NK mediated Antibody Dependent Cellular Cytotoxicity in HIV infections

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2. Antibodies of IgG isotype to target antigen
3. Effector cells (NK cells) with FcγR (gamma receptor)

• Collaboration between innate (NK cells) and adaptive (Ab) immune response
• NK cells kill cells in the absence of MHC while having high specificity mediated by Antibodies
Importance of ADCC against HIV

- **HIV Human Cohort studies:**
  - Low ADCC titres correlate with progression and CD4+ cell decline \( \text{Baum 1996, Forthal 2001,} \)

- **Macaque Cohort Studies:**
  - Animals with higher ADCC responses progress more slowly \( \text{Banks 2002, Ohkawa 1994} \)

- **Macaque Vaccine Studies:**
  - Vaccine elicited ADCC activity correlates with reduced viral load \( \text{Gomez-Roman 2005, Florese 2006,} \)

- **Monkey Passive Transfer Study:**
  - Abrogating Fc binding substantially reduces efficacy of Nab \( \text{Hessell 2007} \)
Novel ICS Assay to detect ADCC

HIV positive human whole blood +
gp140 protein or overlapping HIV peptide pools
(15 aa overlapping by 11 aa)

Brefeldin A
Incubate 37º 5% CO₂

Stain for CD2, CD56, CD3

Stain for intracellular IFNγ

Acquire cells on FACS

gp140 or HIV Peptide pools:
15aa overlapping by 11aa

HIV +ve whole blood

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HIV +ve whole blood
IgG mediates NK cell activation in ICS Assay to detect ADCC

- HIV serum mediated
- Specifically IgG mediated
- Not complement mediated
- Does not require co-stimulation
- Readily maps linear ADCC epitopes
- Can phenotype response
- Measures multiple effector functions

Gated on CD3- lymphocytes

Phenotype of NK effector cells in ADCC ICS Assay

<table>
<thead>
<tr>
<th>Cell Surface Marker</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>–</td>
</tr>
<tr>
<td>CD4</td>
<td>–</td>
</tr>
<tr>
<td>CD8</td>
<td>–</td>
</tr>
<tr>
<td>TCRαβ</td>
<td>–</td>
</tr>
<tr>
<td>TCRγδ</td>
<td>–</td>
</tr>
<tr>
<td>CD19</td>
<td>–</td>
</tr>
<tr>
<td>CD20</td>
<td>–</td>
</tr>
<tr>
<td>CD2</td>
<td>+</td>
</tr>
<tr>
<td>CD16</td>
<td>+/–</td>
</tr>
<tr>
<td>CD56</td>
<td>+/–</td>
</tr>
<tr>
<td>CD85j</td>
<td>+/–</td>
</tr>
<tr>
<td>CD94 (KLRD1)</td>
<td>+/–</td>
</tr>
<tr>
<td>CD158a (KIR2DL2)</td>
<td>+/–</td>
</tr>
<tr>
<td>CD161 (KLRB1)</td>
<td>+/–</td>
</tr>
<tr>
<td>NKB1 (KIR3DL1)</td>
<td>+/–</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Effector Molecule</th>
<th>Expression</th>
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<tbody>
<tr>
<td>IFNγ</td>
<td>+</td>
</tr>
<tr>
<td>Granzyme</td>
<td>+</td>
</tr>
<tr>
<td>Perforin</td>
<td>+</td>
</tr>
<tr>
<td>TNFα</td>
<td>+</td>
</tr>
<tr>
<td>CD107a</td>
<td>+</td>
</tr>
<tr>
<td>IL-2</td>
<td>–</td>
</tr>
<tr>
<td>IL-4</td>
<td>–</td>
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</tbody>
</table>

Effector cells express characteristic NK cell phenotype
Cytolytic functions of NK cells measured by ADCC ICS Assay
Cytolytic functions of NK cells measured by ADCC ICS Assay

![Graphs showing CD107a, Granzyme B, and Perforin levels in different conditions: DMSO, ENV peptide pool, gp140, HIV.](image-url)
Recruit ADCC Cohort

• Currently recruited 80 ART naïve subjects
  – ADCC responses measured by ICS Assay

<table>
<thead>
<tr>
<th>Protein pool</th>
<th>GAG</th>
<th>POL</th>
<th>ENV</th>
<th>RTV</th>
<th>VVN</th>
<th>ANY HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cohort</td>
<td>13</td>
<td>46</td>
<td>53</td>
<td>17</td>
<td>27</td>
<td>70</td>
</tr>
<tr>
<td>% of Cohort</td>
<td>16%</td>
<td>55%</td>
<td>66%</td>
<td>21%</td>
<td>34%</td>
<td>87%</td>
</tr>
</tbody>
</table>

RTV= Rev, Tat, Vpu      VVN= Vif, Vpr, Nef combined peptide pools
What epitopes do ADCC target?

- Use of HIV overlapping peptide pools allows us to readily map linear ADCC epitopes
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- Use of HIV overlapping peptide pools allows us to readily map linear ADCC epitopes
• Identified 37 novel ENV specific linear ADCC epitopes
  • 9 epitopes shared by 2 or more subjects
• Additional epitope in VPU mapped
• Currently mapping additional Pol epitopes
Do these ADCC responses force immune escape?

- Investigate the possibility of viral mutational escape from ADCC activity
  - See if ADCC exerts significant immune pressure upon the virus
Viral sequence variation at ADCC epitopes

Investigated biological relevance of mapped ADCC epitopes
Cloned and sequenced several subjects’ concurrent autologous plasma virus samples across the mapped ADCC epitopes and identified variations from HIV-1 consensus sequence

Identified sequence variation in 96% of ADCC epitopes

Number of amino acid changes within epitope sequence in autologous native virus compared to consensus B HIV
ADCC escape

Concentration of peptide per 200ul well

% of IFNγ secreting cells

- Consensus
- Native
Future Aims

• Functionally compare more consensus and autologous ADCC epitopes
• Conduct a prospective and longitudinal study of ADCC from HIV cohort
  – Identify ADCC epitopes associated with slow progression of disease
• Reach a clearer understanding of significance of ADCC responses in HIV infection
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.