Biology of Innate Immunity: NK cells, Macrophages, PMN, PAMP/TLR

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<table>
<thead>
<tr>
<th>Innate</th>
<th>Adaptive</th>
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<tbody>
<tr>
<td>Immediate response</td>
<td>Delayed response</td>
</tr>
<tr>
<td>Receptors - invariant, germline encoded</td>
<td>Receptors - require somatic genetic recombination</td>
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<tr>
<td>No 'memory' - constant number of precursors, constant response kinetics</td>
<td>Memory' - after primary exposure higher precursor frequency and faster response kinetics</td>
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## Innate vs. Adaptive Immunity

<table>
<thead>
<tr>
<th>Innate</th>
<th>Adaptive</th>
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<tbody>
<tr>
<td>Epithelial cells</td>
<td>T cells</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>B cells</td>
</tr>
<tr>
<td>Monocytes, dendritic cells &amp; macrophages</td>
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<tr>
<td>Mast cells</td>
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<td>NK cells</td>
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Does innate immunity prevent or promote tumor growth?

**Innate immunity promotes tumor growth**

Inflammation - activated macrophages & granulocytes - provide angiogenic factors & growth factors and matrix metalloproteinases that promote tumor spread.

**Innate immunity prevents tumor growth**

NK cells kill tumors and dendritic cells process tumor antigens and prime an adaptive (B & T cell) response.
Inflammation in human breast and prostate cancer

de Visser KE et al. (2006) Paradoxical roles of the immune system during cancer development
Nat. Rev. Cancer. 6: 24-37 doi:10.1038/nrc1782
Innate immunity promotes tumor growth

- Chronic inflammation predisposes to cancer (liver, colon)

- COX2 inhibitors diminish cancer risk

Innate immunity prevents tumor growth

- Direct cell-mediated cytotoxicity
- Cytokine-mediated anti-tumor effects
Innate Cytokines

Epithelial cells ---- Type I interferon, pro-inflammatory cytokines

Granulocytes --- Pro-inflammatory cytokines, reactive oxygen species (ROS), IL-12

Macrophages -- Pro-inflammatory cytokines, ROS, VEGF

Conventional Dendritic Cells -- pro-inflammatory cytokines, IL-12, IL-15

Interferon-producing Dendritic Cells - Type I interferon, IL-12

Mast cells - Pro-inflammatory cytokines, arachidonic acid, IL-4

NK cells - Interferon-γ, TNF, chemokines
What initiates cytokine production by innate immune cells?
The story of the Toll-like receptors begins with insect immunity.

Toll-dependent innate immune responses in *Drosophila* to fungus and Gram+ bacteria.

Lemaitre et al. 1996  *Cell* 86:973

Courtesy Mitch Kronenberg
Mammalian Toll-Like Receptors

TLR recognize conserved structures in microbes

Courtesy Mitch Kronenberg
TLR signaling pathways

From S. Akira Lab Website
Mammalian Toll-Like Receptors

- **Interferon-producing dendritic cells** - TLR 7, TLR9
- **Conventional dendritic cells** - TLR1, 2, 4, 5, 6, 8
- **Resting NK cells** - No functional TLR
- **Activated NK cells** - TLR3, TLR9
TLR-based cancer therapy 100 years ago!

Bacterial infection post-surgery for cancer induces regression and prevention of metastasis
Tumor Immunology and Immunotherapy

First, a little history...

William B. Coley
• Cancer Surgeon at Memorial Hospital (NYC) at the turn of the 20th Century
• Observed that a cancer patient who developed a severe bacterial infection (strep.pyogenes) had spontaneous regression of his tumors.
• Treated over 900 solid tumor patients with a crude bacterial extract (“Coley's Toxin”) and reported a 40% response rate, some leading to long term remissions.
• Approach largely abandoned after his death.
• His daughter, Helen Coley Nauts founded the Cancer Research Institute, which is one of the largest private foundations supporting basic and applied research in tumor immunology.
TLR ligands as cancer therapies

Coley’s Pharma TLR9 agonist CpG effective in Non-small cell lung cancer

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

3M TLR7 agonist imiquimod Approved for superficial basal carcinoma

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
Role of macrophages and granulocytes in innate tumor immunity?

In vivo
- primary tumorigenesis take weeks or months
- no feasible way to deplete granulocytes and macrophages for extended periods

In vitro
- macrophages kill tumors in vitro but receptors (other than FcR) on macrophages & ligands on tumors not defined
Eosinophil-mediated tumor immunity

Renca-IL4

wt

Renca-IL4 Scid or nude
Higher incidence of 3-MCA-induced fibrosarcomas in interferon-α/β receptor-/- mice

Effect of INFα/β probably on host immune cells, rather than tumor

NK cells and tumor immunity

- Identified in '70s as lymphocytes from healthy humans and mice able to kill certain tumors in vitro
- Function in innate immunity to protect against viruses, bacteria, & tumors
- Produce cytokines & kill abnormal cells
Immune surveillance against cancer by NK cells

Mice depleted of NK cells with anti-AsialoGM1 or depleted of NK cells and NKT cells with anti-NK1.1 have higher incidence of 3-MCA-induced sarcomas.
NK Cells Reject Tumors Lacking MHC Class I

Class I+ tumors grow in vivo

Class I- tumors are rejected

Class I- tumors in NK-depleted mice grow in vivo
Mice reject MHC class I-negative RMA/S, but not class I-positive RMA lymphoma
Immune surveillance for ‘Missing Self’

- NK cells preferentially kill cells that have lost MHC class I
- Provides protection against cells escaping T cell recognition
- Predicts existence of inhibitory receptors for MHC class I that spare normal cells from NK cell attack
  - Karre et al Nature 319:675, 1986
Loss of Class I MHC Expression in a Prostate Carcinoma
How are NK cells activated when they encounter tumors or virus-infected cells?
Activating NK receptors - ligands

- Human/mouse CD16-FcεRIγ/ζ, IgG
- Human CD2 ........................................ CD58
- Human 2B4 (CD244)-SAP ................................ CD48
- Human DNAM-1 (CD226) ................................ CD112, CD155
- Mouse PILRβ-DAP12 ....................... PILR-L
- Human NKG2D-DAP10 .......... MICA/B, ULBP
- Mouse NKG2D-DAP10/12 ................ RAE-1,H60, MULT1
- Human/mouse NKp46-FcεRIγ/ζ ........... ?
- Human NKp30-FcεRIγ/ζ ........... ?
- Human NKp44-DAP12 ............... ?
- Mouse NKR-P1c-FcεRIγ ............... ?
Antibody-dependent cellular cytotoxicity
CD16 (Fc\(\gamma\)RIIIA)

Expressed by NK cells and macrophages
CD16 Signaling

Cytotoxicity, cytokine production
FDA-approved therapeutic monoclonal antibodies

CD20  Her2
CD33  CD20  CD52
CD20  CD20  EGF-R
VEGF
Rituxan Pivotal Trial: Treatment of Patients With Relapsed Lymphoma

Rituxan® 375 mg/m² (IV)

Weeks

Monitoring every 3 months x2 years

<table>
<thead>
<tr>
<th>Evaluable Patients</th>
<th>166</th>
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<tr>
<td>Overall Response</td>
<td>80 (48%)</td>
</tr>
<tr>
<td>Complete Response</td>
<td>10 (6%)</td>
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<tr>
<td>Partial Response</td>
<td>70 (42%)</td>
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Polymorphisms in CD16 correlate with therapeutic effects of anti-tumor monoclonal antibodies
CD16 (FcyRIII) mediates Herceptin and Rituxan mediate human tumor elimination in nude mice
### Activating NK receptors - ligands

- **Human/mouse CD16-FcεRIγ/ζ**  \( \text{IgG} \)
- **Human CD2**  \( \text{CD58} \)
- **Human 2B4 (CD244)-SAP**  \( \text{CD48} \)
- **Human DNAM-1 (CD226)**  \( \text{CD112, CD155} \)
- **Mouse PILRβ-DAP12**  \( \text{PILR-L} \)
- **Human NKG2D-DAP10**  \( \text{MICA/B, ULBP} \)
- **Mouse NKG2D-DAP10/12**  \( \text{RAE-1, H60, MULT1} \)
- **Human/mouse NKp46-FcεRIγ/ζ**  \( ? \)
- **Human NKp30-FcεRIγ/ζ**  \( ? \)
- **Human NKp44-DAP12**  \( ? \)
- **Mouse NKR-P1c-FcεRIγ**  \( ? \)
NKG2D
- C-type lectin-like superfamily
- 1 gene, non-polymorphic, conserved mice - humans
- Homodimer expressed on all NK cells, \( \gamma \delta \) T cells, and \( CD8^+ \) T cells
- R in transmembrane associates with D in DAP10 transmembrane

DAP10
- 10 kd homodimer
- Cytoplasmic YINM recruits Grb2 & p85 PI3-kinase
NKG2D ligands in mice and humans

Many genes
Many alleles
NKG2D ligands

- MHC class I-like
  - don’t require peptide or β2-microglobulin
- Bind with nM affinity to NKG2D

- Low levels expressed on healthy tissues
- Induced on virus-infected cells and tumor cells
- Induced by DNA damage
- Elevated in autoimmune diseases
What is the biological role of the NKG2D ligands?

“Danger signals” to alert the immune system to infection
NKG2D on NK cells, γδ T cells and CD8+ T cells detect NKG2D ligands on abnormal cells.
Induction of NKG2D ligands

Replication stress, DNA damage
ATR/ATM/CHK1-dependent Rae-1 / ULBP up-regulation

Healthy cell → Replication stress, DNA damage → ATR/ATM/CHK1-dependent Rae-1 / ULBP up-regulation → Mouse Rae-1 Human ULBP → NKG2D

Tumorigenesis (Infection?)

NK cell

Thanks D. Raulet
Nature 2005
NKG2D ligands (MICA/B) are expressed on many primary human tumors

- Lung tumors
- Prostate tumors
- Ovarian tumors
- Colon tumors

Groh et al. PNAS 96:6879, 1998
Human NK cells kill NK-resistant mouse cells transfected with human NKG2D ligands
NKG2D ligands are expressed on many mouse tumors.
Mouse NK cells kill NK-resistant lymphomas transfected with NKG2D ligands
Mice reject lymphomas transfected with NKG2D ligands

Rejection mediated by NK cells
NKG2D-RAE-1 interaction overrides “self class I-inhibition” *in vivo*

NK Cells Reject RAE-1+ MHC class I+ Tumors!

- Class I+ tumors grow *in vivo*
- RAE-1+ Class I+ tumors are rejected
- RAE-1+ tumors in NK-depleted mice grow *in vivo*
Increased 3-MCA induced tumors in mice treated with anti-NKG2D mAb
If NK cells kill tumors expressing NKG2D ligands - how do the tumors survive?
Shed or secreted NKG2D ligands in the sera of cancer patients
NKG2D and Cancer

- Tumors frequently over-express NKG2D ligands
- DNA-damage induces expression of NKG2D ligands on tumors
- NK cells eliminate tumors expressing NKG2D ligands
- NKG2D ligands on tumors can (sometimes) augment tumor antigen-specific CD8+ CTL
- Tumors shed or secrete soluble NKG2D ligands to act as decoys - immune evasion