Replication Defective Enterovirus Infections: Implications for Type I Diabetes

N. M. Chapman
Department of Pathology & Microbiology
University of Nebraska Medical Center
Enterovirus Genome and Capsid

(Source: ViralZone:www.expasy.org/viralzone, Swiss Institute of Bioinformatics)
Coxsackie B viruses (CVB) are typical human enteroviruses (HEV)

- 4 Human enteroviruses (HEV) species
- More than 100 characterized (acknowledged or identified) HEV serotypes
  - this number goes up much higher when including the human rhinoviruses
- HEV circulate worldwide
  - spread most commonly by a fecal-oral route of transmission
  - aerosol less commonly (although for human rhinoviruses, aerosol is the primary route)
- With increasing hygienic standards, HEV infections are not as frequent as they once were from birth onwards
CVB infections cause...

- myocarditis (in up to 1% of hearts in autopsy studies)
- cardiomyopathy (5.5 cases/100,000 population/year)
- pericarditis
- endocarditis
- pancreatitis
- aseptic meningitis
- fulminant/often deadly neonatal/pediatric infections
- polymyositis
- a trigger of type 1 diabetes
T1D and the HEV connection

• Does such a connection exist?
  – The link is not as firm as in other HEV diseases
    • poliovirus and poliomyelitis
    • CVB and myocarditis or aseptic meningitis
    • rhinoviruses and the common cold

• Nonetheless, data support an HEV etiology in at least some, if not many, cases of human T1D
  – Some cases in which CVB have been isolated at or shortly after T1D onset
  – Other non-CVB HEV (all HEV-B, echovirus types 1,3,4,6,9,30) have been isolated in connection with T1D cases
  – Immunohistochemical evidence of HEV protein in islets
  – Experimental rodent models of virus-induced T1D
  – Ability to infect isolated human or murine islets in culture with HEV
CVB infection of young NOD mice without insulitis reduces T1D incidence relative to mock-infected control mice

Tracy et al., 2002 J. Virol 76:12097
In older mice with existing and increasing insulitis, three basic outcomes can be observed:

- accelerated T1D onset
- or -
- no change from uninfected
- or -
- slowed onset

From Drescher 2004 Virology 329: 384
Enteroviral myocarditis provides a model of how enterovirus persistence plays a role in human disease.

From Kawai C. Circulation. 1999 99:1091-100.
Enterovirus RNA can persist in human heart in the apparent absence of cytopathic or infectious virus

- Enterovirus RNA is detected in about 20-25% of cases of human myocarditis and dilated cardiomyopathy

- It is extremely rare to isolate infectious virus from adult hearts (defined as the ability to lyse cells in culture)


  ... Of the 55 biopsy specimens aseptically collected from the explanted hearts of 55 patients, 21 (38.2%) were positive by RT-PCR microplate assay whereas only 19 (34.5%) were positive by nested RT-PCR assay and none were positive by classical cell culture assays. No enterovirus was detectable by RT-PCR or classical cell culture assays in any of the 55 heart biopsy specimens taken from organ donors without any known heart disease. ...
Expression of coxsackievirus proteins alters cardiomyocyte function, but how can function of the cardiomyocyte be altered by a lytic virus infection?

- Enteroviruses are “hit and run” viruses which lyse the cells they infect (within 8-10 hours in cell culture).
- Normally enterovirus infections are cleared by the generation of enterovirus-specific antibodies.
- How can a cardiomyocyte infected with an enterovirus survive long enough to have effects upon the cytoskeleton?
Enterovirus Replication
Coxsackievirus B3 (CVB3) persists in myocarditis-susceptible mice without cytopathic virus

Homogenize heart, freeze-thaw, clear debris, filter, inoculate cell culture

<table>
<thead>
<tr>
<th>Day p.i.</th>
<th>4</th>
<th>8</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPE</td>
<td>3/3</td>
<td>2/2</td>
<td>2/5</td>
<td>1/3</td>
<td>0/5</td>
<td>0/9</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>3/3</td>
<td>2/2</td>
<td>5/5</td>
<td>3/3</td>
<td>5/5</td>
<td>1/9</td>
</tr>
</tbody>
</table>

From Kim 2005
J Virol 79: 7024
A cardiovirulent CVB3 population evolves to a 5’ deleted virus population in the mouse heart.

Within just a few days, a viral population could develop that appears to completely lack the 5’ end.

Sequence analysis of cloned ends demonstrates 5' terminal deletions

CVB3/28

UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGGCCCAUUGGGCGCUAGCACUCU-

TD8

AGCCUGUGGGUUGAUCCCACCCACAGGGCCCAUUGGGCGCUAGCACUCU-

TD13

GUGGGUUGAUCCCACCCACAGGGCCCAUUGGGCGCUAGCACUCU-

TD18

UUGAUCACCAUAGGGCCCAUUGGGCGCUAGCACUCU-

TD31

UAGGGCCAUUGGGCGCUAGCACUCU-

TD50

GCACUCU-
Coxsackievirus B2 from human myocarditic heart has deleted 5’ terminus

A heart from a fatal case of adult fulminant myocarditis in Japan shows not only in an HEV present but that it lacks the 5’ terminal sequences, as seen in cell culture and in mouse myocarditis models.

2,563 nt of the heart virus sequence were determined from 21 cDNAs generated from RNA purified from formalin-fixed tissue. Primers were designed to amplify conserved CVB or HEV-B sequences; to proceed into the genome, primers were then designed based upon sequence from the heart virus genome.

Chapman 2008 Virol 375: 480
The CVB3 domain I (cloverleaf) in the 5’ NTR. CVB3-TD genomes from heart have been characterized with deletions from 7 to 49 nt (lines show 5’ ends of characterized TD genomes).
CVB3/TD strains encapsidate negative strand RNA

RT-PCR using strand-selected RNA from CsCl purified virions. Negative strand RNA is not detectable at about 1 part in 100, nor in wildtype virus, but it is readily detectable in TD virion RNA.

From Kim 2005 J Virol 79: 7024
CVB3/TD strains produce viral proteins in cell culture replication

Expression of CVB3 proteins in heart cells alters function and causes heart remodeling: persisting enteroviruses express all the viral proteins.

From Kim 2005 J Virol 79: 7024
CVB3/TD produce encapsidated virus: neutralized by anti-CVB3 polyclonal serum

As TD virus makes infectious virus particles further infection of cells can continue

From Kim 2005 J Virol 79: 7024
TD are selected in primary pancreatic cell passaged CVB3-28

By pass 3, TDs have replaced wt

RT-PCR of strand selected RNA from CsCl purified virus

By pass 2, negative strand RNA is detectable in purified virions
How terminal deletions occur...the short version

- Virus infects a quiescent cell which lacks a host cell factor important for positive strand replication in the cytoplasm.
- Positive strand priming sites within domain I are apparently random in this situation.
- If priming occurs between the terminus and stemloop d, the RNA is a TD and it is viable.
- Multiple passages in quiescent cell culture are required to generate a purely TD population, suggesting that a mixed population of TD and wt is generated in these cells.
Summary

- After an acute infection with enteroviruses, some infected cells have selection of a defective virus.
- These viruses replicate at a level too low for detection by cytopathic effect but can be detected by sensitive RT-PCR and immunohistochemistry.
- These viruses produce nearly equal levels of positive strand and negative strand RNA.
- These viruses persist at a stage at which levels of neutralizing antibody should clear enterovirus infection, presumably due to slow replication producing long term intracellular states.
- In the mouse model of CVB3 infection, CVB3 RNA persists in the pancreas at this stage.
Implications

• Defective enterovirus from past infection of the pancreas may persist well after the adaptive immune response to the virus.

• The viral RNA and protein from that persisting virus may be at reduced levels PER CELL compared to levels found with wild type virus.

• Virus may persist in this form at sites other than the pancreas.
Collaborators in this work
Steven Tracy, Ph.D.
Kristen Drescher, Ph.D.
Kyung-Soo Kim, Ph.D.