Detection of Antibodies to the Nonstructural 3C Proteinase of Hepatitis A Virus

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Hepatitis A virus (HAV) infection can stimulate the production of antibodies to structural and nonstructural proteins of the virus. However, vaccination with an inactivated vaccine produces antibodies exclusively to the structural proteins. Current diagnostic assays, such as the Abbott HAVAB test, used to determine exposure to HAV detect antibodies only to the structural proteins and as a result are not able to distinguish between a natural infection and vaccination with an inactivated virus. Therefore, an ELISA was developed that is specific for antibodies to the nonstructural protein 3C of HAV and thus serves to document the occurrence of viral replication. Antibodies to the proteinase were not detected by this assay in serum from HAVAB-seropositive primates that were immunized with inactivated HAV. However, antibodies to the proteinase were detected in the serum of all primates experimentally infected with virulent HAV and in the serum of naturally infected humans.

Hepatitis A virus (HAV) is a picornavirus that causes acute liver disease in humans as well as in nonhuman primates. As with other viruses of the picornavirus family, the genome of HAV can be divided into three regions that encode the proteins of the virus. The structural proteins, which form the viral capsid, are encoded by the P1 region, and the nonstructural proteins, which include a viral proteinase, a polymerase, and other proteins involved in replication, are encoded by the P2 and P3 regions.

During a natural infection, the immune system of the infected individual can produce antibodies to both the structural and nonstructural proteins [1, 2], whereas an inactivated vaccine induces antibodies only to the structural proteins [3]. Therefore, the commercial diagnostic procedures that identify antibodies solely to structural proteins cannot distinguish between an individual who has experienced a natural infection and one who has been vaccinated with the current vaccine, which consists of inactivated whole virus. For diagnostic, safety, and epidemiologic reasons it is important to distinguish between antibodies that are elicited by vaccination and those resulting from infection. Therefore, we have developed an ELISA that can serve as a marker of HAV replication, since it is specific for antibodies to the 3C proteinase, a nonstructural protein of HAV.

The 3C proteinase, which is encoded by the P3 region, is an enzyme responsible for processing the HAV polyprotein. It is the only nonstructural protein of HAV to date that has been expressed from a recombinant vector to yield a functional protein [4–8]. The recombinant 3C proteinase was chosen for the ELISA because it can be recovered from Escherichia coli as a soluble protein that can be purified by standard biochemical methods.

In this study, we determined whether the ELISA could detect anti-3C in sera from chimpanzees and tamarins that had been experimentally infected with virulent HAV and compared the results with those from animals that had been immunized with an inactivated vaccine or immune serum globulin (ISG). We also determined if the ELISA could detect anti-3C in humans who were naturally infected with HAV.

Materials and Methods

Expression and purification of 3C. The plasmid pET-3CD*, which contains the entire 3C gene and 70 N-terminal amino acids of 3D from the HAS-15 strain of HAV, has been described previously [9]. Plasmid pET-3CD* was transformed into competent BL21 cells. After the transformation, individual colonies were selected, and bacteria were cultured in N-Z amine medium containing 50 μg/mL ampicillin. Once the optical density at 595 nm reached 0.5, isopropyl thiogalactopyranoside was added to a final concentration of 0.5 mM. Thirty minutes later, the antibiotic rifampicin was added to reduce bacterial protein expression. Three hours after induction, bacteria were harvested by centrifugation and resuspended in a lysis buffer (50 mM TRIS-HCl, pH 8.5, 2.5 mM EDTA, 2 mM dithiothreitol, 80 μg/mL lysozyme). The suspension was alternately frozen and thawed five times to lyse the bacteria. The lysate was clarified by centrifugation, and the supernatant was incubated overnight at 4°C with DEAE–Sephadex A-25 (Pharmacia, Piscataway, NJ) that was equilibrated in buffer (50 mM TRIS-HCl, pH 8.5, 2.5 mM EDTA, 2 mM dithiothreitol). The supernatant from the Sephadex mixture was collected through a 0.2-μm filter and chromatographed on a CM-Sepharose (Pharmacia, Piscataway, NJ) column. The proteinase was eluted with a linear gradient of 0–1 M NaCl in the above buffer. Fractions were
analyzed for 3C proteinase content by Western blot using rabbit antibodies to the protein. The fractions containing 3C were pooled and concentrated in a Centricon 10 microconcentrator. The proteinase was purified further on a column of Sepharose 300WS (Pharmacia). The protein was eluted off the column in TRIS-buffered saline, pH 7.5. A final purification step involved binding the proteinase on a column of hydroxyapatite (BioRad, Hercules, CA) and eluting it with a linear gradient of 10–400 mM K$_2$PO$_4$. The activity of the 3C proteinase was determined by cleavage of in vitro–generated radiolabeled polyprotein substrate as described by Schultheiss et al. [8]. The protein concentration was determined by a commercially available assay (BioRad).

**ELISA development.** The 3C proteinase was diluted in 50 mM carbonate buffer (pH 9.6) to a final concentration of 1 µg/mL. One hundred microliters of the diluted 3C proteinase was placed in each well of 96-well microtiter plates and incubated overnight at 4°C. After incubation, the wells were washed four times with PBS–1% Tween 20 and then incubated with 100 µL/well blocking buffer (PBS with 10% fetal calf serum and 1% gelatin) for 1 h at 37°C. After removal of the blocking buffer, the wells were washed four times with PBS–1% Tween 20. One hundred microliters of serum from chimpanzees, tamarins, or humans was diluted in blocking buffer, added to the microtiter plate, and then incubated for 1 h at 37°C. After this incubation, the microtiter plate was washed four times with PBS–1% Tween 20. Anti-human IgG or IgM labeled with horseradish peroxidase (HRP) (Organon Teknika Cappel, West Chester, PA) was used to detect antibodies to the HAV proteinase in chimpanzees and humans, while anti–New World monkey immunoglobulin (HRP-labeled by Accurate Chemical and Scientific, Westbury, NY) [10] was required for the tamarins. The final color development was produced by the addition of 2,2'-azino-di-ethylbenzothiazoline-sulfonic acid and incubation of the microtiter plate for 15–20 min at room temperature. Samples with an optical density >0.2 in the ELISA were considered positive. This cutoff value represents 2 SD above the mean value of known negative samples.

**Detection of antibody to HAV structural proteins (HAVAB).** All samples were tested for antibodies to HAV structural proteins with a commercially available competitive binding RIA or EIA (Abbott, Abbott Park, IL). Serum samples were diluted in PBS and incubated with radiolabeled or HRP-labeled anti-HAV in a sample tray. HAV-coated beads were then added to the sample tray. After incubation and washing, the amount of anti-HAV bound to the virus-coated beads was determined by color development or quantification of radioactivity.

**Serum samples.** Serum samples were collected from chimpanzees (Pan troglodytes) and tamarins (Saguinus mystax) that were experimentally infected with wild type or mutant strains of HAV. Chimpanzees 1442 and 1300 were inoculated intravenously with 1000 CID$_{50}$ of wild type HM-175, while chimpanzees 1373 and 1451 were infected orally with 3–30 oral infectious doses of the wild type SD-11 strain. Chimpanzee 1374 was given intravenous inoculations of ISG (SmithKline Beecham, Rixensart, Belgium) prepared from volunteers who had received an inactivated vaccine. Chimpanzees 1396 and 1420 were given commercial preparations of ISG intramuscularly. Chimpanzees 1374, 1396, and 1420 were then challenged by intravenous injection with 1000 CID$_{50}$ of wild type HM-175 2 days after injection of the ISG [11]. Chimpanzees were vaccinated by intramuscular injection of inactivated HAV vaccine (SmithKline Beecham) containing strain HM-175 (1332 and 1380) or by intravenous inoculation of 10,000 TCID$_{50}$ of live attenuated HM-175 (1309 and 1333) and then challenged with 1000 CID$_{50}$ of wild live type HM-175 [11]. Chimpanzees 88A02 and 88A04 were inoculated intravenously with a 10% liver suspension containing live AGM-27 (not titered), a simian strain of HAV; they were later sequentially challenged intravenously with 1000 CID$_{50}$ of wild type HM-175 and 100,000 CID$_{50}$ of wild type SD-11 [12]. Tamarin 682 was inoculated intraperitoneally with infectious RNA encoding a virulent HAV strain, while tamarin 683 was inoculated intravenously with 100,000 CID$_{50}$ of an attenuated strain of HM-175. Human serum samples were collected from persons exposed to HAV during an outbreak of hepatitis A at a military institution [13] and from laboratory staff members who had been naturally exposed to HAV, vaccinated with HAV vaccine, or never exposed to HAV.

Serum samples from inoculated chimpanzees and tamarins were analyzed for alanine aminotransferase (ALT) and isocitrate dehydrogenase (ICD) with commercial assays (Metpath, Rockville, MD). Levels of ALT or ICD equal to or greater than twice the mean prechallenge value were indicative of biochemical evidence of hepatitis. Primates were housed under BL-2 biohazard containment.

**Results**

**Anti-3C proteinase in chimpanzees.** A chimpanzee (1442) infected with wild type HAV, strain HM-175, seroconverted to the structural proteins and produced peak ALT levels at weeks 3 and 4 after inoculation, respectively. IgG antibodies to the nonstructural 3C proteinase appeared later, at week 4 (table 1, figure 1A). The antibodies to the 3C proteinase were detected through week 105 after infection, after which time they decreased below the level of detection. Anti-proteinase antibodies were not boosted following inoculation with the inactivated HAV vaccine at week 75; however, there was a 2-fold boost in antibodies to the structural proteins (data not shown). IgM antibodies to 3C proteinase peaked at week 4 after inoculation but declined rapidly to undetectable levels by week 6 after inoculation (figure 2). Chimpanzee 1300, which was also infected with wild type HM-175, seroconverted to the structural proteins and produced peak enzyme levels at weeks 3 and 4, respectively, after inoculation. Antibodies to 3C proteinase were also detected at week 3 after inoculation and reached a peak titer of 1:1000 by week 5 (table 1).

To determine if the ELISA could measure anti-3C proteinase in chimpanzees infected with an HAV strain other than HM-175, we analyzed serum from animals infected orally with the wild type SD-11 strain. The ELISA detected antibodies to the proteinase in SD-11–infected chimpanzees 1373 (table 1) and 1451 (table 1, figure 1B). As with HM-175–infected chimpanzee 1442, antibodies to the proteinase were first observed after seroconversion to the structural proteins and close to the time of peak ALT elevation. Chimpanzee 1373 produced detectable levels of anti-proteinase on week 8 after inoculation, 1 week...
after seroconversion to structural proteins and peak enzyme production. Chimpanzee 1451, which produced significantly higher levels of anti-proteinase than did chimpanzee 1373, seroconverted to 3C proteinase on week 6 after inoculation, 1 week after seroconversion to structural proteins.

Vaccination with a nonreplicating virus should induce antibodies to the structural proteins but not to nonstructural proteins. Serum from chimpanzees that were immunized with either an attenuated or an inactivated vaccine were tested for an antibody response to 3C proteinase. Two chimpanzees vaccinated with an inactivated HM-175 vaccine (1332 and 1380) seroconverted to the structural proteins after vaccination and did not exhibit elevated serum liver enzyme levels after immunization or after challenge with virulent HM-175 (table 2). Antibodies to the nonstructural protein 3C were not detected in chimpanzee 1332 (figure 1C, table 2) either before or after challenge with HM-175, indicating that the vaccination with the inactivated vaccine in this case protected against significant replication of the virus. However, chimpanzee 1380, which also remained anti-3C-negative after vaccination with inactivated vaccine (figure 1D), did exhibit anti-3C reactivity after intravenous challenge with a high dose of HM-175. Therefore, in this animal, vaccination provided protection against hepatitis but not against infection, since replication of the challenge virus occurred. Chimpanzees 1309 and 1333, vaccinated with an attenuated vaccine, produced antibodies to the structural proteins and mounted a fully protective immune response, since they did not develop hepatitis or become infected after challenge (table 2). Neither animal produced antibody to 3C after vaccination. This suggests that the attenuated virus was able to stimulate production of antibodies to the structural protein, even though replication of the vaccine virus was insufficient to stimulate anti-3C antibodies. The vaccine dose was not sufficient to result in seroconversion to the structural proteins in the absence of replication.

Passive immunization with ISG prevents disease but does not necessarily prevent viral replication [2]. The 3 chimpanzees (1374, 1396, and 1420) (table 1, figure 1E) that were inoculated intravenously with wild type HM-175 seroconverted to the structural proteins but did not exhibit an increase in serum ALT levels and, therefore, did not develop hepatitis. However, all 3 animals produced antibodies to the proteinase, indicating that viral replication had occurred. All 3 chimpanzees developed anti-3C titers ≥ 1:100. Immunization with inactivated HAV vaccine did boost antibodies to the structural proteins (data not shown) but did not increase the anti-3C antibody titer in chimpanzee 1374 (figure 1E).

Infection of chimpanzees 88A02 (figure 1F) and 88A04 (table 2) with the simian strain of HAV, AGM-27, was associated with a low level of viral replication [12] and caused seroconversion to HAV structural proteins in the absence of hepatitis. Although the AGM-27 virus did replicate, albeit to a low level in both animals [12], only chimpanzee 88A04 transiently displayed detectable levels of anti-3C (titer of 1:20) 3 months after inoculation with AGM-27 (data not shown). Following the AGM-27 infection, the chimpanzees were challenged intravenously first with HM-175 and later with SD-11. Neither chimpanzee developed hepatitis A, as indicated by stable ALT levels. However, there did appear to be a boost in antibodies to 3C after the SD-11 challenge in chimpanzee 88A02 (figure 1F) and after both the HM-175 challenge and SD-11 challenge in chimpanzee 88A04 (data not shown), indicating low-level replication of the viruses.

### Table 1. Response of naive or passively immunized chimpanzees to challenge with wild type HAV.

<table>
<thead>
<tr>
<th>Treatment, chimpanzee</th>
<th>Alanine aminotransferase (U/L)</th>
<th>Antibody to 3C proteinase</th>
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<tbody>
<tr>
<td></td>
<td>Before challenge</td>
<td>After challenge</td>
</tr>
<tr>
<td>Naive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1442</td>
<td>48</td>
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<td>56</td>
<td>527</td>
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<tr>
<td>Immune globulin (ISG)</td>
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<td></td>
</tr>
<tr>
<td>1374</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>1396**</td>
<td>45</td>
<td>49</td>
</tr>
<tr>
<td>1420**</td>
<td>38</td>
<td>48</td>
</tr>
</tbody>
</table>

* First week antibodies to 3C proteinase were detected following challenge (week 0) with wild type virus.
† No. of weeks antibody to 3C was detected by ELISA; > samples were not available after this time.
‡ Inoculated intravenously with wild type HM-175 strain.
§ Previously inoculated with attenuated virus but not infected.
¶ Inoculated orally with wild type SD-11 strain.
+ Inoculated intravenously with immune globulin (human) prior to intravenous challenge with wild type virus.
** Inoculated intramuscularly with immune serum globulin (human) prior to intravenous challenge with wild type virus.
1 Present but not detectable by standard assays.
Figure 1. IgG antibody response to 3C proteinase in chimpanzees inoculated with: A, virulent HAV, strain HM-175, and inactivated HAV vaccine; B, virulent HAV, strain SD-11; C, D, inactivated HAV vaccine and virulent HAV, strain HM-175; E, immune serum globulin, virulent HAV, strain HM-175, and inactivated HAV vaccine; F, attenuated HAV, strain AGM-27, and virulent HAV, strains HM-175 and SD-11.
Figure 1. Continued. Antibodies to 3C proteinase and to structural proteins (anti-HAV) were plotted, with alanine aminotransferase levels, as function of time relative to challenge. Antibody to 3C proteinase in 1:20, 1:100, and 1:1000 dilutions of serum was plotted as optical density (OD). Values above cutoff point of 0.2 for 3C proteinase were considered positive.
Anti-3C proteinase in tamarins. To determine if the ELISA could monitor antibody production to 3C in primates other than chimpanzees, we analyzed serum from HAV-infected tamarins. Tamarin 682 had been infected by intrahepatic transfection with RNA transcribed from an HAV cDNA clone (8Y), which encodes a virus with a wild-type phenotype [14]. This tamarin exhibited a peak serum ICD level and seroconverted to the HAV structural proteins at week 5 after inoculation (figure 3A). Antibodies to 3C proteinase were first detected at week 6 after inoculation. Antibody to 3C peaked during week 7 and was still detectable at week 17 after inoculation.

Another tamarin, 683 (figure 3B), was inoculated intravenously with an attenuated virus. This animal seroconverted to the structural proteins, but serum ICD levels were much lower than those in tamarin 682, which had been infected with virulent virus. Even though there were indications of viral replication, such as fecal viral shedding, antibodies to the 3C proteinase did not reach significant levels in this animal.

Table 2. Response of vaccinated chimpanzees to challenge with wild type HAV.

<table>
<thead>
<tr>
<th>Vaccine, challenge</th>
<th>Alanine aminotransferase (U/L)</th>
<th>Antibody to 3C proteinase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before challenge (mean)</td>
<td>After challenge</td>
</tr>
<tr>
<td>Attenuated vaccine³</td>
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<td></td>
<td>1333³</td>
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<td>37</td>
</tr>
<tr>
<td></td>
<td>1380²²</td>
<td>42</td>
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</tbody>
</table>

NOTE: NA, not applicable.
* First week antibodies to 3C proteinase were detected following intravenous challenge (week 0) with wild type virus.
† No. of weeks antibody to 3C was detected by ELISA.
³ Vaccinated prior to challenge with wild type virus.
⁴ Vaccinated intravenously with attenuated strain of HM-175.
⁵ Inoculated intravenously with HAV strain AGM-27.
⁶ First week antibodies to 3C proteinase detected following challenge with SD-11 strain (2nd challenge).
⁷ First week antibodies to 3C proteinase detected following challenge with HM-175 strain (1st challenge).
⁸ Vaccinated by intramuscular injection with inactivated strain of HM-175.
**Figure 3.** Antibody response to 3C proteinase in tamarins infected with: A, virulent HAV, strain HM-175 (8Y); B, attenuated HAV, strain HM-175 (HAV/7). Antibodies to 3C proteinase and to structural proteins (anti-HAV) were plotted, with isocitrate dehydrogenase levels, as function of time relative to challenge. Antibody to 3C proteinase in 1:20, 1:100, and 1:1000 dilutions of serum was plotted as optical density (OD). Values above cutoff point of 0.2 for 3C proteinase were considered positive.

**Anti-3C proteinase in humans.** Serum samples from patients who developed hepatitis A were analyzed to determine if antibodies to the proteinase could be detected in humans. The patients had been exposed to HAV during an outbreak of hepatitis A on a military base [13]. Three patients (B, C, and D) seroconverted to the structural proteins of HAV and exhibited increased serum ALT levels (data not shown). Antibody to the 3C proteinase was detected in the serum of all 3 of these patients (figure 4). However, patient A did not seroconvert to the structural proteins of HAV and was subsequently determined to have chronic non-A, non-B hepatitis. This patient also remained negative for antibody to the 3C proteinase (figure 4).

In a separate study, sera from 17 persons who had tested negative for antibodies to the structural proteins of HAV also tested negative for antibody to the 3C proteinase (data not shown). In addition, sera from 4 human subjects who had been immunized with an inactivated HAV vaccine produced antibodies to the HAV structural proteins but tested negative for antibody to the 3C proteinase (data not shown). The sera from the vaccinees were collected at various times ranging from 11 months to 4 years after the initial inoculation. In contrast, 3 of 9 young adults who were seropositive for antibodies to the structural proteins of HAV because of natural exposure to the virus did test positive for antibody to 3C (data not shown). Eight of the 9 with anti-structural antibodies (including 2 of the 3 with anti-3C antibodies) were from countries where HAV was endemic and had not experienced any clinical signs of disease. Therefore, the exact time of exposure is unknown. However, since subclinical infections are most common in young children, exposure most likely occurred many years previously. The third subject became infected with HAV via exposure in the laboratory. The sample that was tested for antibody to 3C was collected 7 years after that person had initially seroconverted to the structural proteins of HAV.

**Discussion**

The data from this study demonstrated that HAV-infected humans, chimpanzees, and tamarins produced antibodies to the HAV 3C proteinase. Previous studies, based on an immunoprecipitation assay, demonstrated the production of antibodies that reacted with polyproteins encoded by the entire P2 and P3...
regions, but specificity to individual nonstructural proteins was not determined. [3, 15]. Although the immunoprecipitation procedure detects antibodies to the nonstructural proteins, it is not practical because of the time and materials needed to perform the procedure and because of its level of sensitivity. Therefore we developed an ELISA as an alternative method for monitoring antibodies that signal viral replication. We chose the 3C protease as substrate for the ELISA because it is the only nonstructural protein of HAV to date that has been produced in large quantities in a recombinant system and because it can be purified as an enzymatically active and presumably native enzyme [6, 8]. The data from the present study indicated that antibodies that reacted with 3C protease in an ELISA format were produced by humans, chimpanzees, and tamarins following infection with HAV.

The ELISA detected anti-3C at titers of 1:10—1:1000 in serum. The results suggested that, in general, antibodies to the protease did not persist as long as did antibodies to the structural proteins. However, antibodies to 3C were detectable in some chimpanzees for as long as 2 years.

The versatility of the assay was demonstrated by the fact that it recognized antibodies elicited by 3 different HAV strains. Serum from chimpanzees that were infected with strains HM-175, SD-11, or AGM-27 contained anti-protease antibodies that reacted with the 3C protein of a fourth strain, HAS-15, the source of the antigen in the ELISA. One explanation for the wide versatility is that the 3C gene is one of the most conserved regions of the HAV genome. The protease from HAV strain HAS-15 differs from the protease of wild type HAV strain HM-175 by only one amino acid and from the protease of strain AGM-27 by nine amino acids. In addition, the assay detected anti-3C in sera from two lower primate species as well as in sera from humans.

The anti-protease antibodies usually were produced concurrently with or immediately after the elevation in serum liver enzyme levels. This result correlated with previous observations of antibodies to the P2 and P3 proteins as a whole [3]. In addition, our results suggested that, in general, animals that had more severe infections, as measured by higher levels of serum liver enzymes, produced higher titers of anti-protease antibodies. Further investigation will be needed to determine if there is a direct correlation between these two parameters.

The ELISA was able to confirm low levels of viral replication after challenge of 3 passively immunized chimpanzees (1374, 1396, 1420) that did not exhibit signs of clinical disease. In addition, chimpanzee 1380, which developed antibodies to the structural proteins after vaccination with an inactivated HM-175 vaccine, produced low levels (1:20) of antibodies to 3C after challenge with wild type HM-175. The presence of anti-protease indicated that viral replication occurred in the presence of antibodies to the structural proteins. Since there was no increase in serum ALT levels, and polymerase chain reaction analyses of fecal samples were negative for virus excretion, the antibodies to 3C protease were the only indicators that replication of the virus had occurred. The replication of HAV in chimpanzee 1380 may be in part due to the fact that the challenge was one thousand times the dose needed for infection. In addition, the challenge was given intravenously, which allows for a more direct infection of the liver, thereby limiting viral interaction with circulating antibody.

Inoculation with an attenuated HAV vaccine apparently can result in limited viral replication and seroconversion to the structural proteins in the absence of an antibody response to the 3C protease. This was documented in chimpanzees 1309 and 1333, which were both vaccinated with an attenuated strain of HM-175 and did not produce antibodies to 3C after vaccina-
tion or after challenge with a virulent strain of HM-175. Anti-3C was also not detected in tamarin 683, which was inoculated with an attenuated strain of HAV, even though there was evidence of both viral replication and limited disease. The reason for the lack of antibody response to the nonstructural protein is unclear and warrants further investigation.

Limited viral replication after challenge was also documented in chimpanzees 88A02 and 88A04, which were inoculated with a simian virus (AGM-27) and then challenged sequentially with HM-175 and SD-11. Infection of chimpanzees with AGM-27 resulted in a response similar to that seen in animals inoculated with an attenuated vaccine [12]. The chimpanzees seroconverted to structural proteins but did not exhibit any clinical signs of disease. After inoculation with AGM-27, only chimpanzee 88A04 produced limited amounts of antibody to 3C but only after a long incubation period. However, once again, seroconversion to structural proteins did not necessarily prevent infection, since, following intravenous challenge with a high dose of wild type HM-175 or SD-11, there was a slight increase in anti-proteinase activity. One can assume, however, that the level of replication was low because all clinical signs of disease remained negative, and fecal shedding was not detected by polymerase chain reaction.

Finally, our preliminary data with sera from HAV-infected humans correlates with the results from the HAV-infected chimpanzees and tamarins. Individuals who were naturally infected with HAV produced antibody to the 3C proteinase, while individuals who were vaccinated with an inactivated vaccine were exposed but not infected remained anti-3C−negative. Even though our initial studies indicate that the antibody to 3C can persist for years in humans, further studies must be done to determine the duration of antibody to 3C in humans and the role this antibody plays in the human immune response to HAV infection.

Our data indicate that the ELISA using HAV 3C proteinase as the antigen can accurately detect antibodies to a nonstructural protein and therefore distinguish an immune response to active infection from that resulting from vaccination with an inactivated vaccine. In addition, the ELISA appears to provide a useful and simple method for the detection of limited replication in cases in which virus is not excreted to detectable levels and disease does not occur. This assay may facilitate a better understanding of the role of the nonstructural antibodies in the immune response to HAV infection.

Acknowledgment

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References