The conjunctiva as a site for investigation of human mucosal immunology in situ – elucidating the mechanisms of immune escape in adenovirus-induced epidemic keratoconjunctivitis (EKC)

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Inflammation of the ocular mucosal surface caused by adenovirus infection – EKC (epidemic keratoconjunctivitis)

Human NK cell populations and their regulation

Profiling NK cells in the conjunctiva mucosa over the course of EKC

Elucidating the mechanisms of immune subversion by adenoviruses
Group D human adenoviruses (HAdV) cause epidemic keratoconjunctivitis (EKC)

Subepithelial keratitis

Severe conjunctivitis

Pseudomembrane

Human adenovirus types are classified into seven groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Type</th>
<th>Clinical diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12, 18, 31</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3, 7, 11, 14…</td>
<td>Conjunctivitis, Pharyngitis, Pneumonia</td>
</tr>
<tr>
<td>C</td>
<td>1, 2, 5, 6</td>
<td>Pharyngitis, Pneumonia</td>
</tr>
<tr>
<td>D</td>
<td>8, 9, 19, 37, 53, 54…</td>
<td>EKC</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>Conjunctivitis, Pneumonia</td>
</tr>
<tr>
<td>F</td>
<td>40, 41</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>G</td>
<td>52</td>
<td>Gastroenteritis</td>
</tr>
</tbody>
</table>
Clinical features of epidemic keratoconjunctivitis (EKC)

Severe and prolonged inflammation
Group D Human adenoviruses (HAdV) cause EKC

Severe conjunctivitis  Pseudomembrane

Mechanisms?

Inflammation

0 7

Conjunctivitis (non EKC)  Conjunctivitis (EKC)
Natural Killer cells provide initial protection against virus infections and prime adaptive immunity.

- **Innate immunity**
  - NK cells
  - Cytotoxicity
  - Cytokine production

- **Adaptive immunity**
  - T cells
    - Cytotoxicity
    - Cytokine production
  - B cells
    - Immunoglobulin
  - Prime Th1 anti-viral response

Graph showing the timeline of viral load, IgM, IgG, and the transition from innate to adaptive immunity.
NK cells are controlled through a balance in signaling from inhibitory and activating factors.

**Inhibitory**

<table>
<thead>
<tr>
<th>Cell contact factors</th>
<th>Killer Cell Immunoglobulin-like Receptors (KIR2DL1/2/3, 3DL1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NKG2A</td>
</tr>
<tr>
<td></td>
<td>LILRB1</td>
</tr>
<tr>
<td></td>
<td>2DS1, 3DS1</td>
</tr>
<tr>
<td></td>
<td>NKG2C</td>
</tr>
<tr>
<td></td>
<td>NKG2D</td>
</tr>
<tr>
<td></td>
<td>DNAM-1</td>
</tr>
<tr>
<td></td>
<td>NKp30/46</td>
</tr>
<tr>
<td></td>
<td>CD16</td>
</tr>
<tr>
<td>Soluble factors</td>
<td>IL-10</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
</tr>
<tr>
<td></td>
<td>IL-12, IL-15, IL-18</td>
</tr>
<tr>
<td></td>
<td>IFN-α</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
</tr>
</tbody>
</table>

Soluble factors

- IL-10
- TGF-β
- IL-12, IL-15, IL-18
- IFN-α
- IL-2
HLA class I-specific inhibitory receptors create NK cell heterogeneity

**Inhibitory receptors**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR2DL1</td>
<td>HLA-C2</td>
</tr>
<tr>
<td>KIR2DL2/3</td>
<td>HLA-C1</td>
</tr>
<tr>
<td>KIR3DL1</td>
<td>HLA-B Bw4</td>
</tr>
<tr>
<td>KIR2DL4</td>
<td>HLA-G</td>
</tr>
<tr>
<td>KIR2DL5A,5B</td>
<td>?</td>
</tr>
<tr>
<td>KIR3DL2</td>
<td>HLA-A3/11</td>
</tr>
<tr>
<td>KIR3DL3</td>
<td>?</td>
</tr>
</tbody>
</table>

**Activating receptors**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR2DS1,2,3,4,5</td>
<td>HLA-C?</td>
</tr>
<tr>
<td>KIR3DS1</td>
<td>?</td>
</tr>
<tr>
<td>KIR2DL4</td>
<td>HLA-G</td>
</tr>
<tr>
<td>NKG2C</td>
<td>HLA-E</td>
</tr>
<tr>
<td>NKG2D</td>
<td>MICA&amp;B/ULBP</td>
</tr>
<tr>
<td>CD16</td>
<td>Fcy</td>
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<tr>
<td>CD160</td>
<td>HLA-C</td>
</tr>
<tr>
<td>2B4</td>
<td>CD48</td>
</tr>
<tr>
<td>NKp30</td>
<td>BAT3</td>
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<tr>
<td>NKp44</td>
<td>HA</td>
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<tr>
<td>NKp46</td>
<td>HA</td>
</tr>
<tr>
<td>DNAM-1</td>
<td>CD112/155</td>
</tr>
</tbody>
</table>

**Variegated expression**

**Homogeneous expression**

*Yawata et al. Blood 2009*
‘Missing-self’ response is unique to NK cells

Reduced inhibition of NK cells through cognate KIR-HLA class I interaction

Normal cells

Activating ligand

HLA class I/HLA-E (inhibitory ligand)

Activating receptor

NK

Abnormal cells

NK

cytokine production

Normal cells

virally infected cells

cancer cells

cytolysis

iKIR/NKG2A
### Human natural killer cell subsets have distinct function

<table>
<thead>
<tr>
<th></th>
<th>Mature</th>
<th>Immature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxicity</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Cytokine production by monokines</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Chemokine receptors</td>
<td>CXCR1, CX3CR1</td>
<td>CXCR3, CCR5, CCR7</td>
</tr>
<tr>
<td>HLA specific inhibitory receptors</td>
<td>KIR, NKG2A</td>
<td>NKG2A</td>
</tr>
<tr>
<td>ADCC</td>
<td>CD16^{++}</td>
<td>CD16^{-}</td>
</tr>
</tbody>
</table>
NK cell populations in human organs

- **Lung**: CD56^{dim}
- **Tonsil**: CD56^{dim} < CD56^{bright}
  - IL-22 & IFN-g
- **Lymph node**: CD56^{dim} < CD56^{bright}
- **PBMC/spleen**: CD56^{dim} > CD56^{bright}
  - IFN-g
- **Intestine**: CD56^{bright}
  - IL-22
- **Decidua**: CD56^{bright}
  - Angiogenic cytokines
- **Lower reproductive tract**: CD56^{dim}
- **The eye**: ?
Mature CD56\textsuperscript{dim} NK cells are the dominant type in the conjunctiva

Expression frequencies on CD56\textsuperscript{dim} NK cells (%)

<table>
<thead>
<tr>
<th></th>
<th>PBMC</th>
<th>Conjunctiva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead cell</td>
<td>51.7</td>
<td>0.08</td>
</tr>
<tr>
<td>CD45</td>
<td>72.8</td>
<td>0.08</td>
</tr>
<tr>
<td>CD56</td>
<td>97.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The ratio of CD56\textsuperscript{bright}/CD56\textsuperscript{dim} NK cells

PBMC: n=8
Conjunctiva: n=8

Yawata et al., Mucosal Immunology 2015
Ocular surface NK cells produce anti-viral cytokines, similar to those in peripheral blood.

Yawata et al., Mucosal Immunology 2015
CD56<sup>bright</sup> NK cells increase in acute adenovirus-induced conjunctivitis

**Mild conjunctivitis (HAdV-3)**
- **Acute phase**
- **Convalescent phase**

**Severe conjunctivitis (HAdV-8)**
- **Acute phase**
- **Convalescent phase**

Yawata et al., Mucosal Immunology 2015
CD56\textsuperscript{bright} NK cells increase in adenovirus-induced conjunctivitis

Yawata et al., Mucosal Immunology 2015
Elevated levels of chemokines that recruit $\text{CD56}^{\text{bright}}$ NK cells are identified in the tear fluid in the acute phase of adenovirus-infected conjunctiva.

**Chemokines attracting $\text{CD56}^{\text{bright}}$ NK cells** (CXCR3, CCR5)

![Bar charts showing levels of chemokines in the acute phase compared to other phases.]

**Chemokines attracting $\text{CD56}^{\text{dim}}$ NK cells** (CXCR1, CXCR2, CXCR4)

![Bar charts showing levels of chemokines in the acute phase compared to other phases.]

- **Acute phase**
- **Convalescent phase**
- **Healthy control**

n=13 Multiplex beads assay

Yawata et al., Mucosal Immunology 2015
The CD56\textsuperscript{bright}, immature NK cells express the receptors CXCR3 and CCR5 in the acute phase of viral inflammation
The conjunctiva NK cells recruited to the conjunctiva in adenovirus infection (CD56\textsuperscript{bright} NK cells and NKG2A\textsuperscript{+}NK cells) display are functionally suppressed.
Summary from *ex vivo* study

1. Mature NK cells are the dominant NK cell type in the conjunctiva.
2. Immature NK cells are recruited during adenovirus infection.
3. NKG2A⁺NK cells increase in severe conjunctival inflammation.
4. Mature NKG2A⁻NK cells display an activated state.
CD56<sup>dim</sup> NK cells produce IFN-γ against HAdV-infection

![Diagram showing the process of CD56<sup>dim</sup> NK cells producing IFN-γ against HAdV-infection.](image-url)
Cell contact-dependent NK cell activation in HAdV infection

Inhibitory receptor–ligand interactions?
Activating receptor–ligand interactions?

Yawata et al., Mucosal Immunology 2015
Weaker IFN-g production by NKG2A⁺NK cells
NKG2A⁺ NK cells showed lower response against HAdV-infected cells
Weaker IFN-γ production by NKG2A⁺NK cells

Activation < inhibition

Self specific iKIR or NKG2A

HLA class I

HLA-E

Normal cells

Abnormal cells

Mock

HAdV-3

HAdV-8

HLA class I

Ligand for KIR

HLA-E

Ligand for NKG2A
Lower IFN-g response by NK cells against group D HAdVs

Yawata et al., Mucosal Immunology 2015
DNAM-1 and NKG2D are key for NK cell activation against HAdV infection

![Graph showing intracellular IFN-γ (%)](image)

* * 

n=3

NKG2A⁺NK cells  NKG2A⁺NK cells

Yawata et al., Mucosal Immunology 2015
Ligands for DNAM-1 are reduced in HAdV-8 infection

- **CD155** (Ligand for DNAM-1)
- **CD112** (Ligand for DNAM-1)
- **MICA/B** (Ligand for NKG2D)
- **ULBP-1** (Ligand for NKG2D)
- **ULBP-2** (Ligand for NKG2D)
- **ULBP-3** (Ligand for NKG2D)

Isotype control
- Mock
- HAdV-3
- HAdV-8
Group D HAdVs down-regulate specific activating ligands on infected epithelia and dampen NK cell responses

Yawata et al., Mucosal Immunology 2015
Summary

- Immature CD56\textsuperscript{bright} NK cells and NKG2A\textsuperscript{+} mature CD56\textsuperscript{dim} NK cells are inhibited by up-regulated inhibitory ligand on HAdV-infected epithelium.

- Group D HAdV escape from mature NK cell anti-viral response by down-regulating activating ligands on infected cells.
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