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Viral evolution in infant HIV infection

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Perinatal HIV infection

- High mortality, fast disease progression
- High peak VL, slow decline over years
- High CD4 counts in infancy, modest decline with HIV

Possible causes of poor viral control:
- Abundance of HIV susceptible cells
- HLA concordance with mother who is transmitting virus
- Deficiencies in HIV specific T cell responses
Parent Study Cohort

- Study on infant T cell responses and prevention of MTCT
- Nairobi, Kenya. 1999-2002
- HIV+ve women recruited during pregnancy
- Received AZT from 34-36 weeks until delivery
- Follow-up with infants until 2 years

- MTCT rate 18%
- Infant mortality 52% at 2yr
- HIV specific T cell responses measured in infants
  - No relationship between magnitude or breadth of T-cell responses and clinical parameters - are these T-cells fully functional?

John-Stewart et al, JID 2009
Current project aims and methods

• Assess timing and strength of cellular immune response in early infancy by looking at virus evolution

• Determining when T cells become functional in infancy will inform timing of vaccines or immunotherapies, e.g. “cure” strategies

• Infants infected close to birth chosen, based on availability of stored plasma samples from several timepoints

• Viral clonal sequences of \textit{gag} and \textit{nef} generated using molecular cloning
### Sequences generated

<table>
<thead>
<tr>
<th></th>
<th>No of infants</th>
<th>Total no of sequences</th>
<th>Mean no of sequences per timepoint</th>
<th>Mean no of timepoints per infant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nef sequences</strong></td>
<td>13</td>
<td>1275</td>
<td>22.8 (13-24)</td>
<td>3.9</td>
</tr>
<tr>
<td><strong>Gag sequences</strong></td>
<td>13</td>
<td>1210</td>
<td>21.6 (10-24)</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>14</td>
<td>2485</td>
<td>22.2</td>
<td>3.7</td>
</tr>
</tbody>
</table>

### Clinical data

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td><strong>Peak viral load</strong></td>
<td>2.4E+07</td>
<td>8.6E+05 – 1.4E+08</td>
</tr>
<tr>
<td><strong>CD4 %</strong></td>
<td>21%</td>
<td>7-35%</td>
</tr>
<tr>
<td><strong>Peripartum transmission</strong></td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td><strong>Mortality at 2yrs</strong></td>
<td>30%</td>
<td></td>
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</table>

- Phylogenetic analysis of viral sequences taken longitudinally allow study of viral evolution
  - Determine selection pressures acting on virus
Positive selection pressure found at some codon sites in *gag* and *nef*

- Ratio of non-synonymous vs synonymous substitution rate (dN/dS) measures selection pressure
  - dN/dS ratio =1: no selection pressure
  - dN/dS ratio <1: negative selection
  - dN/dS ratio >1: positive selection
- Most codons in *gag* and *nef* were under negative or neutral selection
- Few codons under positive selection
  - could indicate immune pressure acting at that site
  - sites differed between infants
  - 1/13 infant’s *nef* and 1/13 infant’s *gag* had no codons under positive selection
Sites under positive selection matched to known T cell epitopes

- Sites under most positive selection in *gag* and *nef* in each infant matched to known T cell epitopes
- In most infants at least one position under positive selection could be matched to known epitopes restricted by that infant’s HLA type
- Those positions studied further by determining what and when amino acid change occurs
- Changes consistent with T cell immune pressure first become evident by 3 to 12 months of age

<table>
<thead>
<tr>
<th>ID</th>
<th>Infant HLA</th>
<th>HXB2</th>
<th>Study HLA</th>
<th>Infant sequence</th>
<th>aa change</th>
<th>1mth</th>
<th>3mth</th>
<th>6mth</th>
<th>9mth</th>
<th>12mth</th>
<th>15mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>B231</td>
<td></td>
<td></td>
<td>VPIRPMTY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>26 35 4</td>
<td>53 4</td>
<td></td>
<td>VPLRPMTY</td>
<td>Y</td>
<td>100%</td>
<td>21%</td>
<td>0%</td>
<td>25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>34 35 4</td>
<td></td>
<td></td>
<td>VPIRPMTf</td>
<td>F</td>
<td>0%</td>
<td>46%</td>
<td>61%</td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>34 53 4</td>
<td></td>
<td></td>
<td>VPIRPMTf</td>
<td>H</td>
<td>0%</td>
<td>33%</td>
<td>39%</td>
<td>65%</td>
<td></td>
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</tr>
</tbody>
</table>
Conclusion

• Evidence of positive selection on virus at some codon sites in both gag and nef genes in most infants

• Many sites under most significant positive selection could be matched to known T cell epitopes – immune driven selection

• Evidence of escape mutations in these epitopes evident from as early as three months of age in a small number of infants

• Data suggests an effective T cell response against HIV can be generated from early infancy

• Encouraging for prospects of immune-based therapies for paediatric HIV infection
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