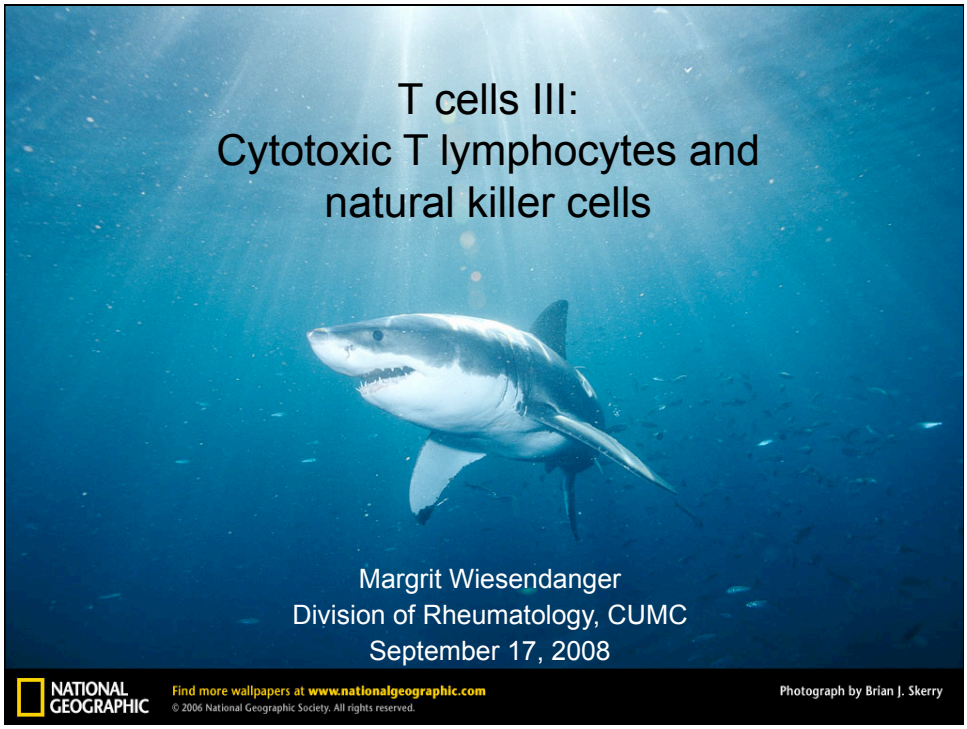


Killer cells: CD8$^+$ T cells (adaptive) vs. natural killer (innate)

Shared purpose:

protect the host from viral, bacterial and parasitic infection

recognize and destroy malignant cells

Shared mechanisms *of cytotoxicity and similar cytokine secretion profiles*

Distinct modes of target recognition

Cytotoxic T lymphocytes

- Express CD8 (potentiates interaction with class I MHC molecule)
- Each T cell expresses a unique receptor, within a highly diverse repertoire generated by V(D)J recombination
- Scan MHC class I-peptide complexes, searching for pathogen or tumor-encoded antigens
- Preactivation and differentiation required

Natural killer cells

- Invariant activating and inhibitory receptors
- Recognize 'missing self': the absence of class I MHC on the cell surface triggers NK attack (viral or tumor strategy to evade immune surveillance by CD8+ T cells)
- No preactivation required, but significantly potentiated by cytokines

Clinical relevance of cytotoxic cells according to Goldilocks

Too Hot

- Autoimmune diseases:
  - seronegative spondyloarthropathies,
  - type I diabetes
- Hypersensitivity reactions
- Graft versus host disease
- Transplant rejection

Too Cold

- Immunodeficiency syndromes with decreased NK function:
  - Chediak-Hidashi syndrome (CHS1 gene)
  - Griscelli syndrome (Rab27a gene)
  - Hermansky-Pudlak syndrome (HPS1 gene)
  - Familial Hemophagocytic Lymphohistiocytosis: (perforin gene defect)

Just Right

- Host defense against:
  - Viruses (HSV, EBV, CMV)
  - Bacteria (Listeria monocytogenes)
  - Parasites (Plasmodium falciparum and Toxoplasma gondii)
  - Primary and metastatic tumors
- Graft versus leukemia effect
- NK cells in placenta: vascularization and inhibition of fetal rejection

Examples are provided for illustrative purposes: do not memorize!

Cytotoxic effector cells: armed and very dangerous

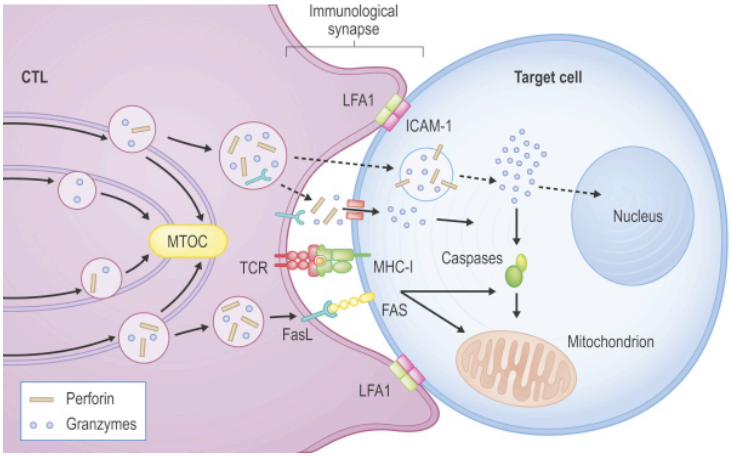
Cytotoxicity:

- Granzyme/perforin pathway
- Death receptor pathway:
  - Fas/Fas ligand
  - TNF-Related Apoptosis-Inducing Ligand (TRAIL)

Immune modulation:

- Production of inflammatory cytokines:
  - interferon-γ
  - tumor necrosis factor (TNF)
- Chemokine secretion:
  - CCL3 (MIP1α)
  - CCL4 (MIP1β)
  - CCL5 (RANTES)
- Immunomodulatory cytokines:
  - Interleukin-10
  - Granulocyte and Monocyte Colony Stimulating Factor (GM-CSF)

Mechanisms of cytotoxic T lymphocyte (CTL)-induced cell death: a form of 'assisted suicide'



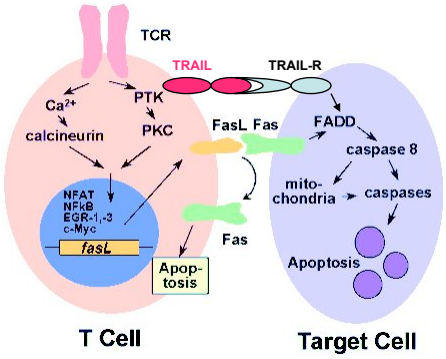
Perforin: disrupts cell membrane

Granzyme A: cleaves nuclear proteins and facilitates double-stranded DNA breaks

Granzyme B: activates the pro-apoptotic molecule BID

Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18, p. 272 (2008)

Mechanisms of cytotoxic T lymphocyte (CTL)-induced cell death: Fas/FasLigand and TRAIL/TRAIL receptors



Fas expressed on target cell: enables killing via Fas/FasL pathway

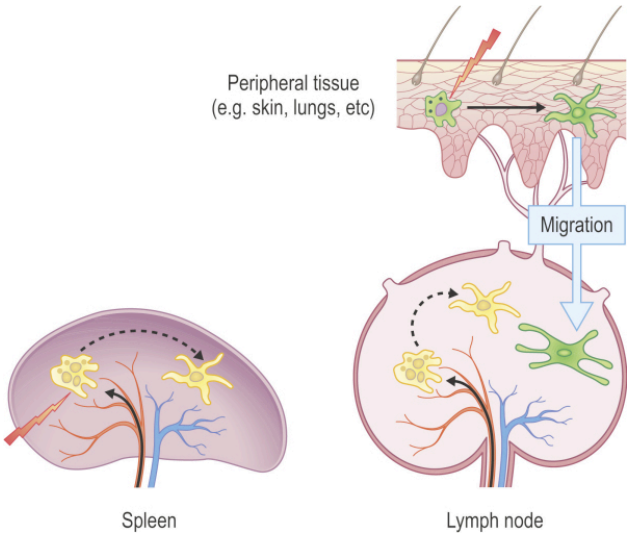
Fas expressed activated T cell: provides mechanism for downregulating the immune response by T cell 'fratricide' (activa

TRAIL (TNF-related apoptosis inducing ligand) expressed on activated T cell:

TRAIL: enables killing (apoptosis) via signaling through the receptor expressed by the target cell -- tumor cells may be particularly sensitive to this death pathway

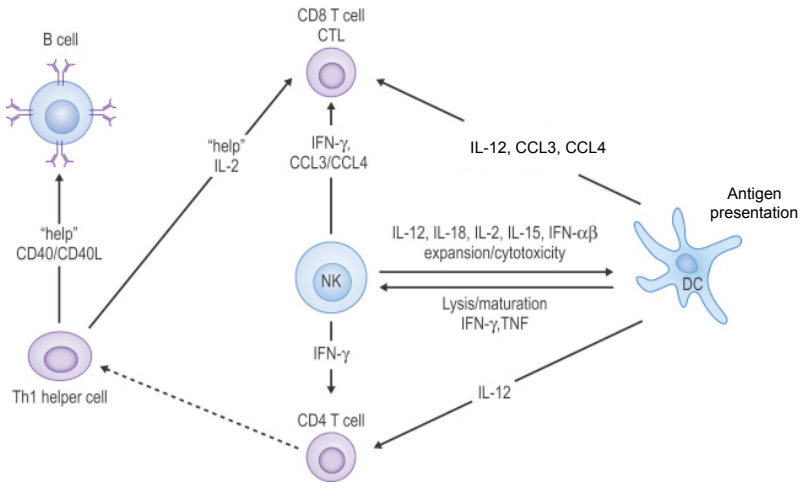
Thomas Brunner lab website,
Institute of Pathology, University of Bern, CH

How T cells become activated: life cycle of the dendritic cell



José A. Villadangos, Louise J. Young
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 7 (2008)

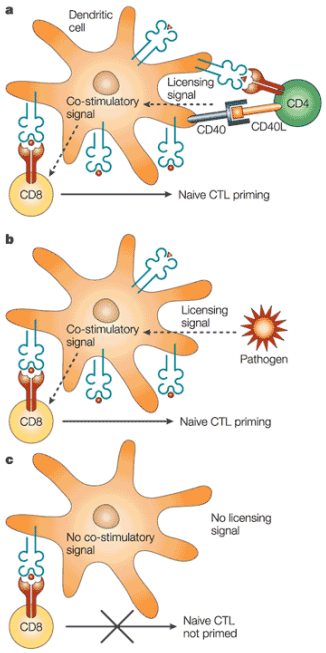
How T cells become activated:
Cellular interactions during an immune response in the lymph node



Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18 (2008)

007: license to kill

In order to be able to efficiently prime naïve CD8+ T cells, dendritic cells must first be 'licensed'



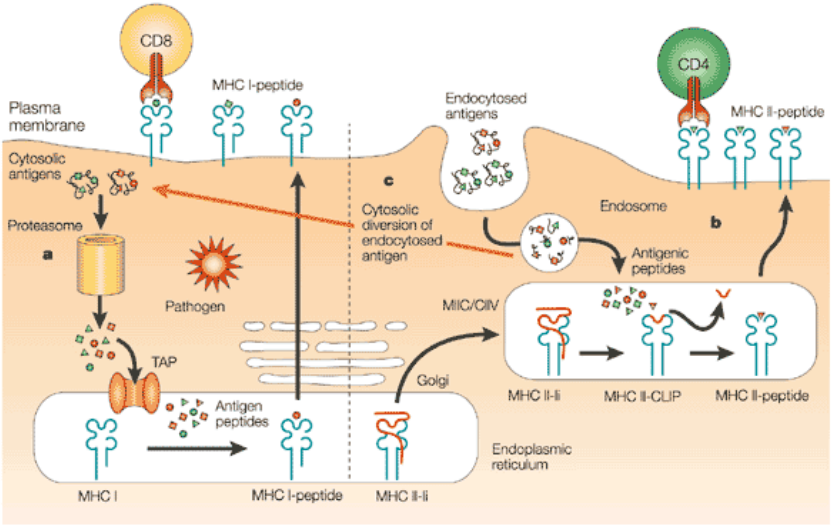
CD4+ T cell help: licensing via CD40/ CD40L

Virulent pathogen: licensing via Toll-like receptors (TLR)

No license -> no priming

William Heath, Francis Carbone
Nature Reviews in Immunology 1: 126 (2001)

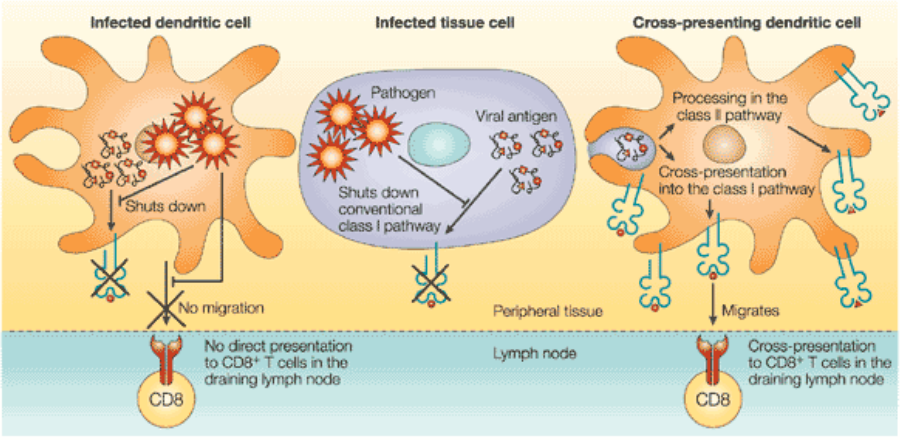
Different antigen processing pathways for MHC class I and class II molecules



William Heath, Francis Carbone
Nature Reviews in Immunology 1: 126 (2001)

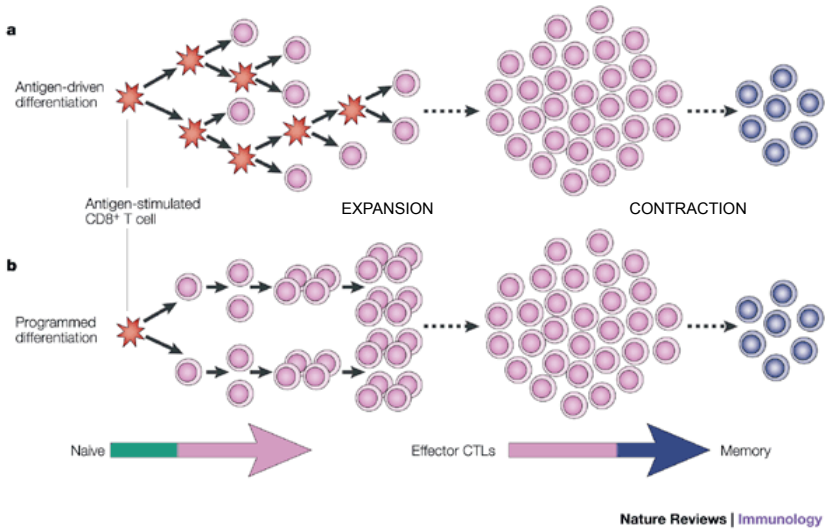
What if a virus directly infects and shuts down the antigen-presenting cell?

Cross-presentation pathways can take over



William Heath, Francis Carbone
Nature Reviews in Immunology 1: 126 (2001)

Generation of an effector response and memory CTL:
2 models for the role of antigen stimulation in T cell proliferation



Susan Kaech, E. John Wherry, Rafi Ahmed
Nature Reviews in Immunology, 2:251 (2002)

Generation of memory CTL: CD8+ T cells need 'help' to remember

CD4+ T cell help: directly or indirectly produces cytokines that promote the survival, proliferation and programming of the memory CTL.

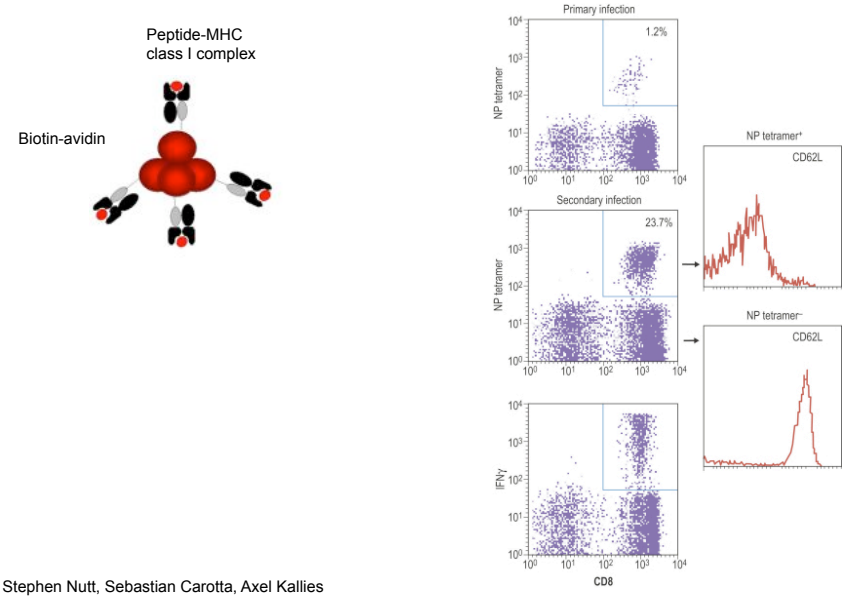
CD4 T cell-deficient mice: a model for the study of 'helpless' CD8+ T cells, which resemble CTL in chronic infections in which pathogens are not cleared despite a robust CTL response.

Two molecules have been found to mediate the defects in helpless CTL responses:

1. Re-stimulation of 'helpless' CTL leads to an abortive response due to AICD that is mediated by TRAIL.
2. PD-1 (programmed death 1), an inhibitory member of the TNFR family, is expressed on both helpless CTL and on CTL cells during chronic infections. Blocking the interaction of PD-1 with its ligands greatly enhances the numbers and functions of the impaired CTL.

Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18 (2008)

Detection and analysis of CTL function



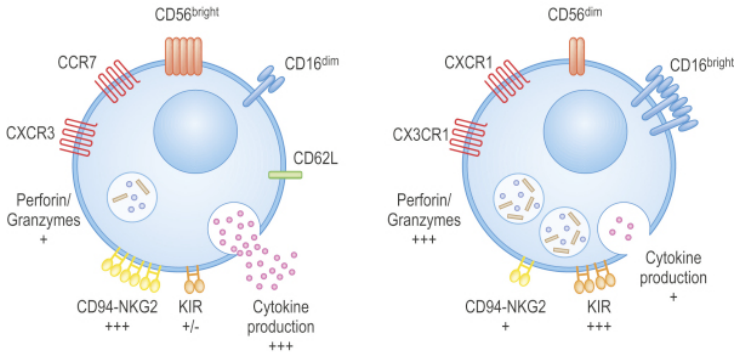
Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18 (2008)

Natural Killer Cells

- Surveillance function: NK cells are found in:
  - Peripheral blood
  - Secondary lymphoid organs: bone marrow, spleen, activated lymph nodes
  - Peripheral tissue: liver, lung and the decidual lining of the uterus
- Key cytokines:
  - Interleukin-15: required for NK cell development
  - IL-12, IL-18: promote activation, cytotoxicity, IFN-γ production
- Key surface markers:
  - CD16 (FcγRIII), binds IgG and promotes the antibody-dependent cytotoxicity (ADCC) of NK cells
  - CD56 (adhesion molecule),
  - Killer cell Immunoglobulin-like Receptor (KIR): recognize MHC class I molecules (HLA-A, B, C). A specific allele (KIR3DS1) can recognize HIV peptide in HLA-Bw4 and is associated with slow progression to AIDS.

Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18, p. 277 (2008)

2 subsets of human natural killer cells



$CD56^{bright}$ $CD16^{dim}$ $KIR^{+/-}$
predominant NK population in secondary lymphoid organs,
highly proliferative,
greater cytokine production

$CD56^{dim}$ $CD16^{bright}$ KIR^{+}
predominant NK cells in peripheral blood,
highly cytotoxic

Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18, p. 272 (2008)

Natural Killer cells vs. Cytotoxic T cells: target recognition

	NK cell	Cytotoxic T cell
Receptor type	NK receptor (numerous activating or inhibitory)	T cell receptor
Ligand type	Class I MHC, MICA/B, immune complexes, etc.	Peptide-MHC class I complex
Absence of class I MHC results in…	Immediate cytotoxicity ('missing self')	Lack of recognition
Presence of class I MHC results in…	Inhibitory signal to NK cell	TCR engagement

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NK cell receptors

Inhibitory receptors:

- Recognize mostly MHC class I ligands with high affinity
- Signal via ImmunoTyrosine Inhibitory Motifs (ITIM)
- Recruit phosphatases (SHP and SHIP) to prevent a cytotoxic response
- Required for NK cell licensing

Activating receptors:

- Ligands include viral molecules and stress induced proteins
- Do not bind MHC class I molecules with high affinity
- Signal via ImmunoTyrosine Activating Motifs (ITAM)
- Use several signaling adaptors, including DAP12

Note: most NK cell receptors can also be expressed by some T cells after activation

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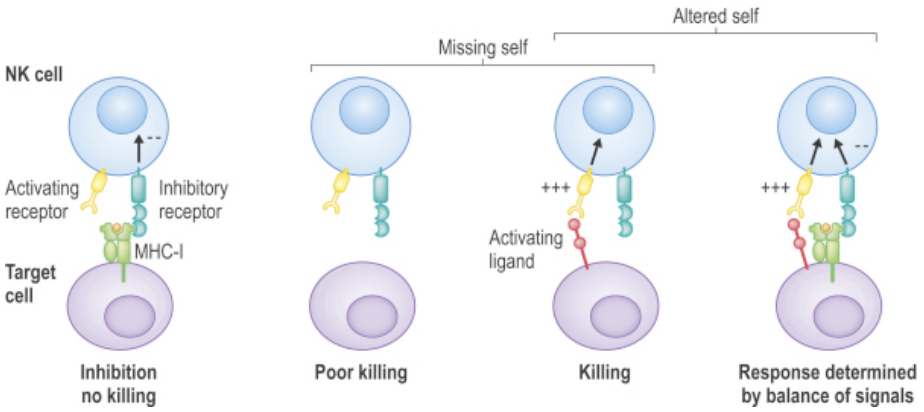
NK cell recognition of target cells



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Specific NK cell functions (I)

- Control of viral infections:
  - Patients with selective NK deficiencies suffer from recurrent herpes simplex and cytomegalovirus infections
  - NK cells can lyse HIV-infected target cells either directly or by ADCC (Antibody-Dependent Cellular Cytotoxicity)
  - NK cells secrete large quantities of chemokines (CCL3, CCL4, CCL5) which are the ligands for CCR5 and inhibit CCR5-dependent entry of HIV into target cells
  - (however, HIV-infected T cells selectively downregulate a subset of HLA genes, thus evading immune control while remaining resistant to NK cell cytotoxicity)

Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18, p. 282 (2008)

Specific NK cell functions (II)

Control of malignant cells:

- A long-standing hypothesis: NK cells function in protective tumor immune surveillance (by killing tumors that have downregulated MHC class I to evade recognition and cytotoxicity by T cells)
- Difficult to test this theory in humans, but NK cells can reject tumors in mouse models
- NK cells activate dendritic cells by producing IFN-γ (thus enhancing tumor immunogenicity), and also by providing DC with increased access to tumor antigens by killing activity

Stephen Nutt, Sebastian Carotta, Axel Kallies
Clinical Immunology: Principles and Practice, 3rd ed., Elsevier, ch. 18, p. 282 (2008)

Specific NK cell functions (III)

- Role in hematopoeitic stem cell transplantation:
  - Allogeneic bone marrow transplantation (BMT): the “graft vs. leukemia” effect cures leukemia via killing of residual malignant cells by donor cytotoxic T cells
  - However: transferred donor T cells can also mediate graft vs. host disease.
  - Proposal (controversial): BMT from a haplo-identical donor (eg from parent, where one-half of MHC is shared between parent and child) may provide allogeneic NK cells with an HLA haplotype that would potentiate the graft vs. leukemia effect (while minimizing graft vs. host effect).

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Specific NK cell functions (IV)

- NK cells and pregnancy:
  - During pregnancy, maternal and paternal (nonself) antigens are expressed in the embryo and placenta
  - Implantation site: uterine NK cells are the predominant leukocyte population.
  - Features of uNK cells: low cytotoxicity, but do secrete IFN-γ, TNF and angiogenic factors ('immune deviation'?)
  - Model: maternal NK cells interact with the trophoblast for physiologic placental development

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How viruses and tumors evade cytoxicity

- Latency: minimizing viral gene detection (HSV, EBV, HIV)
- Antigenic variation: rapid mutation of viral genome (HIV) or tumor markers
- Infection of 'immune privileged sites': central nervous system (HSV)
- Production of 'immunoevasins': adenovirus and Epstein-Barr virus produce proteins that hinder Fas or TNF-mediated killing, or inhibit cytokine function.
- EBV also produces homologs of the Bcl-2 anti-apoptotic molecule.
- Modulation of molecules involved in target recognition: viruses interfere with antigen processing, presentation, or MHC class I expression.

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Take Home Messages

1. $CD8^+$ T cells (adaptive immunity) and Natural Killer cells (innate immunity) cooperate to protect the host from viruses, intracellular bacteria and parasites, and in tumor surveillance

2. Mechanisms of cellular cytoxicity shared between $CD8^+$ T cells and NK cells include triggering apoptosis in the target cell via the perforin/granzyme pathways or cell surface receptors (Fas, TRAIL)

3. Target recognition relies on either specific peptide presented in MHC class I (for $CD8^+$ T cells) or the lack of MHC class I (for NK cells).

4. $CD8^+$ T cells require a licensing step (by activated dendritic cells) in order to acquire cytotoxic function and generate memory.

5. Cross-presentation allows the priming of CD8+ T cells against viruses that attempt to evade the immune response by shutting down antigen presentation

6. NK cell activation is determined by the 'balance' of positive and negative signals received through an array of surface receptors.