CASE STUDY: JANUARY 2013

High frequency of herpesvirus-specific clonotypes in the human T-cell repertoire can remain stable over decades with minimal turnover

BACKGROUND
• Select T-cell clonotypes emerge to engage infectious agents. Often these T-cell expansions are further amplified by persistent pathogens that provide a constant stream of antigen to the cellular compartment
• The effect of long-term persistent infection on the T-cell repertoire is not well defined

AIM
To profile herpes-specific clonotypes from peripheral blood mononuclear cells (PBMCs) over time

METHODS
Cryobanked PBMCs from two healthy individuals were obtained. Sample collection time points included the years 1993, 1997, 2008, and 2011 for each individual

For each time point:
Cryobanked PBMCs → Isolation of CD8+ T cells via fluorescence-activated cell sorting (FACS) → gDNA extraction → immunoSEQ™

RESULTS
• The 40 most frequent T-cell receptor beta (TCRB) chain sequences from 2011 were observed in 2008, while 37 and 32 of the sequences were observed in 1997 and 1993, respectively
• TCRB-chain sequences and longitudinal analysis of Epstein-Barr virus (EBV)-specific clonotypes within the CD8 compartment of one individual were investigated:

<table>
<thead>
<tr>
<th>CDR3 sequence</th>
<th>TRBV gene(s)</th>
<th>TRBJ gene</th>
<th>1993</th>
<th>1997</th>
<th>2008</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAGSGGVPMTGEYFF</td>
<td>29-1</td>
<td>2-2</td>
<td>1,446</td>
<td>614</td>
<td>ND</td>
<td>29,800</td>
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<tr>
<td>CASSLMEYQFF</td>
<td>28</td>
<td>2-5</td>
<td>44,058</td>
<td>33,700</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>CASPPGFPPOYF</td>
<td>12-3/12-4</td>
<td>1-5</td>
<td>15,106</td>
<td>17,427</td>
<td>12,318</td>
<td>10,528</td>
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<tr>
<td>CSASGDIFEOYF</td>
<td>20-1</td>
<td>2-7</td>
<td>ND</td>
<td>ND</td>
<td>11,049</td>
<td>1,383</td>
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<tr>
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<td>2-7</td>
<td>ND</td>
<td>ND</td>
<td>4,011</td>
<td>ND</td>
</tr>
<tr>
<td>CASSYDGSFGYEQF</td>
<td>20-1</td>
<td>2-7</td>
<td>ND</td>
<td>ND</td>
<td>28,921</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Frequency rank of beta-chain sequence among all sequences identified within the CD8+ subset using high-throughput sequencing. ND, not determined.

• 13 EBV-specific beta-chains that corresponded to sequences from the immunoSEQ analysis of the total CD8 subset were identified
• These included three highly prevalent beta-chains that were within the 40 most frequently observed sequences from the 1993 or 2011 immunoSEQ data

CONCLUSIONS
• High-frequency T-cell clonotypes, including herpes-specific T-cell clonotypes, can remain stable for up to 18 years, with minimal inflation, deflation, or turnover
• In spite of exposure to a barrage of micro-organisms over the course of life, the dominant clonotypes in the mature peripheral T-cell repertoire can remain surprisingly stable

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