materials and Methods*, including details concerning the care of chimpanzees at the Southwest National Primate Research Center (48) and the HAV and HCV infections. The methods for transcriptome analysis using Affymetrix microarrays (accession no. GSE27850; *SI Materials and Methods*) and ABI TaqMan assays (26, 27) and for immunohistochemical staining of liver sections (49) have been published previously.

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