IgM Antibody Response in Acute Hepatitis C Viral Infection

By John M. Clemens, Suhas Taskar, Kurt Chau, David Vallari, J. Wai-Kuo Shih, Harvey J. Alter, Joseph B. Schleicher, and Larry T. Mimms

IgM antibody against hepatitis C virus (IgM anti-HCV) was measured in serial samples from 15 transfusion recipients in whom posttransfusion chronic non-A, non-B hepatitis (NANBH) developed and three plasmapheresis donors during acute HCV infection using recombinant proteins derived from three immunodominant regions: core, NS-3, and NS-4 (c100). IgM anti-HCV core was detected in 13 of 15 posttransfusion patients. Nine of these patients had transient, acute-phase IgM anti-HCV core detected coincidentally or earlier than active IgG anti-HCV core response. The average duration of IgM anti-HCV core reactivity was 8.1 ± 3.7 weeks. One patient lacking an IgM anti-HCV core response had detectable IgM anti-HCV NS-3 during the acute phase. Passive transfer of IgM anti-HCV was not observed in these posttransfusion cases, in contrast to the high frequency observed for IgG anti-HCV. Late IgM anti-HCV was detectable against core, c100, and NS-3 in three, two, and one posttransfusion patients, respectively. These data indicate that IgM anti-HCV core is a useful acute-phase marker in HCV infection.

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based on a 50-member antibody-negative population; sample to
cutoff ratio (S/CO) values greater than 1.0 were considered
reactive. Effective removal of high titer IgG anti-HCV by the
preincubation procedure was demonstrated by control incubation
in which biotinylated goat anti-human IgG was substituted for the
biotinylated goat anti-human IgM.

MATRIX HCV IgG test was conducted as described previously.1

RESULTS

Posttransfusion NANBH patients. Fifteen transfusion re-
cipients in whom NANBH developed were assayed for the
presence of IgM anti-HCV. In this study, serial bleeds were
obtained from 10 patients over a short period (up to 66
weeks following surgery) and from five patients over a
longer period (up to 10 years following surgery). The timing of
posttransfusion IgG and IgM seroconversion is summa-
rized in Table 1. IgG antibody reactivities to the recombi-
nant HCV proteins for 14 of these patients were described
previously by Vallari et al.4

Seroconversion to IgG anti-HCV was detected in all 15
patients by recombinant c100 and NS-3 antigens, and in 13
cases by core.4 Anti-HCV IgG was detected by core polypep-
tide as the earliest (n = 6) or simultaneously with the
earliest marker (n = 4) in most cases. Detection of anti-
body by NS-3 or clOO as the earliest marker was observed in one
case and was coincident with the earliest in five, and by c100
as the earliest or coincident with the earliest in two and
three cases, respectively. Passive transfer of IgG antibody
donors was evident in 13 patients.1 Serological profiles of
two of these patients are shown in Fig 1B and C. A
serological profile of one of the two patients in which
passive transfer was not observed is shown in Fig 1A. Also,
in all five long-term patients (no. 11 to 15, Table 1), IgG was
detected by all recombinant HCV proteins and persisted
throughout the monitoring period, and up to 10 years in one
patient.

Patterns and timing of IgM HCV reactivity differ signifi-
cantly from the IgG response. Anti-HCV core was the only
detectable IgM response in 12 patients and one patient
showed only IgM anti-HCV NS-3 reactivity. In only two
patients was an IgM response to multiple HCV epitopes
evident. IgM anti-HCV responses were transient and oc-
curred during acute illness in nine recipients who devel-
oped IgM anti-HCV core and in the single patient in whom
IgM anti-HCV NS-3 was evident (Fig 1B). The first detection
of IgM anti-HCV core coincided with IgG seroconversion
in seven patients; IgM anti-HCV core was detected
during the decline of passively transferred IgG anti-HCV
in two patients. The mean interval to IgM anti-HCV serocon-
version was 10.1 weeks after transfusion or 1.8 weeks from
the onset of hepatitis (measured as the first elevation in
serum ALT levels). The average duration of the IgM
anti-HCV core response was 8.1 ± 3.7 weeks during the
acute phase.

The acute phase IgM anti-HCV NS-3 response was
coincident with first detection of IgG anti-HCV NS-3 and
was 40 weeks in duration (Fig 1B).

Late onset of IgM anti-HCV core was evident in three of
15 patients. Two were transient and one (no. 13) had
intermittent reactivity but remained positive at the time of
the last bleed 364 weeks posttransfusion (Fig 1C). This
patient was also reactive for IgM anti-HCV c100 at the last
bleed date. Also, one patient (no. 12) showed late onset of
IgM anti-HCV c100 and NS-3 at 37.6 weeks posttransfu-
sion, but IgM titers had decreased to undetectable levels by
158 weeks posttransfusion.

Interestingly, no passive transfer of IgM anti-HCV was
observed in these 15 patients. Overall titers of IgM anti-
HCV core (usually S/CO < 10) were low, although pa-

Table 1. Weeks Posttransfusion of Anti-HCV IgG and IgM Seroconversion

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ALT+</th>
<th>Matrix IgG*</th>
<th>Matrix IgM</th>
<th>Core†</th>
<th>Matrix IgG</th>
<th>Matrix IgM</th>
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<tr>
<td></td>
<td></td>
<td>c100†</td>
<td>33c†</td>
<td>Core†</td>
<td>c100†</td>
<td>33c†</td>
</tr>
<tr>
<td>1</td>
<td>10.0</td>
<td>15.7</td>
<td>15.7</td>
<td>10</td>
<td>10.0</td>
<td>19.0-30.0</td>
</tr>
<tr>
<td>2</td>
<td>12.4</td>
<td>17.9</td>
<td>14.9</td>
<td>13</td>
<td>13.0</td>
<td>17.9-30.0</td>
</tr>
<tr>
<td>3</td>
<td>8.1</td>
<td>19.7</td>
<td>15.7</td>
<td>15.7</td>
<td>15.7-26.15</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>8.3</td>
<td>36.9</td>
<td>19.3</td>
<td>&gt;50.7</td>
<td>11.3</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>14.7</td>
<td>14.7</td>
<td>23.7</td>
<td>&gt;23.7</td>
<td>3.7-23.7</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>6.9</td>
<td>20.0</td>
<td>6.9</td>
<td>3.7</td>
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<td>7</td>
<td>10.1</td>
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<td>26.1</td>
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<td>—</td>
</tr>
<tr>
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</tr>
<tr>
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<td>12.6</td>
<td>17.4</td>
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<td>38-160</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>11.1</td>
<td>18.1</td>
<td>24.6</td>
<td>24.6</td>
<td>366.3</td>
<td>—</td>
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</table>

*Data from Vallari et al.4 Patients 8 through 15 correspond to patients 7 through 14, respectively, in ref 4.
†First date of active antibody response.
‡Interval of reactivity is indicated with hyphenated numbers, isolated number indicates only reactive draw in which IgM was detected.
§Indicates last available draw.
IgM RESPONSE TO HCV

Acute HCV plasmapheresis donors. Serial bleeds obtained from three plasmapheresis donors who developed acute HCV infection as measured by increased liver enzyme levels and seroconversion to anti-HCV were tested for the presence of specific IgM anti-HCV. All three donors developed IgM anti-HCV core, whereas negligible or no IgM anti-HCV reactivity was detected against NS-3 or c100. In two cases, IgM anti-HCV core was detected coincident with IgG anti-HCV core and, in one case, IgM anti-HCV core was detected in a draw before seroconversion to IgG anti-HCV core (Fig 2). IgM anti-HCV core was detectable coincident with an increase in ALT in two donors and 20 days after an ALT increase in one donor. Some decline in IgM core titers were observed with time, but all three donors were still reactive for IgM core at the last bleed obtained (50 to 85 days after the first ALT elevation).

DISCUSSION

Previous data have shown that addition of recombinant HCV core and NS-3 polypeptides to IgG anti-HCV assay containing c100 nearly eliminates the interval between the onset of hepatitis and the first detection of IgG antibody to HCV in posttransfusion NANBH patients. These studies also demonstrated that passive transfer of antibodies to HCV was primarily responsible for IgG anti-HCV positivity from 0 to 14 weeks after transfusion. Because of high level of passively transferred IgG anti-HCV in these patients, time of seroconversion and production of active IgG anti-HCV production resulting from HCV infection could only be assessed by measuring increasing levels of IgG anti-HCV. Since no passive transfer of IgM anti-HCV was detected in these patients, presence of IgM anti-HCV gave a clear indication of HCV infection and anti-HCV seroconversion (Table 1, Fig 1A to C).

The primary IgM anti-HCV response was against the core polypeptide in 13 of 15 posttransfusion NANBH cases and was also the only detectable IgM anti-HCV response in three plasmapheresis patients with acute HCV infection.

Fig 1. IgG and IgM anti-HCV detected by recombinant core, NS-3, and c100 proteins in sequential bleeds from three representative posttransfusion NANBH patients. Patients no. 6, 11, and 13 from Table 1 are shown in A, B, and C, respectively. These three blood recipients showed markedly different patterns and timing of anti-HCV seroconversion. IgG anti-HCV (open symbols, dashed lines) and IgM anti-HCV (closed symbols, solid lines) were measured as described in the Methods. Days indicate days posttransfusion. Arrow indicates the first observation of ALT elevation. S/CO greater than 1.0, indicated by the horizontal line, are considered reactive.

Fig 2. Serial bleeds from a plasmapheresis donor during the acute phase of HCV infection. In the donor IgM anti-HCV core was detected in one bleed earlier than IgG anti-HCV detection. Days indicate days after entry into the study. Arrow indicates the first observation of ALT elevation. Symbol legend shown in Fig 1A.
Only one posttransfusion patient had detectable IgM anti-
HCV NS-3 during the acute phase and none had detectable
IgM anti-HCV c100. IgM anti-HCV core was the first
marker for active antibody response and seroconversion in
three posttransfusion NANBH patients and in one acute-
phase HCV plasmapheresis donor. However, appearance
of IgM and IgG anti-HCV core usually occurred coinciden-
tially. Data showed that IgM anti-HCV is not always limited
to the acute phase of the disease, since some long-term
chronic patients had protracted periods of IgM anti-HCV
reactivity (Table 1). Similar results have been observed in
chronically infected hepatitis B patients during reactivation
of viral replication and exacerbation of liver disease.10,11

The duration of IgM anti-HCV core detectability was 8.1
weeks on average, but was considerably shorter in some
patients. This window of IgM detectability may be missed in
some acute-phase patients if specimens are not collected at
short intervals.

These data indicate that IgM anti-HCV core is a useful
marker for acute HCV infection in posttransfusion NANBH
and in acute HCV infections in which the mode of transmis-
sion is unknown. However, the IgM response does not
generally precede the IgG response, and thus detection of
IgM is unlikely to narrow the window of seronegative
reactivity that exists between the time of exposure and the
first appearance of antibody.

Further study is required to determine the frequency of
IgM anti-HCV in chronic HCV patients and whether IgM
anti-HCV markers might be a useful measure of response
to antiviral therapy in chronic patients.

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REFERENCES

1. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW,
Houghton M: Isolation of a cDNA clone derived from a blood
borne non-A, non-B viral hepatitis genome. Science 244:359, 1989

2. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG,
Purcell RH, Miyamura T, Dienstag MJ, Alter MJ, Stevens LE,
Testeiner GE, Bonino F, Colombo M, Lee W-S, Kuo C, Berger K,
Shuster JR, Overby L, Bradley DW, Houghton M: An assay for
circulating antibodies to a major etiologic virus of human non-A,

3. Choo Q-L, Richman KH, Han JH, Berger K, Lee C, Dong C,
DW, Kuo G, Houghton M: Genetic organization and diversity of
the hepatitis C virus. Proc Natl Acad Sci USA 88:2451, 1991

4. Vallari DS, Jett BW, Alter HJ, Mimms LT, Shih JW:
Serological markers of post transfusion hepatitis C viral infection. J
Clin Microbiol (in press)

IK, Zeldis J: Specificity of anti-HCV ELISA assessed by reactivity
to three immunodominant HCV regions. Lancet 336:1590, 1990

6. Van der Poel CL, Cuypers HT, Reensnick HW, Weiner AJ,
Quan S, DiNello R, Van Boven JJ, Winkel I, Mulder-Folkerts D,
Exel-Oehler PJ, Schaasberg W, Leent vaar-Kypers A, Polito A,
Houghton M, Lelić PN: Confirmation of hepatitis C virus infection
by new four-antigen recombinant immunoblot assay. Lancet 337:
317, 1991

7. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M,
Choo QL, Kuo G: Detection of antibody to hepatitis C virus in
prospectively followed transfusion recipients with acute and chronic

J, Mattimiro C, Puttermian C, Stalder A, Defreeze J: Enzyme

9. Bolling TJ, Mandeckl W: An Escherichia coli expression
vector for high-level production of heterologous proteins in fusion

10. Davis GL, Hoofnagle JH: Reactivation of chronic type B
hepatitis presenting as acute viral hepatitis. Ann Intern Med
102:762, 1985

H, Furuta S, Kameko M, Kanai M: Significance of IgM antibody to
hepatitis B core antigen for the differential diagnosis of acute and
chronic hepatitis B virus infection and for the evaluation of the
inflammatory activity of type B chronic liver diseases. Gastroen-
terol Jpn 21:601, 1986
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