HCV structure

envelope proteins  →  nucleocapsid

E2  E1

Core protein

Enzymes co-factors

viral genome (RNA)

PG, AKI, Zürich 2002
PHYLOGENETIC RELATIONSHIP OF THE FLAVIVIRIDAE FAMILY

Pestiviruses
BVDV
CSFV

Flaviviruses
YFV
JEV
DENV

Hepaciviruses
HCV
1a, 1b, 2a, 2b
3a

GBV-A
GBV-B
GBV-C
HGV
HCV genome

- Single stranded RNA (positive polarity)
- 9600 nucleotides
- open reading frame (C, E1, E2, NS2-5) → encoding for polyprotein
- ev. small open reading frame © → encoding for AFR *
- 5' non-coding region (NCR)
  → containing IRES (internal ribosomal entry site)
  → translation of RNA
- 3' non-coding region (NCR)
  → co-regulates viral replication

* ARF = alternative reading frame protein/frameshift: 160AA

PG, AKI, Zürich 2002
Genetic organisation of HCV

HCV-RNA (genome)

Polyprotein

Structural and non-structural proteins

* Cleavage of the polyprotein by: ▲ endoplasmatic reticulum signal peptidase
  ▲ NS2-3 protease and NS3 protease

RNA-dependent RNA-polymerase
Cleavage products of HCV polyprotein

• Structural proteins

  HCV envelope *
  - composed of 2 glycoproteins E1 (gp31) and E2 (gp70) which associate to noncovalent heterodimers
  - only limited sequences are highly conserved
  - E2 contains 2 hypervariable regions: HVR1 and 2
  - E2 also contains the binding site for CD81
  - little or no surplus production of HCV envelope proteins

  HCV nucleocapsid *
  - core protein (p21)
  - fairly conserved sequences
  - core protein might interact with a variety of cellular proteins
  * processed from the HCV polyprotein by the host’s endoplasmatic reticulum signal peptidase

• non-structural, regulatory proteins

  NS2/3 autoprotease

  NS3 serine protease + NS4A co-factor
  → both proteases process polyprotein (non-structural part)

  RNA helicase (NS3)

  RNA dependent RNA-protease
  → essential for viral replication

  NS5A encoded protein
  → interferon sensitivity
Legend
Life cycle
1. binding of HCV to a cell surface receptor
2. cytoplasmic release and uncoating of the viral RNA genome
3. IRES-mediated translation
4. polyprotein processing by cellular and viral proteases
5. RNA replication
6. packaging and assembly
7. virion maturation
8. release from the host cell

Structures for defense (viral clearance)
a) occupation of receptor leads to signal transduction (anti-viral status)
b) binding of NK cells leads to destruction of infected cell
c) T cell epitopes of HCV presented on the MHC molecules target the infected cells for the attack by HCV-specific cytotoxic T-cells

PG, AKI, Zürich 2002
HCV life cycle

8 Steps

1. Binding of HCV to a cell surface receptor complex
   → internalisation
   • components of surface receptor
     - CD81 protein, a tetraspanin
     - low density lipoprotein receptor
     - other candidates

2. Cytoplasmatic release and uncoding of viral genome
   • interaction of HCV-IRES with 40S ribosomal unit

3. IRES mediated translation
   → polyprotein

4. Processing of polyprotein
   • Host cell proteases → envelope glycoproteins E1, E2, core protein
   • viral proteases → regulatory enzymes/co-factors

5. RNA replication

6. Packaging and assembly

7. Virion maturation

8. Virion release from the host cell
CD81 - a Binding Partner for E2

Kitadokoro K et al. EMBO J 2001;20:12-18.
Interaction of the HCV IRES with the 40S Ribosomal Subunit

Structure of the HCV NS3-4A Complex

Structure of the Hepatitis C Virus NS3 Serine Protease Domain

Structure of the HCV RNA-Dependent RNA Polymerase

**Viral dynamics**

- viral half-life: few hours - 1 day
- average daily production and clearance rate: up to $10^{12}$ copies
- surplus liver cell death/replacement rate: ?

---

**Peripheral viral load**

- measured by RNA/DNA amplifying methods
- results given as HCV-RNA copies/ml
- rough statistics of Zurich untreated patients (more than 10'000 measurements)

<table>
<thead>
<tr>
<th>Viral Load</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>500-1000</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>1000-10,000</td>
<td>5-20%</td>
</tr>
<tr>
<td>$10^5$-$10^6$</td>
<td>60-75%</td>
</tr>
<tr>
<td>$10^7$ and more</td>
<td>&lt; 5%</td>
</tr>
</tbody>
</table>

---

**Total viral mass**

- multiple of viral load?

---

**Viral reservoir**

- hepatocytes, B-lymphocytes, ev. other cells with
  - latent infection?
  - abortive infection?

PG, AKI, Zürich 2002
HCV genotypes and subtypes
(according to the nucleotide sequences of the HCV NS5B region; according to P. Simmonds)
### World wide distribution of HCV genotypes

<table>
<thead>
<tr>
<th>Country</th>
<th>Main genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA and Canada</td>
<td>1a, 1b, 2a, 2b, 3a</td>
</tr>
<tr>
<td><strong>South America</strong></td>
<td>1a, 1b, 2, 3a</td>
</tr>
<tr>
<td><strong>Northern Europe</strong></td>
<td>1a, 1b, 2b, 3a</td>
</tr>
<tr>
<td><strong>Western Europe</strong></td>
<td>1a, 1b, 2a, 2b, 3a</td>
</tr>
<tr>
<td><strong>Southern Europe</strong></td>
<td>1b, 2c (Italien, Span)</td>
</tr>
<tr>
<td><strong>Eastern Europe</strong></td>
<td>1b</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
<td></td>
</tr>
<tr>
<td>- Turkey</td>
<td>1b</td>
</tr>
<tr>
<td>- Middle East</td>
<td>4</td>
</tr>
<tr>
<td><strong>China</strong></td>
<td>1b, 2a, 2b</td>
</tr>
<tr>
<td><strong>Africa</strong></td>
<td></td>
</tr>
<tr>
<td>- parts Northern Central Africa</td>
<td>4</td>
</tr>
<tr>
<td>- Egypt</td>
<td>4a</td>
</tr>
<tr>
<td>- South Africa</td>
<td>1, 2, 3, 5a</td>
</tr>
<tr>
<td><strong>Pacific</strong></td>
<td></td>
</tr>
<tr>
<td>- Australia</td>
<td>1a, 1b, 2a, 2b, 3a.</td>
</tr>
<tr>
<td>- Taiwan</td>
<td>1b, 2a, 2b</td>
</tr>
<tr>
<td>- Japan</td>
<td>1a, 2a, 2b</td>
</tr>
<tr>
<td>- Hong Kong</td>
<td>6a, 1b, 2a, 2b</td>
</tr>
<tr>
<td>- Thailand</td>
<td>1b, 2, 3, 6</td>
</tr>
<tr>
<td>- Malaysia</td>
<td>1b, 2, 3</td>
</tr>
<tr>
<td>- Vietnam</td>
<td>1b, 2, 6</td>
</tr>
</tbody>
</table>

According P. Simmonds, P. Marcellin

---

PG, AKI, Zürich 2002
### Distribution of HCV genotypes in Switzerland

<table>
<thead>
<tr>
<th>HCV genotypes</th>
<th>Zürich 1)</th>
<th>Geneva 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>172 (51.9%) 3)</td>
<td>185 (52.9%)</td>
</tr>
<tr>
<td>2</td>
<td>35 (10.6%) 4)</td>
<td>35 (10.0%)</td>
</tr>
<tr>
<td>3</td>
<td>100 (30.2%)</td>
<td>92 (26.3%)</td>
</tr>
<tr>
<td>4</td>
<td>22 (6.7%)</td>
<td>34 (9.7%)</td>
</tr>
<tr>
<td>5</td>
<td>1 (0.3%)</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>6</td>
<td>1 (0.3%)</td>
<td>0</td>
</tr>
<tr>
<td>mixed types</td>
<td>0</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>331</td>
<td>350</td>
</tr>
</tbody>
</table>

1) Tested by the Clinical immunology, University Hospital Zürich between Aug. 99 and Jan. 2000 using the “line-probe assay” [INNO-LiPA, Innogenetics, Ghent, Belgium]

2) Tested by the Gastroenterology and Hepatology, University Geneva between June 98 and Jan. 2000 using “restriction fragment length polymorphism”

3) Subtypes 1a: 64, 1b: 98, other subtypes 1: 10

4) Subtypes 2a/2c: 29, other subtypes 2: 6
Co-infections with multiple HCV-geno-/subtypes

• most infected individuals: 1 geno-/subtype
• < 1-3% 2 or more geno-/subtypes

in multiple infections
• 1 geno-/subtype often prevails
  - genotype 1 over the others
  - subtype 1a over 1b

• all infected individuals develop quasi-species
Measurable markers for HCV infection

- **anti-HCV** (against biogenetically produced antigens)
  - screening test formats
  - confirmatory test formats
  - No distinction between ongoing and past infection
  - Anti-HCV might disappear decades after end of infection (under estimation of HCV prevalence)
  - Immuno-compromised individuals with ongoing HCV infection might have no anti-HCV

- **HCV-RNA** (measured by RNA/DNA amplifying methods)
  - commercial tests available with lowest detection limits
    - 600 copies/ml (quantitative test format)
    - 50 copies/ml (qualitative test format)
  - Low-grade HCV infection is not detectable

- **HCV core antigen**

- **HCV components in cryoglobulins**

- **Autoantibodies** (against cell nuclei, mitochondria etc)
Natural disease course of HCV infection

Initial infection:
- Asymptomatic: ca. 60% (50-80%)
  - Spontaneous resolution within 6-12 months: ca. 30% (10-40%)
- Anicteric hepatitis: ca. 10-20%
  - Spontaneous resolution within 6-12 months: ca. 30% (10-40%)
- Icteric hepatitis: ca. 10-20% (5-50%)
  - Spontaneous resolution within 6-12 months: ca. 30% (10-40%)
- Fulminant hepatitis: ca. 1%
  - Spontaneous resolution within 6-12 months: ca. 30% (10-40%)

Chronic infection:
- Chronic hepatitis minimal/mild: ca. 30-50%* (spontaneous resolution frequent)
- Chronic hepatitis intermediate/severe: ca. 20-50%* (spontaneous resolution possible)
  - Cirrhosis (irreversible): ca. 10-20% (5-40%)*
  - Hepatocellular carcinoma: ca. 5-10% (2-20%)*

* of those individuals with chronic infection

PG, AKI, Zürich 2002
Infection course and pathogenic mechanisms

- The course of infection and the eventual clinical sequelae are very variable, the clinical sequelae being:
  - surplus liver cell replacement/turnover *
  - liver inflammation *
  - liver fibrosis *, liver cirrhosis *, HCC
  - extrahepatic manifestations *: cryoglobulinopathy/vasculitis

* These events do often take an independent course. The individual course of infection is not predictable.

- Also the crucial events of infection must have complex self-tuning and interaction mechanisms, the crucial events being:
  - viral replication, viral clearance

- HCV is not essentially cytopathogenic. Immune reactions are thought to be essential for:
  - viral elimination
  - pathogenic events leading to the clinical sequelae

- The essential immune reactions are:
  - HCV-specific reactions:
    - cytotoxic T lymphocytes, T1 and T2 helper lymphocytes (cytokine release),
    - B lymphocytes/plasma cells producing anti-HCV
  - non-specific reactions (co-activated lymphocytes, macrophages, other inflammatory cells) leading to a surplus production of cytokines

- The immune reactions leading to the pathogenic events seem antigen/HCV driven.

PG, AKI, Zürich 2002
HCV and speculative defense mechanisms

- HCV-infected hepatocytes
- Viral replication
- Viral clearance
- Cell excess turnover
- NK-cells (innate immunity)
- HCV-specific response (adoptive immunity)
  - HCV-spec. cytotoxic T-lymphocytes (MHC class I restricted)
  - HCV-spec. T1 and T2 helper lymphocytes (MHC class II restricted), cytokine release
  - Co-activated, non-spec. T-lymphocytes, macrophages, other inflammatory cells, surplus cytokine release
- Cytokines e.g. IFN
- Liver inflammation
- Extrahepatic manifestations
- Fibrosis
- Cirrhosis
- HCC

○ = associated with destruction of infected hepatocytes
R's = receptors for natural killer cells (NK) and interferons/other cytokines as well as MHC class I molecules presenting HCV epitopes

PG, AKI, Zürich 2002
## Factors decisive for the outcome of a HCV-infection

<table>
<thead>
<tr>
<th>Influencing factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>virus dependent</td>
<td>• infection dose</td>
</tr>
<tr>
<td></td>
<td>• HCV replication rate</td>
</tr>
<tr>
<td></td>
<td>• “aggressivity” of HCV</td>
</tr>
<tr>
<td></td>
<td>• escape mutations (e.g. quasi-species)</td>
</tr>
<tr>
<td></td>
<td>• viral reservoir (abortive and latent infections)</td>
</tr>
<tr>
<td></td>
<td>• resistance to anti-virals</td>
</tr>
<tr>
<td>host dependent</td>
<td>• innate immunity (natural killer cells, complement [alternative pathway]) etc</td>
</tr>
<tr>
<td></td>
<td>• specific immunity to HCV (antigen presentation on MHC class I and II molecules, HCV-specific cytotoxic and helper [type 1 and 2] T-cells, B-lymphocytes [antibody formation], cytokine production)</td>
</tr>
<tr>
<td></td>
<td>• non-specific immune response (co-activated T and B cells, macrophages, dendritic cells, surplus cytokine release etc)</td>
</tr>
<tr>
<td></td>
<td>• genetics (e.g. MHC class I, II dependent) at various levels</td>
</tr>
<tr>
<td></td>
<td>• sex, age at infection</td>
</tr>
<tr>
<td></td>
<td>• risk behaviour e.g. alcohol intake</td>
</tr>
<tr>
<td></td>
<td>• viral co-infections e.g. with HIV, HBV, GBV-C/HGV, HAV</td>
</tr>
<tr>
<td>environmental</td>
<td>• nutritive etc</td>
</tr>
</tbody>
</table>
HCV - Superinfection with HAV

Patients with chronic HCV infection, superinfected with HAV → increased risk of fulminant hepatitis

• negative reports: Leino et al. 1997, Battegay et al. 1998
  Helbling et al. 1998, Mele et al. 1998

• confirmation: Pramoolsinsap et al. 1999 (Thailand)
HCV - Co-infection with HBV

• Fulminant hepatitis: HBV-related fulminant hepatitis; HCV co-infection might often be implicated (Feray et al. 1993)

• Chronic co-infections HBV/HCV:
  - **viral level:**
    - HBsAg is lost → „anti-HBc alone“ (HBV-DNA pos.: 2-80%) (Jilg et al. 1995, Grob et al. 2000)
    - HBsAg and anti-HBc are lost (HBV-DNA pos.) = occult HBV-infection (Cacciola et al. 1999)
    - HBV-DNA and HCV-RNA levels are lower in single than in double infections (Jardi et al. 2001)

  - **clinical level:**
    Patients with double infections
    - more aggressive liver disease
    - HCC is more frequent
    - less response to therapy (Brechot et al. 1998, Chiaramonte et al. 1999, Tagger et al. 1999)
**HCV and HBV**

Co-infections with HBV of patients with chronic HCV-infection might be underestimated (Cacciola 1999).

200 patients with chronic HCV-infection  
- HBsAg neg.  
→ 100 patients with „anti-HBc alone“  → 46 (46%) HBV-DNA pos.  
→ 100 patients without any HBV markers  → 20 (20%) HBV-DNA pos.

Total: 200 patients  → 66 (33%) HBV-DNA pos.

<table>
<thead>
<tr>
<th>HCV-RNA</th>
<th>HBV-DNA</th>
<th>n</th>
<th>Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>66</td>
<td>22 (33%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>134</td>
<td>26 (20%)</td>
</tr>
</tbody>
</table>

PG, AKI, Zürich 2002
HCV and HIV

• Simultaneous infection with HCV and HIV
  (Eyster et al. 1993; 223 hemophiliacs, Yee et al. 2000; 310 hemophiliacs, Garcia-Samaniego 1997, Soto 1997)
  - accelerates the progression of liver disease including HCC
  - liver disease develops earlier
  - liver related death is more frequent

• Simultaneous infection with HCV and HIV (and low CD4 counts)
  (Di Martino et al. 2001)
  - worsened outcome of liver damage
  - HCC occurs earlier
  - increased level of HCV-RNA
  - decreased response to interferon therapy
GBV-C/HGV and HIV infection


197 HIV-infected patients
- 33 (16,8%) GBV-C/HGV-RNA pos.
- 112 (56,9%) anti-E2 pos.
- 52 (26,4%) no markers


362 HIV-infected patients
- 144 (39,8%) GBV-C/HGV-RNA pos.

41/144 (28,5%) GBV-C/HGV-RNA pos. patients died
123/218 (56,4%) GBV-C/HGV-RNA neg. patients died

Main conclusions of both papers
GBV-C/HGV-infection of HIV-infected individuals results in:
- reduced mortality
- slower progression to AIDS
- longer survival with AIDS
- lower viral load, higher CD4 lymphocytes

Independent of age, sex, risks, and concentrations of CD4 lymphocytes

• Data remained controversial. Very preliminary results: HCV infection is mandatory

PG, AKI, Zürich 2002
Experimental systems

• Chimpanzees, only animal susceptible to HCV infection. Limitations/protection
• Newer test systems

  - HCV-infection in immunodeficient mize reconstituted with human hepatocytes (Lechner 2000)

  - Replicon system (Blight 2000, Lohmann 1999)
    In vitro transcribed HCV-RNA „plasmid“ constructs containing IRES is transfected into HuH-7 human hepatoma cells. Clones with replicating subgenomic HCV-RNA are then selected.
Future therapeutics and vaccines

• **Therapeutics**: e.g. phase I and phase II clinical trials with inhibitors of
  - NS3 serine protease
  - HCV RNA helicase
  - HCV RNA-dependent polimerase

• New vaccines
  - peptide and protein vaccines
  - dentritic cell based vaccines
  - vaccines with virus-like particles
  - DNA vaccines

A phase II clinical trial (therapeutic vaccination) is currently ongoing with a HCV E1 recombinant vaccine