Protection against Chronic Hepatitis C Virus Infection after Rechallenge with Homologous, but Not Heterologous, Genotypes in a Chimpanzee Model

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An open question for hepatitis C virus (HCV) vaccine development is whether the various genotypes of this virus protect against the development of chronic infection after heterologous infection with different genotypes. We approached this question by challenging chimpanzees that had recovered from HCV genotype 1a or 1b infection with 6 heterologous genotypes as well as with a homologous genotype (for chimpanzees originally infected with genotype 1a). All 9 chimpanzees rechallenged with a homologous genotype developed self-limited infections. Of 11 chimpanzees challenged with 100 chimpanzee infectious doses of heterologous genotypes, 6 developed self-limited infections, with peak viral loads in acute-phase serum that were ~5-fold lower than those seen during primary infections. One chimpanzee (which had recovered from genotype 1b infection and was rechallenged with genotype 6a) did not develop viremia but did show an anamnestic cell-mediated immune response after rechallenge. Four of the 11 chimpanzees rechallenged with heterologous genotypes developed chronic infections with the genotypes used for rechallenge. These findings suggest that a universally protective HCV vaccine may need to incorporate epitopes from multiple genotypes.

Hepatitis C virus (HCV) has been classified into 6 major genotypes, subtypes, and quasispecies [1–3]. It has been well established in studies with chimpanzees that HCV infection does not confer resistance to reinfection with either homologous or heterologous genotypes after rechallenge [4–12]. Reinfection has also been observed after superinfection of chronically infected chimpanzees [9, 12].

In homologous challenges, infection is generally self-limited, although a recent report describes 2 chimpanzees that developed chronic infections after rechallenge with the same monoclonal strain of HCV [13]. Limited data on the results of rechallenge of recovered chimpanzees with heterologous genotypes or subtypes are available. Seven such cross-challenges have been described, all of which appeared to result in self-limited infection [4, 6, 14], although in some cases follow-up may not have been long enough to reveal the reappearance of viremia and the development of chronicity [15]. By contrast, Okamoto et al. have described 3 chimpanzees that were chronically infected with genotype 1b and were rechallenged with genotype 1a, at least 2 of which became chronically infected with genotype 1a [9]. Furthermore, De Mitri et al. have described a patient who had recovered from infection with genotype 4 and then became chronically reinfected with genotype 3 [16].

Clearly, the goal of a prophylactic vaccine for HCV...
Table 1. Summary of homologous rechallenges resulting in self-limited infections.

<table>
<thead>
<tr>
<th>Chimpanzee</th>
<th>Interval, a years</th>
<th>Duration of viremia, weeks</th>
<th>Peak viral load after rechallenge, log_{10} RNA molecules/mL</th>
<th>ELISPOT response, total IFN-γ spots/1 × 10^6 PBMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After initial infection</td>
<td>After rechallenge</td>
<td>Before rechallenge</td>
</tr>
<tr>
<td>213</td>
<td>18</td>
<td>13</td>
<td>8</td>
<td>5.2</td>
</tr>
<tr>
<td>291</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td>16</td>
<td>1.2</td>
<td>ND</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>33</td>
<td>3</td>
<td>ND</td>
<td>6</td>
<td>4.8</td>
</tr>
<tr>
<td>50</td>
<td>1.2</td>
<td>ND</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>53</td>
<td>1.2</td>
<td>ND</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>59</td>
<td>3.5</td>
<td>ND</td>
<td>1</td>
<td>3.4</td>
</tr>
<tr>
<td>235</td>
<td>5</td>
<td>13</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>238</td>
<td>5</td>
<td>11</td>
<td>5</td>
<td>4.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>11.2 ± 2.4^b</td>
<td>4.0 ± 2.5^b</td>
</tr>
</tbody>
</table>

NOTE. ELISPOT, enzyme-linked immunospot; IFN, interferon; ND, not done (samples were no longer available); PBMCs, peripheral-blood mononuclear cells.

a Interval between initial infection and rechallenge.

b Significance of difference: P<.1.

is the prevention of chronic infection, because of its risk of cirrhosis and hepatocellular carcinoma. Acute infection is unlikely to be substantially preventable; however, such infections are usually of minor clinical significance.

The present study was designed to gain further information on the role played by genotype in immunity to the development of chronic infection, because this information is vital to the rational design of an HCV vaccine. Twenty chimpanzees that had recovered from infection with genotype 1a or 1b were rechallenged with 100 chimpanzee infectious doses (CID_{50}) of homologous or heterologous genotypes or subtypes and were followed for 2 years. The 2 chimpanzees that were challenged homologously in this study, as well as 7 of 7 from previous challenge experiments [10] that were recently reanalyzed by polymerase chain reaction (PCR) to provide long-term follow-up data, developed self-limited infections after rechallenge. By contrast, 4 of 11 chimpanzees rechallenged with heterologous genotypes or subtypes developed chronic infections with the types used for rechallenge. These findings suggest that a universally effective HCV vaccine may need to incorporate epitopes from multiple genotypes.

MATERIALS AND METHODS

Chimpanzees (Pan troglodytes). Chimpanzees were housed at the New York Blood Center’s primate research facility, Vilab II, at the Liberian Institute for Biomedical Research (LIBR) in Robertsfield, Liberia. The animals were housed in groups of at least 2 in spacious outdoor enclosures under conditions that were in accordance with those outlined in the Guide for the Care and Use of Laboratory Animals [17]. All protocols were approved by the institutional animal care and use committees of the New York Blood Center and the LIBR. As is shown in tables 1 and 2, the chimpanzees in this study were initially infected with HCV genotype 1a or 1b. Serum samples and peripheral-blood mononuclear cells (PBMCs) were collected weekly for 8 weeks, then at 2-week intervals for 6 months, and then monthly for 2 years after rechallenge. Serum samples were stored at −70°C until examined.

Challenge viruses. The following challenge viruses were used in this study: genotype 1a, Hutchinson strain (Genbank accession number M67463); genotype 1b, HC-74/91 strain (Genbank accession number D10750); genotype 2b, HC-I8 strain (Genbank accession number D10988); genotype 3a, S52 strain (Genbank accession number M84837); genotype 4a, ED43 strain (Genbank accession number Y11604); genotype 5a, SA13 strain (Genbank accession number AF064490); and genotype 6a, HK6a strain (Genbank accession number Y12083). The genotypes of viruses that resulted in chronic infection after rechallenge were determined by L. Ganova-Raeva at the Centers for Disease Control and Prevention by sequencing the NS5b region, amplified by nested PCR.

Isolation of PBMCs. PBMCs collected at different intervals after rechallenge were purified by use of ficoll-hypaque and then were cryopreserved at 1°C/min in the presence of 10% dimethyl sulfoxide and 20% fetal bovine serum. PBMCs were thawed by swirling in a 37°C water bath, washed, and counted before use in assays. Viability was assessed by inclusion of a phytohemagglutinin (PHA)–stimulated group in enzyme-linked immunospot (ELISPOT) assays. Samples with <2500 interferon (IFN)–γ spots/1 × 10^6 PBMCs were not included in the analysis.

Quantitation of HCV RNA. HCV RNA was quantitated by a real-time PCR assay with molecular beacon technology,
Table 2. Summary of heterologous challenges.

<table>
<thead>
<tr>
<th>Category, chimpanzee</th>
<th>Initial infection/rechallenge genotype</th>
<th>Interval, a years</th>
<th>Duration of viremia, weeks</th>
<th>Peak viral load after rechallenge, ( \log_{10} ) RNA molecules/mL</th>
<th>ELISPOT response, total IFN-( \gamma ) spots/1 ( \times ) 10^6 PBMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>After initial infection</td>
<td>After rechallenge</td>
<td>Before rechallenge</td>
</tr>
<tr>
<td>Development of chronic infection</td>
<td></td>
<td></td>
<td>After rechallenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>1a/3a</td>
<td>11</td>
<td>25</td>
<td>&gt;156</td>
<td>5.0</td>
</tr>
<tr>
<td>146</td>
<td>1a/1b</td>
<td>1.5</td>
<td>20</td>
<td>&gt;155</td>
<td>5.4</td>
</tr>
<tr>
<td>227</td>
<td>1a/5a</td>
<td>1.5</td>
<td>26</td>
<td>&gt;127</td>
<td>6.2</td>
</tr>
<tr>
<td>296</td>
<td>1a/6a</td>
<td>1.5</td>
<td>16</td>
<td>&gt;154</td>
<td>4.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21.7 ± 4.6b</td>
</tr>
<tr>
<td>Development of self-limited infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>147</td>
<td>1a/4a</td>
<td>20</td>
<td>26</td>
<td>121</td>
<td>5.2</td>
</tr>
<tr>
<td>228</td>
<td>1b/6a</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>Nonef</td>
</tr>
<tr>
<td>238</td>
<td>1a/2b</td>
<td>17</td>
<td>11</td>
<td>5</td>
<td>4.7</td>
</tr>
<tr>
<td>275</td>
<td>1a/2b</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>4.8</td>
</tr>
<tr>
<td>353</td>
<td>1b/1a</td>
<td>11</td>
<td>14</td>
<td>52</td>
<td>5.5</td>
</tr>
<tr>
<td>317</td>
<td>1b/1a</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>3.6</td>
</tr>
<tr>
<td>358</td>
<td>1a/1b</td>
<td>1.5</td>
<td>8</td>
<td>4</td>
<td>5.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.2 ± 6.1b</td>
</tr>
</tbody>
</table>

**NOTE.** ELISPOT, enzyme-linked immunospot; IFN, interferon; PBMCs, peripheral-blood mononuclear cells.

a Interval between initial infection and rechallenge.

b Significance of difference: \( P > .1 \).
c Significance of difference: \( P > .1 \).
d Significance of difference: \( P > .1 \).
e Significance of difference: \( P > .1 \).
f Sterile immunity (see figure 1).
as described elsewhere [18, 19], with modifications. Briefly, RNA was extracted from plasma by use of Qiagen HCV RNA kits. Reverse transcription (RT) PCRs were set up with a Beckman Biomek 2000 pipetting station in a laminar flow hood in a room dedicated to PCR setup. The assay permits quantitation of both high and low viral loads by comparison with a standard curve derived from serial dilutions of synthetic HCV RNA. The method is sensitive to ~100 RNA molecules/mL with a synthetic genotype 1b RNA standard and gives linear results between $1 \times 10^{12}$ and $1 \times 10^7$ RNA molecules/mL. This method has 2 major advantages: the ability to quantitate high and low viral loads from a single dilution and the fact that the amplified samples are not used for other assays (gel or ELISA), which can serve as sources of contamination. Quality control was routinely performed by inclusion (as external negative and positive controls, respectively) of 4–10 HCV-negative serum samples and 2–4 HCV-positive serum samples during each PCR run, to monitor the extraction and amplification efficiencies. Assays in which positive control quantities were outside the mean ± 2 SDs for all assays run (quality assurance curve) were discarded.

Selected samples were also tested by a recently developed high-sensitivity universal molecular beacon assay (L. Andrus et al., unpublished data), which has a sensitivity of <15 RNA molecules/mL and detects all of the genotypes used in this study with similar sensitivity. To increase the sensitivity of this assay, RNA was extracted from 400 µL of plasma by use of an SDS–proteinase K lysis buffer for 25 min at 58°C; ethanol was added to 35% vol/vol, and RNA was collected on glass fiber membranes. After the membranes were washed with AW2 washing buffer (Qiagen), RNA was eluted with 65 µL of nuclease-free water, and 50 µL of the eluate was used for the universal molecular beacon assay. To minimize the effect of sequence variation in molecular beacon probe binding sites—and thus permit detection of all genotypes—the universal molecular beacon assay (described in detail elsewhere [20]) uses 2 relatively long primers (20–27 mer) located head-to-tail on adjacent sequences in the target region. The probe is directed to the sequences of portions of both primers.

### Table 3. Peak acute-phase alanine aminotransferase (ALT) levels and viral loads.

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak ALT level, mean ± SE, SFUs/mL</th>
<th>Peak viral load, mean ± SE, log_{10} RNA molecules/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary infection $^a$ (n = 38)</td>
<td>103 ± 13$^b$</td>
<td>5.8 ± 0.1$^{c,d}$</td>
</tr>
<tr>
<td>Homologous rechallenge (n = 9)</td>
<td>52 ± 6.1</td>
<td>4.1 ± 0.62$^d$</td>
</tr>
<tr>
<td>Heterologous rechallenge (n = 11)</td>
<td>40 ± 11$^b$</td>
<td>5.0 ± 0.2$^d$</td>
</tr>
</tbody>
</table>

*NOTE.* SFUs, Sigma-Frankel units.

$^a$ Data from a previous study [25].

$^b$ Significance of difference: $P<.01$.

$^c$ Significance of difference: $P<.01$.

$^d$ Significance of difference: $P<.01$.

### HCV ELISA. The Ortho HCV 3.0 ELISA test system (Ortho Diagnostic Systems), serial dilutions of chimpanzee serum samples, and a positive control serum sample were used to quantitatively measure anti-HCV antibody responses, in accordance with the manufacturer’s instructions, with some modifications. The level of anti-HCV antibodies is expressed as units in comparison to that of the positive control serum sample. One unit is equivalent to the dilution of the positive control serum sample at 50% binding.

### IFN-γ ELISPOT assay. This assay was conducted in accordance with the instructions included with the IFN-γ ELISPOT kit (Mabtech), with modifications. Briefly, 96-well nitrocellulose-bottomed Millititer plates (Millipore) were coated with murine anti–human IFN-γ monoclonal antibody at a concentration of 15 µg/mL in PBS and were incubated at 4°C. After 24 h, the plates were washed and blocked with 10% human AB+ serum for 1 h at 37°C. For the present study, unexpanded PBMCs were added at different concentrations (1 × 10^5–1 × 10^6 PBMCs/well) in a 100-µL volume of complete medium (RPMI 1640 containing 10% AB+ serum). The target cells, either HCV recombinant vaccinia– (see below) or parental vaccinia–infected autologous PBMCs, were added at a concentration of 1 × 10^5 PBMCs/well in a 100-µL volume of complete medium. After a 24-h incubation at 37°C, the plates were washed extensively by suction by use of an ELISPOT plate washer (Millipore), and biotinylated anti–human IFN-γ monoclonal antibody (clone 4S.B3) was added for 2 h at 37°C. Plates were then washed again, and a 1:1000 dilution of streptavidin peroxidase was added and incubated at room temperature for 2 h, followed by washing 4 times with washing buffer and addition of substrate 3-amino-9-ethyl carbazole reagent (Sigma) in substrate buffer. After the spots were developed for 10–15 min, the plates were washed with distilled water and air dried. The number of spots was enumerated using a computer-assisted ELISPOT image analyzer (Zeiss Axiosplan 2 imaging system) and KS ELISPOT software (version 4.2; Zeiss), which are designed to detect spots by predetermined criteria on the basis of size, shape, and colorimetric density. Data were derived on the basis of the following equation: HCV antigen–specific IFN-
Figure 1. Alanine aminotransferase (ALT) levels, viral loads, and immune responses in chimpanzee 228, which was rechallenged with hepatitis C virus (HCV) genotype 6a after recovering from infection with genotype 1b, showing sterile immunity. The cutoff for the enzyme-linked immunospot (ELISPOT) assay was calculated as the mean plus 2 SDs of the no. of interferon (IFN)–γ spots per 1 × 10^6 peripheral-blood mononuclear cells (PBMCs) observed with parental vaccinia–infected target cells. Abs, antibodies; C–NS3, HCV genotype 1b capsid through NS3; NS3–NS5, HCV genotype 1b NS3 through NS5; SFUs, Sigma-Frankel units.

Generation of HCV recombinant vaccinia viruses for ELISPOT assays. Recombinant vaccinia viruses expressing C–NS3, NS3 alone, and NS3–NS5 in the L variant of the WR strain were provided by M. Perkus at Virogenetics, who constructed them as described elsewhere [21]. These constructs have been used successfully in our laboratory to infect target cells for use in ELISPOT assays [22–26].

Statistical analysis. For unpaired data, levels of significance were determined by Student’s t test. The significance of differences in proportions was tested by Fisher’s exact test.

RESULTS

As is summarized in tables 1 and 2, the rechallenges conducted in the present study were as follows: 1a/1a (homologous), chimpanzees 213 and 291; 1a/1b, chimpanzees 146 and 358; 1a/2b, chimpanzees 238 and 275; 1a/3a, chimpanzee 280; 1a/4a, chimpanzee 147; 1a/5a, chimpanzee 227; 1a/6a, chimpanzee 296; 1b/6a, chimpanzee 228; 1b/1a, chimpanzees 317 and 353. In each case, the chimpanzees were inoculated intravenously with 1 mL of PBS and 10% normal chimpanzee serum containing an estimated 100 CID_{50} of the indicated strains. The estimates of
infectivity titers, based on titrations in chimpanzees, were provided by J. Bukh (National Institutes of Health), as were the chimpanzee-titrated stocks of the different HCV genotypes.

**Homologous rechallenges.** As is summarized in table 1, all of the chimpanzees rechallenged with the homologous strain of HCV used for the initial infection developed acute-phase viremia; however, in each case, these infections resolved completely. Three of the chimpanzees developed viremia that was detectable for only 1 week after rechallenge, and the viremia was detectable only by the high-sensitivity universal molecular beacon assay described above.

Two of these rechallenges (those for chimpanzees 213 and 291) were done as part of the present study. The remaining 7 homologous rechallenges were done as part of a previous study [10], before the establishment of assays for cell-mediated immunity in our laboratory. The peak acute-phase alanine aminotransferase (ALT) levels and the peak acute-phase viral loads were lower than those seen during primary infections (table 3). The ELISPOT assays, the results of which are expressed as the sum of the number of IFN-γ spots from 3 separate assays with target cells respectively expressing C–NS3, NS3 alone, and NS3–NS5, revealed that both of the tested chimpanzees had moderate CD8+ T cell immunity before rechallenge and that this appears to have been boosted after homologous rechallenge (P > .1). The duration of viremia seen after the first challenge (mean ± SD, 11.2 ± 2.4 weeks) was longer than that seen after homologous rechallenge (mean ± SD, 4.0 ± 3.4 weeks); however, this difference was not statistically significant.

**Heterologous rechallenges.** The results of heterologous rechallenges are shown in table 2 and, in more detail, in figures 1–3. In contrast to the results of the homologous rechallenges, 4 (36%) of these 11 chimpanzees developed chronic infections, as is shown in figures 2 and 3. This rate of chronicity is not significantly different than what we have observed for primary infections (17/38 [45%]; P = .5) [25]. As was the case for the homologous rechallenges, peak ALT levels and viral loads were significantly lower than those seen for primary infections (table 3). For 3 of the 4 chimpanzees that developed chronic infections, viremia was detected in the sample collected 1 week after rechallenge, suggesting that it derived from the challenge.
Figure 3. Alanine aminotransferase (ALT) levels and viral loads in 3 additional heterologously challenged chimpanzees (296, 227, and 146), showing development of chronic infection. HCV, hepatitis C virus; SFUs, Sigma-Frankel units.

virus. This was confirmed by genotyping: the virus appearing in chimpanzee 146 was genotype 1b; that appearing in chimpanzee 227 was genotype 5a; that appearing in chimpanzee 280 was genotype 3a; and that appearing in chimpanzee 296 was genotype 6a.

It is interesting that the duration of viremia during primary infection was lower in the chimpanzees that developed self-limited infections after rechallenge (mean ± SD, 13.2 ± 6.1 weeks) than in those that developed chronic infections after rechallenge (mean ± SD, 21.7 ± 4.6 weeks). Although this difference was not statistically significant, it may indicate that the former group had intrinsically stronger cell-mediated immune responses. Furthermore, the duration of viremia after homologous rechallenge (mean ± SD, 4.0 ± 3.4 weeks) was lower than that after heterologous rechallenge (mean ± SD, 29.7 ± 43 weeks). Again, this difference was not statistically significant; however, it supports the conclusion, drawn from the rate of chronicity in this group, that immunity is weaker after heterologous rechallenge.

One chimpanzee (228) developed sterile immunity, as is shown in figure 1. Although detectable viremia did not develop (not even that detectable by the high-sensitivity universal molecular beacon assay), it is likely that this animal developed a mild infection after rechallenge, because there was a doubling ($P > .1$) of T cell reactivity in the ELISPOT assay 2 weeks after rechallenge and a increase in anti-HCV antibody titer between 4 and 8 weeks after rechallenge. It is noteworthy that this chimpanzee had what was by far the strongest ELISPOT response seen in the prechallenge samples from any of the chimpanzees in this study.

Six chimpanzees developed typical self-limited infections. Interestingly, 2 of these had completely negative ELISPOT results at the time of rechallenge. One of these chimpanzees (147) developed ELISPOT reactivity 2 weeks after rechallenge; the other (358) remained negative 2 weeks after rechallenge but showed 200 IFN-γ spots/10^6 PBMCs 6 weeks after rechallenge. Blas
togenesis responses were weak in all chimpanzees, possibly reflecting an adverse effect of cryopreservation on reactivity of cells in this assay ([27] and unpublished data), and are therefore not shown (the HCV proteins used in the blastogenesis assay were provided by M. Houghton [Chiron]).

Before heterologous rechallenge, the mean ELISPOT reactivity of the chimpanzees that developed sterile immunity or self-limited infections (mean ± SD, 557 ± 305 IFN-γ spots/
1 × 10⁶ PBMCs) was ~2-fold higher than that of the 4 chimpanzees that developed chronic infections (mean ± SD, 265 ± 314 IFN-γ spots/1 × 10⁶ PBMCs). This difference, however, was not statistically significant (P = .57). Two weeks after rechallenge, the ELISPOT responses had increased 1.6-fold in the chimpanzees that developed chronic infections and 2.2-fold in those that developed self-limited infections. As has been previously reported [24], anti-HCV antibody responses were strongest in the chimpanzees that developed chronic infections.

**DISCUSSION**

The main finding of the present study is that, whereas 9 of 9 homologous rechallenges resulted in self-limited infections only, 4 of 11 heterologous rechallenges resulted in chronic infections. This difference was of borderline statistical significance by Fisher’s exact test (2-sided P = .09). This finding is in accordance with those of previous reports indicating the development of chronic infection after cross-genotype rechallenge of 2 chimpanzees [9] and in human reinfec-tions [2, 28]. These and the present findings suggest an important role for genotype in conferring immunity against chronic HCV infection.

Almost all reports of the results of homologous rechallenges have shown self-limited infection after challenge [4–8, 10, 29, 30], as was observed in the present study. The only exception, to our knowledge, is the recent report by Bukh et al. showing the development of chronic infection after rechallenge with a homologous monoclonal strain of genotype 1a [13]. Whether this surprising observation reflects the unusual virulence of this monoclonal strain is not known.

The only chimpanzee in the present study that developed apparent sterile immunity after heterologous rechallenge showed what was by far the highest level of T cell immunity, as revealed by ELISPOT assay. This is in accordance with the findings of numerous reports that have indicated an association between levels of cell-mediated immunity and control of HCV replication in chimpanzees [24, 29, 30] and in humans [31–35]. Our results also support a role for cell-mediated immunity in the control of HCV replication, in that pre- and postexposure ELISPOT responses were higher in the chimpanzees that developed self-limited infections than in those that developed chronic infections (table 2).

Two chimpanzees that were negative by ELISPOT assay at the time of rechallenge developed self-limited infections, and 1 developed a chronic infection. This is not dissimilar from the rate of chronicity for primary infection when there is no cell-mediated immunity at the time of challenge.

It is clear that both homologously and heterologously rechallenged chimpanzees developed some degree of immunity from their primary infections. This is indicated by their significantly lower peak ALT levels and viral loads, compared with those seen during primary infections. Nevertheless, this immunity was not sufficient to prevent chronic infection in 4 of 11 chimpanzees after heterologous rechallenge. This suggests that, optimally, a universally protective vaccine for HCV should incorporate epitopes from multiple genotypes.

**Acknowledgments**

We gratefully acknowledge the skillful assistance of Mussah Konneh, John Zeonyuwe, and Joseph Thomas in the conduct of the chimpanzee experiments and of Liping Li, Patricia McCormack, George Saycohay, Etmonia Davis, and Venus McCausley in the performance of laboratory assays and procedures.

**References**