Increased Usage of a Public CD8 T Cell Clonotype in Spontaneously Resolved HCV Infection

Sabrina Mazouz, Maude Boisvert, Julie Bruneau, Naglaa H. Shoukry
CRCHUM, Montréal

HCV-specific CD8+ T Lymphocyte

• Polyfunctionality
• Cytotoxicity

(Gruner NH et al. J.Infect Dis. 2000)
CD8+ T Cell Receptor (TCR)

αCD8β

(Vγ, Dγ1, Jγ1, Cγ1, Dγ2, Jγ2, Cγ2)

βVβ-N-Dβ-N-Jβ-Cβ

CDR1 CDR2 CDR3

TCR repertoire parameters

- **Diversity**: The number and abundance of each unique TCR sequence (i.e. clonotype) forming the repertoire.
  
  - **Pauciclonal / Narrow repertoire**: Limited number of TCR clonotypes.
  
  - **Broad repertoire**: Large number of TCR clonotypes.

- **Public clonotype**: Found among several persons.

- **Private clonotype**: Unique to an individual.

A focused repertoire is associated with spontaneous resolution during HCV reinfection

Public repertoire associated with a better control of HCV?

Hypothesis

Spontaneous resolution will be associated with a narrow TCR repertoire comprised of highly functional clonotypes.

Public clonotypes will be preferentially expanded in SR as compared to CI.

Sub-aims

To compare the HCV-specific (HLA-A2 restricted NS3-1073 epitope) CD8+ T cell repertoire in spontaneous resolvers and chronically infected individuals to evaluate:

1) TCR diversity

2) The presence of public versus private clonotypes associated with spontaneous clearance.
Montréal Hepatitis C Cohort (HEPCO)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at infection Mean (range) (Years)</th>
<th>Gender (M/F)</th>
<th>HCV Genotype 1/3/NA</th>
<th>Time points analyzed (≤ 6 months / &gt; 6 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolver (n=9)</td>
<td>35 (21-45)</td>
<td>6/3</td>
<td>6/0/3</td>
<td>5 / 4</td>
</tr>
<tr>
<td>Chronic (n=9)</td>
<td>34 (19-48)</td>
<td>7/2</td>
<td>8/1/0</td>
<td>9 / 0</td>
</tr>
</tbody>
</table>

ND: Not Done; NA: Not Available

Experimental design

Plasma

Autologous NS3 1073 epitope sequences
To compare the HCV-specific (HLA-A2 restricted NS3-1073 epitope) CD8+ T cell repertoire in **spontaneous resolvers** and **chronically infected** individuals to evaluate:

1) TCR diversity

2) The presence of public versus private clonotypes associated with spontaneous clearance

**Comparable TCR repertoire diversity between resolvers and chronics**

**Simpson diversity**: The index takes into account the number and frequency of each clonotype

0: High diversity (broad repertoire)
1: No diversity (narrow repertoire)
Comparable TCR repertoire diversity between resolvers and chronics

Shannon entropy: The index takes into account the number of different clonotypes as well as their relative proportion.

0: No diversity (narrow repertoire)
1: High diversity (broad repertoire)

In both groups:
- Broad repertoire
- Comparable TCR repertoire diversity
Sub-aims

To compare the HCV-specific (HLA-A2 restricted NS3-1073 epitope) CD8+ T cell repertoire in spontaneous resolvers and chronically infected individuals to evaluate:

1) TCR diversity

2) The presence of public versus private clonotypes associated with spontaneous clearance
Increased usage of the unique public TCR clonotype

![TCR clonotype diagram](image)

Patient I.D. | Time Point | Genotype | Public clonotype (%) | NS3 1073 Epitope
--- | --- | --- | --- | ---
SR 6 | 347 | 1 | 0.21 | CINGVCWTV (H77/1a) (4/4)
SR 7 | 29 | 1a | 0.63 | (1/6) (5/6)
SR 10 | 168 | 1a | 2.65 | (4/5) (1/5)
CI 1 | 77 | 1a | 0.77 | (8/8)
CI 3 | 68 | 1a | 1.65 | (8/8)
CI 4 | 91 | 1a | 0.68 | (6/6)
CI 8 | 83 | 1a | 0.65 | (5/5)

Autologous epitope sequences in resolvers and chronics
Repertoire evolution of TCRBV gene usage among dominant clonotypes

Acute Phase (<6 months)
- SR-1: 9.7%
- SR-7: 10%
- SR-9: 29.6%
- SR-10: 47.7%

Follow-up (~18 months)
- SR-1: 17.6%
- SR-7: 13.7%
- SR-9: 18.8%
- SR-10: 64.7%

Persistence of the unique public clonotype after HCV spontaneous resolution

CASSQEPGAPNTGELFF

TCRBV04-02
TCRBJ02-02

Public clonotype % of repertoire

Early acute Follow-up
Conclusions

• The TCR repertoire diversity does not distinguish SR/CI

• Higher frequency of a unique public clonotype correlates with SR and is not associated with a specific variant of this epitope

• This unique public clonotype is preserved within the memory pool after resolution of the infection

Future directions

• Characterize the functional avidity and polyfunctionnality of this public clonotype

• Correlate the expansion of this public clonotype with an efficient vaccine strategy
Acknowledgements

**Laboratory**
- Dr. Naglaa H. Shoukry
- Nathalie Bédard
- Sarah Tran
- Dr. Maude Boisvert
- Thomas Fabre
- Manuel Flores
- Mohamed Abdelnabi
- Nicolas Tremblay

**Flow cytometry platform**
- Dominique Gauchat
- Annie Gosselin

**University of Pennsylvania**
- Dr. Mohammed Abdel-Hakeem

**Clinical collaborators**
- Dr. Julie Bruneau
- Cohorte Montreal HEPCO
- Montreal HepCo cohort and the participants

**Funding:**

- Réseau SIDA du PRPQ
- CIHR
- IRSC
- CanHepC

**Unique public TCR clonotype among dominant clonotypes**

<table>
<thead>
<tr>
<th>CDR3β region</th>
<th>Patients</th>
<th>Frequency</th>
<th>nt additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGT GCC AGC AGC CAA GAG CCT GGG GCT CCC AAC ACC GGG GAG CTG TTT TTT</td>
<td>SR-2</td>
<td>1.88</td>
<td>8</td>
</tr>
<tr>
<td>TGT GCC AGC AGC CAA GAG GCA GGG GCC CCC AAC ACC GGG GAG CTG TTT TTT</td>
<td>SR-1</td>
<td>1.70</td>
<td>7</td>
</tr>
<tr>
<td>TGT GCC AGC AGC CAA GAG GCA GGG GCC CCC AAG ACC GGG GAG CTG TTT TTT</td>
<td>SR-2</td>
<td>1.72</td>
<td>5</td>
</tr>
<tr>
<td>TGT GCC AGC AGC CAA GAG GCA GGG GCC CCC AAG ACC GGG GAG CTG TTT TTT</td>
<td>SR-8</td>
<td>1.85</td>
<td>5</td>
</tr>
</tbody>
</table>