THE IMPACT OF HCV DIVERSITY ON DIAGNOSIS
TOOLS FOR HCV INFECTION

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HCV COLLABORATIVE TEAM OF RIIPIA
HCV infection is a major health problem worldwide.

- HCV is a hepatotropic RNA virus and it is a major causative agent of human liver disease.
- HCV infects over 170 million people worldwide and causes 476,000 deaths per year.
- Blood borne virus transmitted mainly parenterally.
- Current therapies for HCV infection (combination of pegylated IFN-α and ribavirin) show only limited efficacy and the development of a vaccine remains a major challenge.
HCV is a small enveloped RNA virus that is classified within the flaviviridae family.

Exceptional degree of genetic heterogeneity

High propensity to establish chronic infection
HCV can be classified into six genetically distinct genotypes/clades (Seq. divergence ~30%)

- HCV genotypes can be further subdivided into at least 90 subtypes (Seq. divergence ~20%)

- Different HCV genotypes may exhibit differing phenotypic properties
  - Severity of liver disease
  - Response to antiviral therapy
  - Antigenic heterogeneity/Impact in diagnostic assays and vaccine development

- Functional properties of viral proteins.

Evolutionary tree of known HCV genotypes/subtypes
WORLDWIDE DISTRIBUTION OF HCV GENOTYPES
To assess the impact of the HCV genotypes (particularly HCV-4 and HCV-6) on the detection of anti-HCV antibodies in human sera
EXPERIMENTAL OBJECTIVES

- To produce home-made HCV antigens for core, NS3 and NS4B from HCV isolates of selected HCV genotypes
  - Peptides
  - Recombinant proteins
- To generate a sera panel
- To develop home-made ELISA assays and assess the antibody reactivity in HCV patient sera using type-homologous and type-heterologous antigens
CAMBODIAN PANEL: 90 plasma from Cambodian blood donors

58-Positive [RNA HCV (+) and Ab HCV (+)]
  28 HCV-6
  9  HCV-1a
  20 HCV-1b
  1  HCV-2

3 False Negative [RNA HCV (+) but Ab HCV (-)]

29 HCV-Negatives [RNA HCV (-) and Ab HCV (-)]

2 Controls (-)

1 HBsAg (+) HCV (-)
CORE PEPTIDE ANTIGENS

Peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Core</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV-21</td>
<td>1b</td>
<td>3-75aa</td>
</tr>
<tr>
<td>HCV-26</td>
<td>2a</td>
<td>11-45aa</td>
</tr>
<tr>
<td>HCV-34</td>
<td>1b</td>
<td>11-45aa</td>
</tr>
</tbody>
</table>
PROKARYOTIC EXPRESSION OF THE HCV ∆ CORE AND ∆ CORE+1 PROTEINS TYPE 1a OR 6e

Genotype 1a

171 aa core type 1a control
146 aa core+1 type 6e
146 aa core+1 type 1a
120 aa core type 1a
120 aa core type 6e

Genotype 6e

29
24
20
14,4

Type 6e
HCV-34 (11-45aa/1b)
57/57 +ves (100%)
28/29 -ves (97%)
3/3 false -ves

HCV-26 (11-45aa/2a)
57/57 +ves (100%)
28/29 -ves (97%)
3/3 false -ves

HCV-21 (3-75aa/1b)
55/57 +ves (96%)
16/29 -ves (55%)
3/3 false -ves
CONCLUSIONS

- All three core peptides are immunoreactive with all HCV positive sera

- All three core peptides are immunoreactive with the 3 false-negative sera

- The shorter core peptides (aa 11-45) exhibit better specificity
NS4 PEPTIDE ANTIGENS

Peptides tested:
HCV-6    NS4:1b    1921-1940 aa
HCV-45   NS4:1b    1689-1738 aa
HCV-452  NS4:2a    1688-1740 aa
HCV-645  NS4:1b    1689-1738 aa +
                1921-1940aa
Hydrophobicity profile of NS4B

Prediction of transmembrane domains

- Phob: Probability for an a.a. to be in a transmembrane domain
- Phil: Prediction of transmembrane domains

Residue position

Cytoplasm ER lumen COOH

86%

45%

77%
Detection of antibodies using NS4b peptides

% of positive sera

(1921-1940) 72%
(1689-1738) 67%
(1688-1740) 28%
(1689-1738+1921-1940) 81%

NS4b peptides (a.a.)
Detection of antibodies using NS4b peptides

<table>
<thead>
<tr>
<th>NS4b peptides (a.a.)</th>
<th>Sera type 6</th>
<th>Sera type 1a</th>
<th>Sera type 1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1921-1940)</td>
<td>71%</td>
<td>67%</td>
<td>75%</td>
</tr>
<tr>
<td>(1689-1738)</td>
<td>57%</td>
<td>44%</td>
<td>95%</td>
</tr>
<tr>
<td>(1688-1740)</td>
<td>18%</td>
<td>33%</td>
<td>35%</td>
</tr>
<tr>
<td>(1689-1738+1921-1940)</td>
<td>75%</td>
<td>67%</td>
<td>95%</td>
</tr>
</tbody>
</table>
Detection of antibodies using NS4b peptides

- a.a.1921-1940
- a.a.1689-1738
- a.a.1689-1738 + 1921-1940
- a.a.1688-1740

sera of genotype 6
Detection of antibodies using NS4b peptides

[Graph showing antibody detection results for different genotypes.]

- Red: a.a.1921-1940
- Yellow: a.a.1689-1738
- Light blue: a.a.1689-1738 + 1921-1940
- Green: a.a.1688-1739

Sera of genotypes 1a (29-37), 1b (38-57) or 2 (58)
CONCLUSIONS

- NS4 peptides recognize both HCV-1 and HCV-6 genotypes

- NS4 peptide HCV-645 (aa 1689-1738+1921+1940/1b) has the highest positivity rate (80%)
Implications of HCV diversity in the diagnosis and pathogenesis of virus infection in SE Asia, Eastern Europe and Central Africa (PTR126)