Natural Killer Cells: Development, Diversity, and Applications to Human Disease

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Columbus, Ohio, USA

Human large granular lymphocytes or natural killer (NK) cells

- The role of cytokines in NK cell development
- Secondary lymphoid tissue and human NK cell development
- Human NK cell subsets
- Clinical application of NK cell receptor biology

Human natural killer cell development: 1993: what was known??

But, is IL-2 the real growth factor for NK cells in vivo??

- IL-2+/ NK
- IL-2Rα-/- NK
- IL-2Rβ-/- NO NK
- IL-2Rγc-/- NO NK
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**Natural Killer Cells**

- Development,
- Diversity,
- and Applications to Human Disease

**Dr. Michael A. Caligiuri**

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**IL-15 induces human NK cell differentiation from CD34+ HPCs**

- IL-15 induces human NK cell differentiation from CD34+ HPCs

**CD56**

- CD3

**IL-15**

- CD34+ HPC

**IL-15**

- CD56+ NK

**CD56**

- CD3

**What are the other factors responsible for NK cell development?**

A clue: the CD56bright human NK cell subset expresses the RTK c-kit

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KL or Flt3 ligand (FL) enhance IL-15-mediated development of CD56⁺CD3⁻ NK cells from CD34⁺ HPCs in vitro.}

**Figure 1:**
- CD56-PE
- CD3-FITC
- Mononuclear cells
- CD56⁺ NK cells

**Figure 2:**
- How does KL or FL assist IL-15 in NK cell development?

**Figure 3:**
- How does KL (SCF) assist IL-15 in NK cell development?

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**Text:**

- KL or FL ligand (FL) enhance IL-15-mediated development of CD56⁺CD3⁻ NK cells from CD34⁺ HPCs in vitro.

**Table:**

<table>
<thead>
<tr>
<th>Medium</th>
<th>FL</th>
<th>IL-15</th>
<th>FL + IL-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold Increase in Mononuclear Cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

**Figure:**

- Medium FL IL-15 FL +
- Absolute Num. of CD56⁺ Cells (x 1000)
- 0 | 50 | 100 | 150 | 200 | 250 |

**How does KL or FL assist IL-15 in NK cell development??**

Both KL and FL induce expression of CD122 (IL-2/15Rβ) protein on CD34⁺ HPCs making the CD34⁺ HPC more responsive to IL-15.

**How does KL (SCF) assist IL-15 in NK cell development?**

**Figure:**

- Phospho-ERK1/2
- Actin

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Human natural killer cell development

NK progenitor

NK precursor

Mature NK cell

CD34+ CD38+ flt3+ c-kit+

Flt3L

CD34+

KL

IL-2/15Rβ+
c-kit+

CD56 bright NK cell precursor,
now identified as CD34 dim CD45RA(+),
has unique high expression of L-selectin and β7.

Only CD34 dim CD45RA(+) HPC differentiate into CD56 bright NK cells

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The CD34dimCD45RA(+)/β7bright NK precursor is selectively enriched in human lymph nodes

Peripheral blood
~6%

Lymph node
>95%

Freud, et al., Immunity, 2005

The CD34dimCD45RA(+)/β7bright NK precursor is selectively enriched in human lymph nodes and differentiates to a CD56bright NK cell

Lymph node
>95%

CD34

CD45RA

IL-15

IL-2

CD56

CD3/14

CD45RA

Hypotheses: secondary lymphoid tissue might be the site of human NK development


CD56+ cells in the parafollicular T-cell rich regions of human lymph nodes

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NK cells are in human lymph nodes and are CD56brght

FACS from fresh human lymph node

Putative stages of human NK cell differentiation in nodes/tonsils

Human NK cell development occurs in secondary lymphoid tissues


How NK cell development fits in human lymphopoiesis

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IL-15 and human NK cell survival: low concentrations of IL-15 maintain human NK cell survival in serum free medium in vitro

Adoptive transfer of NK cells into IL-15-deficient mice

IL-15 is required for NK cell survival in vivo: adoptive transfer of NK cells into IL-15 KO mice
IL-15 and NK cell development and survival

- IL-15 is critical for NK cell development, and human NK development appears to occur in secondary lymphoid tissue.
- IL-15 is required for NK cell survival in vivo, and so are dendritic cells.
- Collectively, these data point towards IL-15 as a key mediator of NK cell homeostasis in vivo.

IL-15 transgene

1. Multiple AUG's removed
2. Replacement of signal peptide
3. C-terminus stabilization

NK cell expansion in IL-15tg mice

- IL-15 transgenic mice show a significant increase in absolute NK cell numbers compared to wild-type controls.
- The expansion is predominantly due to the upregulation of CD3 and Ly49D expression.

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IL-15tg mice: 3-7 months: lymphoblastic leukemia

Clinical signs and findings (20-30% of mice):
- Progressive alopecia
- Decreased activity, weight loss, labored breathing
- Massive hepatosplenomegaly
- Dramatic WBC elevation blood with massive lymphocytosis (see below)
  (mean 188,000/uL blood, range 47,000-606,000/uL blood, n = 22)
- Premature death (19 weeks) likely secondary to respiratory insufficiency

Bone marrow
- CD3-FITC 13%
- DX5-PE 64%

Spleen
- CD3-FITC 10%
- DX5-PE 46%

Blood (476,000 WBC/uL)
- CD3-FITC 10%
- DX5-PE 63%

IL-15tg mice: acute T-NK lymphoblastic leukemia

CD3+ T Cells
- TCR Vβ 53%

AML/ALL
- FLT3 ITD
- FL
- IL-15

NK progenitor
- CD34+
- CD38+
- FLT3+
- c-kit+

T-NK precursor
- CD34−/−
- IL-2/15Rβ−
- c-kit+

Mature NK cell
- CD56+ NK Cells
- CD16+/−
- NKR+
- c-kit+/−
- IL-2/15Rβ+
Summary of human NK cell subsets

Peripheral blood lymphocytes

<table>
<thead>
<tr>
<th>Receptor</th>
<th>CD56^{bright}</th>
<th>CD56^{dim}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD16</td>
<td>Dim/neg</td>
<td>bright</td>
</tr>
<tr>
<td>IL-2Rα</td>
<td>pos</td>
<td>neg</td>
</tr>
<tr>
<td>KIR</td>
<td>&lt; 10%</td>
<td>&gt; 85%</td>
</tr>
<tr>
<td>L-selectin</td>
<td>pos</td>
<td>neg</td>
</tr>
<tr>
<td>c-Kit</td>
<td>pos</td>
<td>neg</td>
</tr>
</tbody>
</table>

Effector functions

- ADCC + + +
- Natural cytotoxicity + + +
- Cytokine production + + +

Do NK cell subsets have unique immunoregulatory roles?

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Macrophage and NK co-culture: LPS activation
1. Pathogen
2. Monokines

NK cells provide critical IFN-γ for early monocyte/macrophage control of infection

NK cell-derived IFN-γ in the innate immune response
- Early NK-derived IFN-γ is requisite for the elimination of intracellular pathogens and activation of monocytes
- Genetic deficiencies in NK cells lead to fatal viral infections
- Genetic deficiencies in IFN-γ lead to fatal infections by obligate intracellular pathogens

Macrophage and NK co-culture: LPS activation (2)
1. Pathogen
2. Monokines

CD56\textsuperscript{bright} NK

CD56\textsuperscript{dim} NK

???
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CD56\textsuperscript{bright} NK cells are the primary source of NK-derived IFN-γ

- Contribution of the CD56\textsuperscript{bright} NK cells in the human immune response

**Lymph node**

- EARLY RESPONSE
- LATE RESPONSE

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Emerging model for human NK cell subsets: innate immunoregulator, CD56^{bright}

- CD56^{bright} NK Cell
- Low cytokine production
- Effector functions
  - +++ ADCC
  - +++ LAK
  - +++ natural cytotoxicity

Emerging model for human NK cell subsets: innate cytotoxic effector, CD56^{dim}

- CD56^{dim} NK Cell
- Low cytokine production
- KIRs
  - +++ KIR
  - + CD94/NKG2A
- Effector functions
  - +++ ADCC
  - +++ LAK
  - +++ natural cytotoxicity

How do inhibitory NKR work?

Inhibitory signal in the absence of activating ligand blocks autologous NK killing activation of normal cells

- NK
- KIR 2DL2 Inhibitory R
- HLA-Cw3
- SELF
- HPC
How does KIR work?

- KIR sees and engages MHC class I
- Negative signal via KIR blocks NK killing activation
- Leukemia cells that lack MHC class I are rare, as they are most likely killed by NK cells
- Certain infectious agents (e.g., CMV) can downregulate MHC class I to avoid T-cell immune surveillance; These agents would presumably be destroyed by NK cells

How do inhibitory NKR work? (2)

Lack of negative signal (absent or defective MHC class I): NK cell kills tumor cell target if tumor expresses ligand to activating receptor

Human NK cells have inhibitory receptors that bind to specific epitopes that are shared among some different MHC class I molecules, but not others

- HLA-Cw2, 4, 5, and 6 (Group 2 HLA-C epitopes)
- HLA-Cw1, 3, 7, and 8 (Group 1 HLA-C epitopes)
Human NK cells have inhibitory receptors that bind to specific epitopes that are shared among some different MHC class I molecules, but not others (2).

Donor has KIR on some NK cells that bind to Cw3.

That KIR is called KIR 2DL2.

KIR 2DL2 recognizes an epitope shared by group 1 HLA-C, that includes HLA-Cw1, 3, 7, and 8.

If the recipient AML blast lacks group 1 HLA-C, and expresses a ligand to an activating receptor, lysis occurs.

KIR 2DL2 expressed on some donor NK cells recognizes an epitope shared by HLA-Cw1, 3, 7, 8.

The AML blast from recipient expresses HLA-Cw2: no engagement of KIR.
**Haplo-mismatch BMT (3)**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient (leukemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A24, B35, Cw2</td>
<td>A24, B35, Cw2</td>
</tr>
<tr>
<td>A3, B62, Cw3</td>
<td>A1, B14, Cw8</td>
</tr>
</tbody>
</table>

- Donor has KIR on some NK cells that binds to HLA-Cw3
- That KIR is called KIR 2DL2
- KIR 2DL2 recognizes an epitope shared by group 1 HLA-C that includes HLA-Cw1, 3, 7, and 8
- If the recipient AML blast expresses group 1 HLA-C, NO lysis

**Haplo-mismatch BMT (4)**

**NK**

KIR 2DL2 expressed on donor NK cells recognizes an epitope shared by HLA-Cw1, 3, 7, 8

The AML blast from recipient expresses HLA-Cw8:

- Engagement of KIR
- NO Lysis

**NK**

Leukemia Target

Inhibitory KIR2DL2 (group 1 specific)

HLA-Cw2 (group 2)

- No kill

Leukemia Target

Inhibitory KIR2DL2 (group 1 specific)

HLA-Cw8 (group 1)

- Kill
Role of NK cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation

Conclusions
- In mismatched transplants, a clinical graft-versus-leukemia effect, independent of T-cell-mediated graft-versus-host disease, appears to positively impact on relapse rate when NK cell KIR epitope incompatibility is in the graft-versus-host direction
- Additional study of donor-versus-recipient NK cell alloreactivity in mismatched hematopoietic stem cell transplants has confirmed these results

Summary from today
- The role of cytokines in NK cell development
- Secondary lymphoid tissue and human NK cell development
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