

Cytotoxic T Cell Functions

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Micro 204, 11/05/18, CL220

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Recommended Reading:

Textbook:

- Janeway's 9th Edition: Chapters 9 and 11.

Reviews:

- Kaech and Cui. Transcriptional control of effector and memory CD8+ T cell differentiation (2012) *Nat Rev Immunology* 12: 749.
- Farber et al. Human memory T cells: generation, compartmentalization and homeostasis. (2014) *Nat Rev Immunology* 14: 24.
- Bantug et al. The spectrum of T cell metabolism in health and disease. (2018) *Nat Rev Immunology* 18: 19.

Primary literature:

- Araki et al. mTOR regulates memory CD8 T-cell differentiation. (2009) *Nature* 460: 108.
- Lim et al. Neutrophil trails guide influenza-specific CD8+ T cells in the airways. (2015) *Science* 349: aaa4352.
- Schenkel et al. T cell memory. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. (2014) *Science* 346: 98.

The Course of a Typical Acute Infection That is Cleared by an Adaptive Immune Response

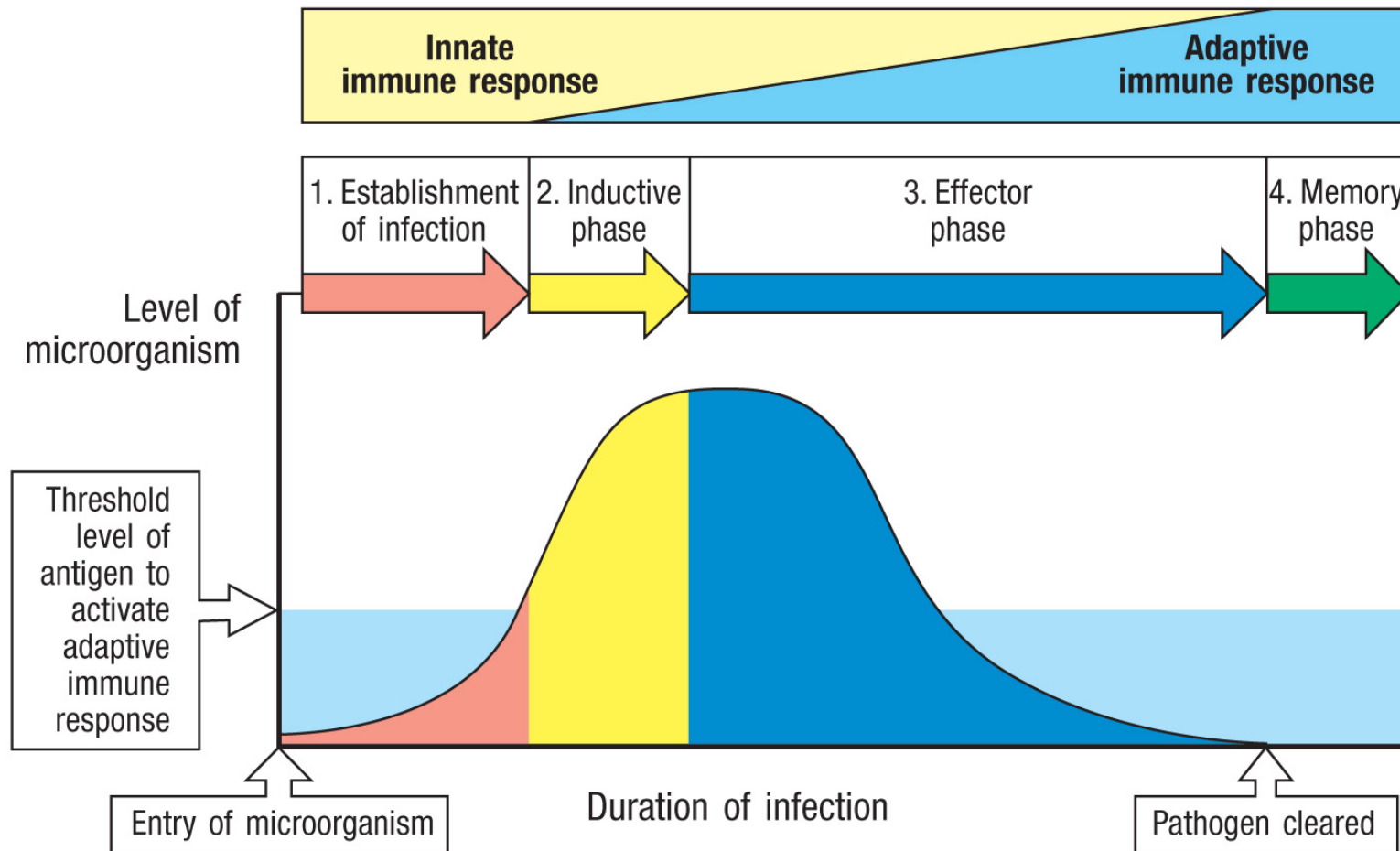


Figure 11.1 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

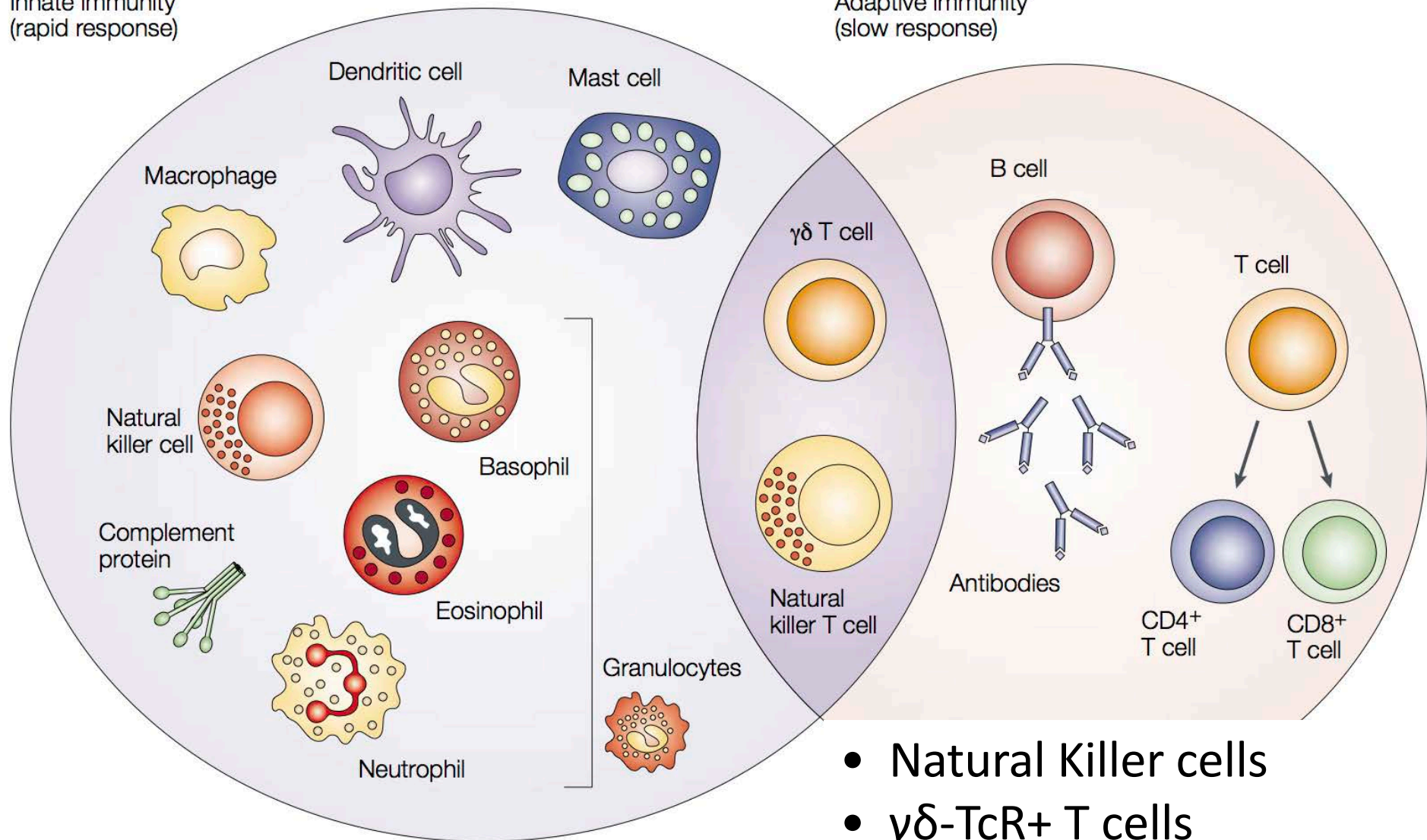
Ultimate goal:

- 1) clear pathogen with minimal damage to host
- 2) form immunological memory

Both Innate and Adaptive Cells are Involved in Cytotoxicity

Innate immunity
(rapid response)

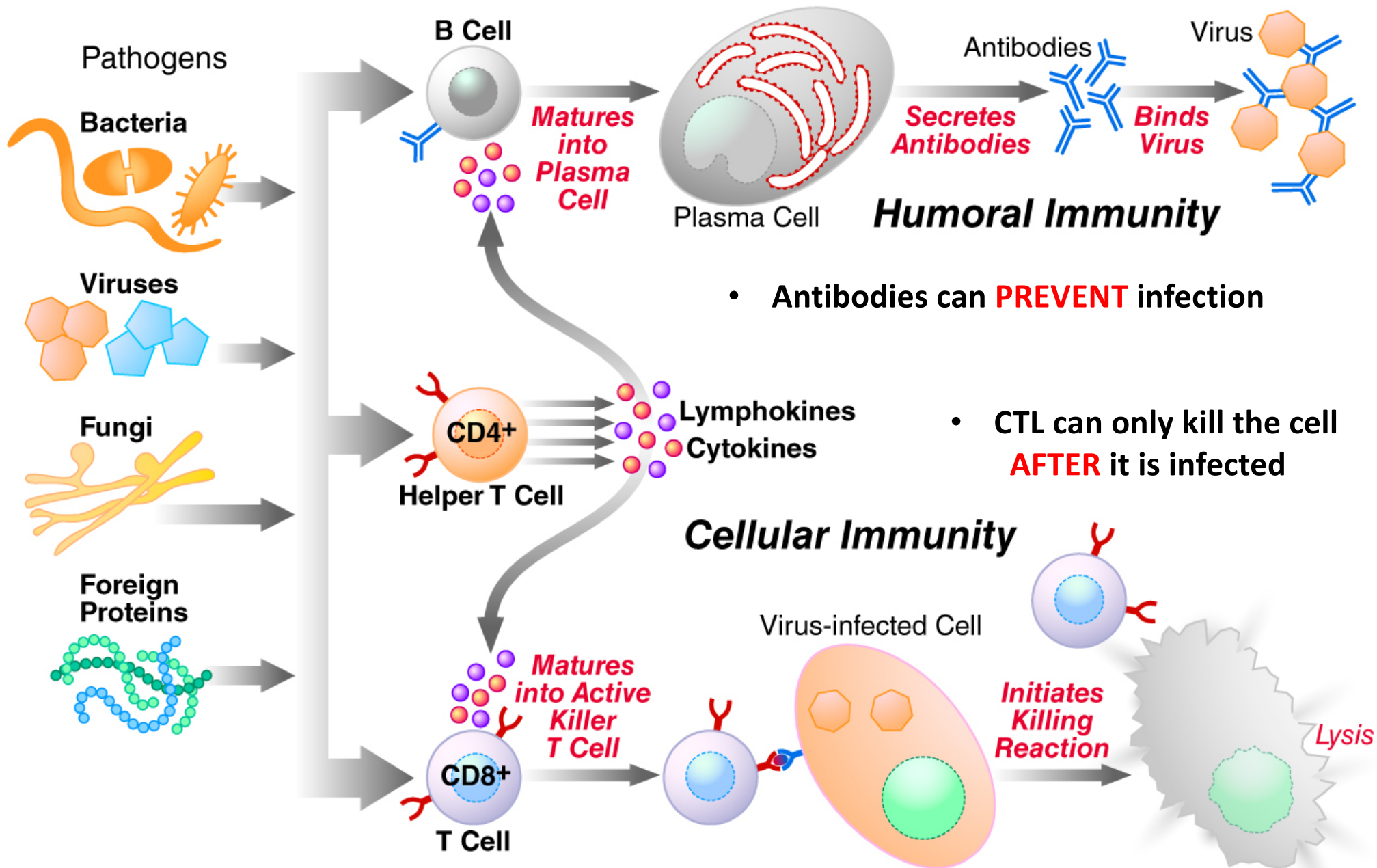
Adaptive immunity
(slow response)



Dranoff (2004) *Nature Reviews Cancer*

- Natural Killer cells
- $\gamma\delta$ -TcR⁺ T cells
- CD4⁺ $\alpha\beta$ -TcR⁺ T cells
- **CD8⁺ $\alpha\beta$ -TcR⁺ T cells**

Activation of Adaptive Immunity

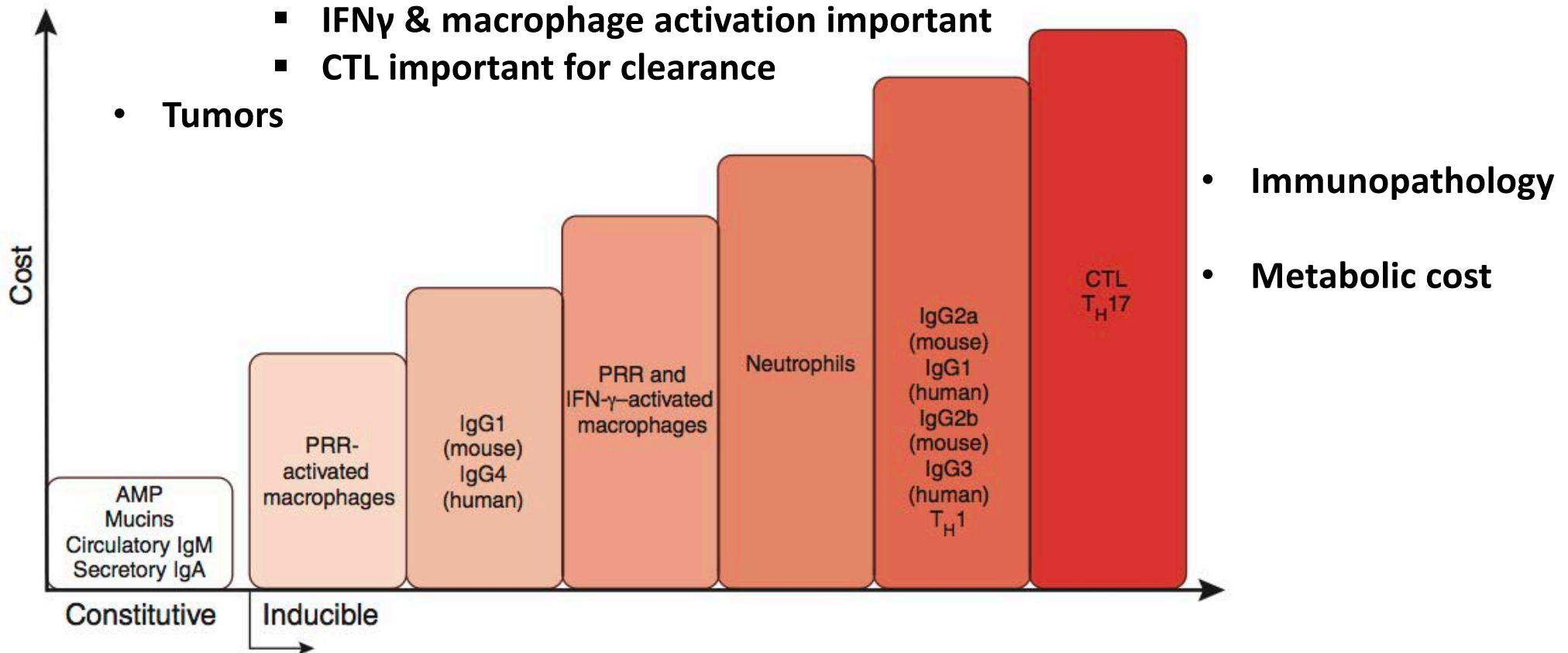


- Antibodies can **PREVENT** infection

- CTL can only kill the cell **AFTER** it is infected

Layered Immune Response to Minimize Cost and Provide Sufficient Protection

- Viral infections
 - ✓ Acute lytic viruses (polio, vaccinia, influenza)
 - ✓ Persistent non-lytic viruses (CMV, HSV, EBV, HIV) that need control FOREVER
- Intracellular bacteria & parasites
 - ✓ Listeria, Mycobacteria, Toxoplasma
 - IFN γ & macrophage activation important
 - CTL important for clearance
- Tumors

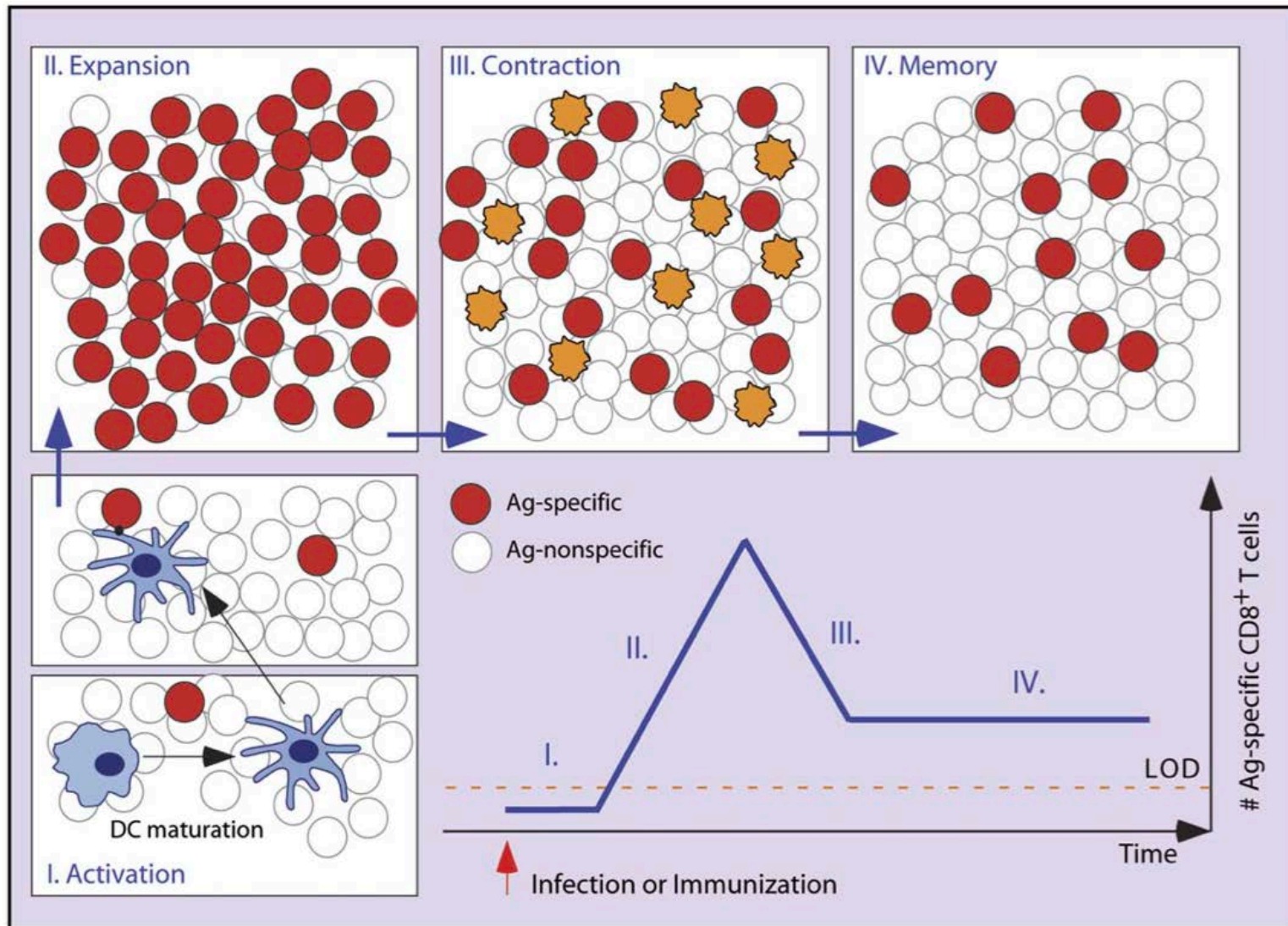


Order of engagement

Iwasaki and Medzhitov *Nature Immunology* 2015

Stages of CD8 T Cell Activation, Differentiation, and Memory Formation

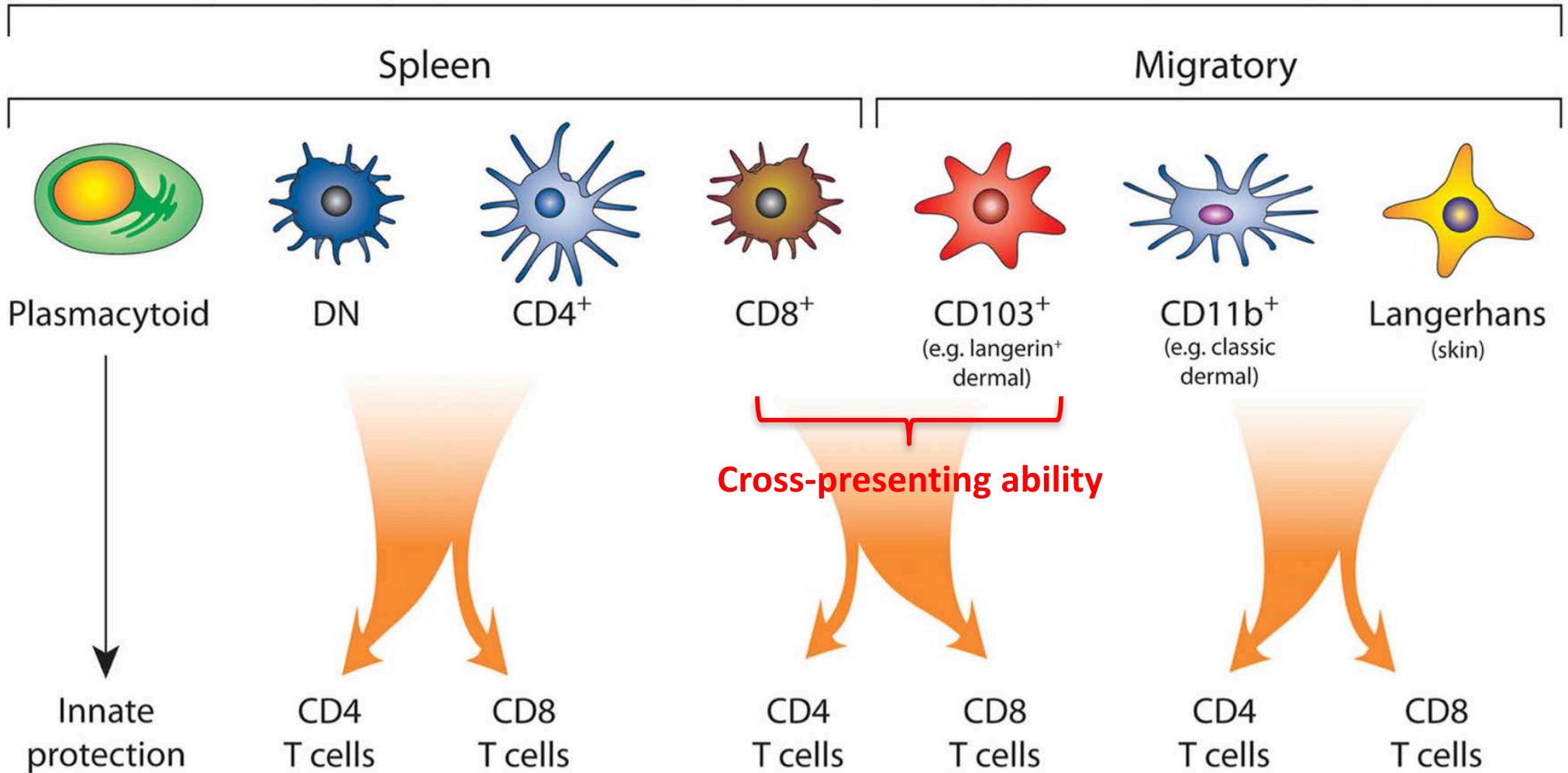
SLECs (KLRG1^{hi}) MPECs (IL-7R α ^{hi})



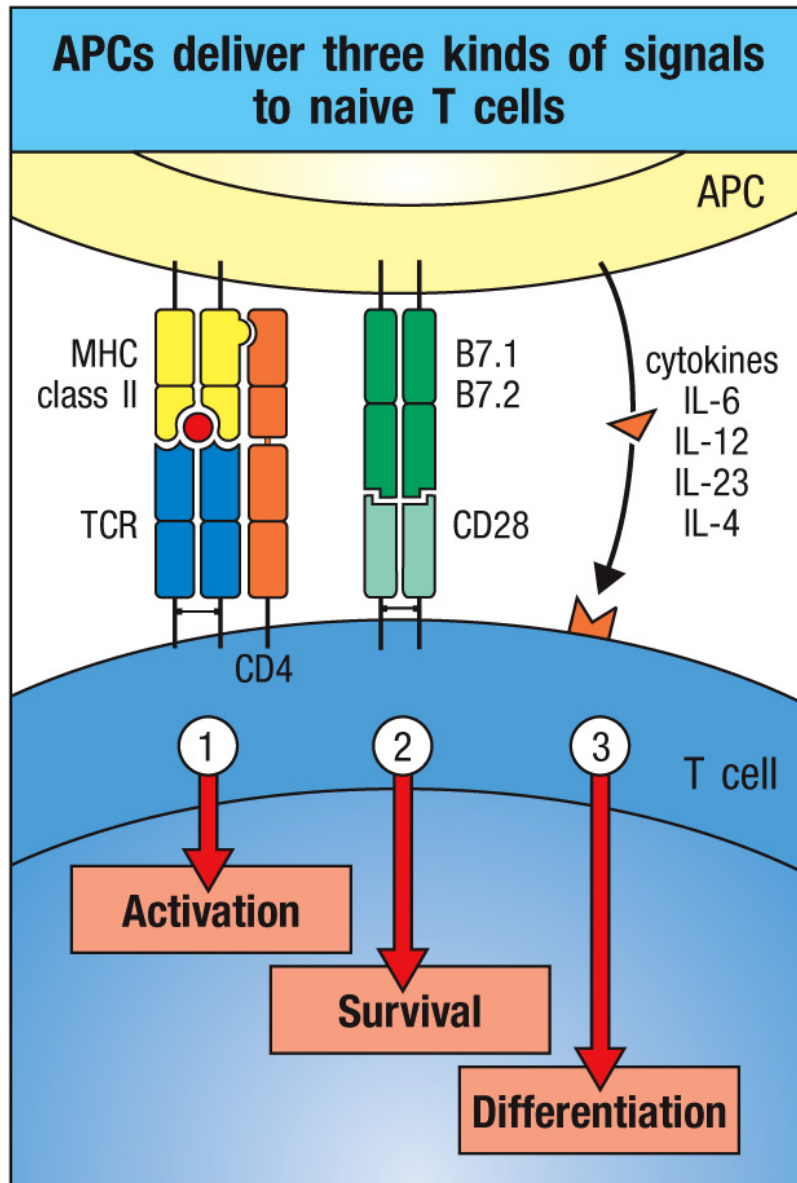
I. T Cell Activation

Specialized DCs for CD8 T Cell Priming

Lymph nodes



Three Signals are Required to Activate Naïve T Cells



1) TCR activation: (Foreign-peptide:self MHC complex & T-cell receptor)
→ Activation

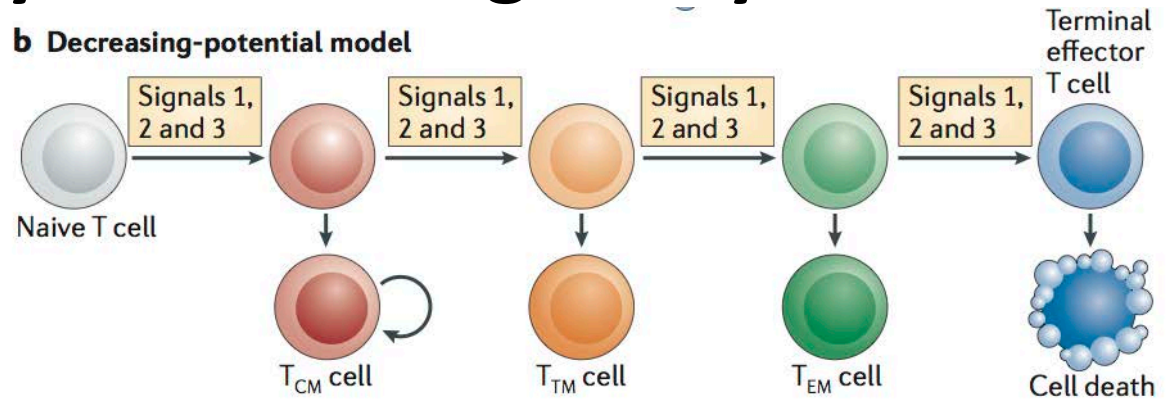
2) Co-stimulation: (B7.1/B7.2 & CD28)
→ Survival and proliferation (IL-2)
→ Priming of mitochondria for memory

3) Inflammation: (cytokines & corresponding receptor)
→ Differentiation
→ Inflammatory environment can affect CD8 T cell differentiation into SLECs (i.e. IL-2, IL-12, IFN γ) vs. MPECs (i.e. IL-10, IL-21, TGF- β)

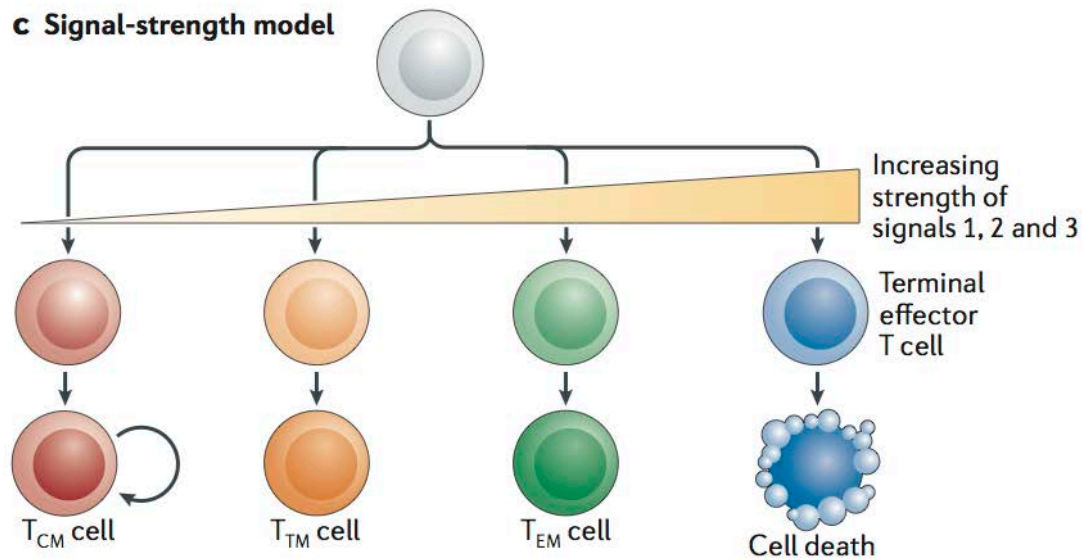
Figure 9.22 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Models for Generating Effector and Memory Cell Heterogeneity

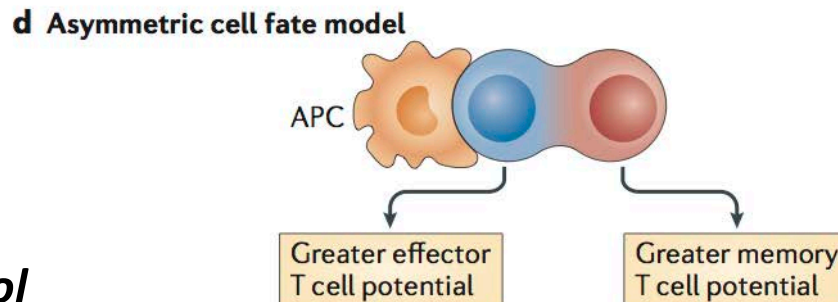
Cumulative history of signals.



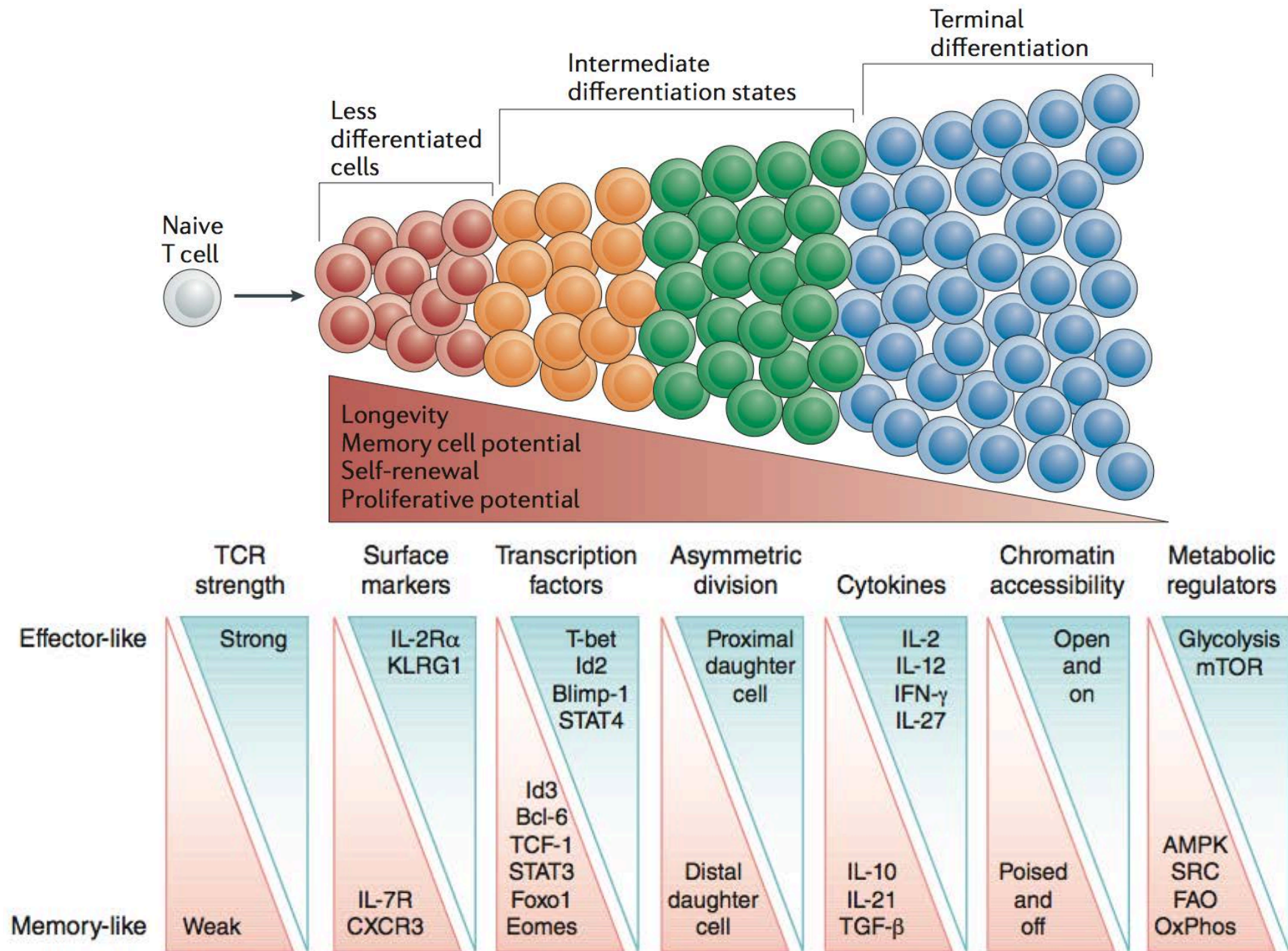
Overall strength of the signals.



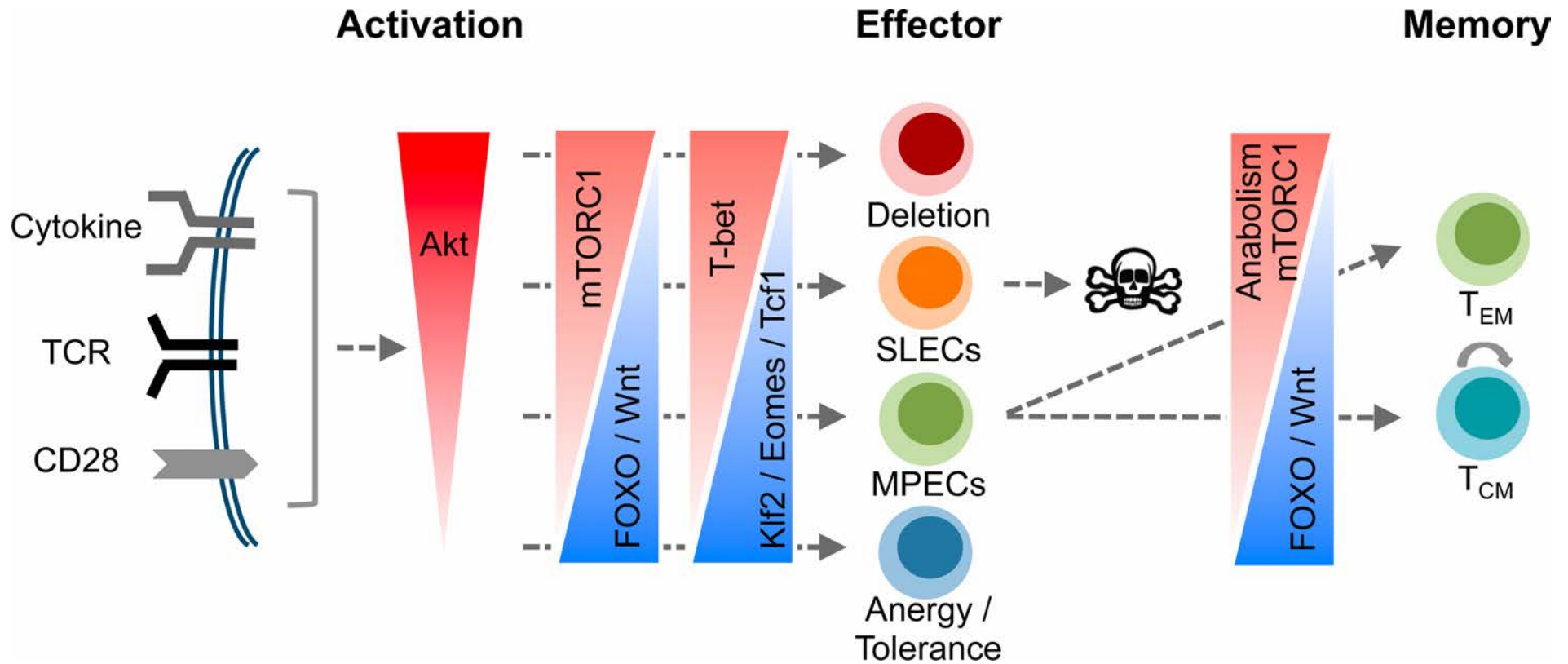
Fate rises from first cell division.



Signals Contributing to the Diversity of Memory Cells

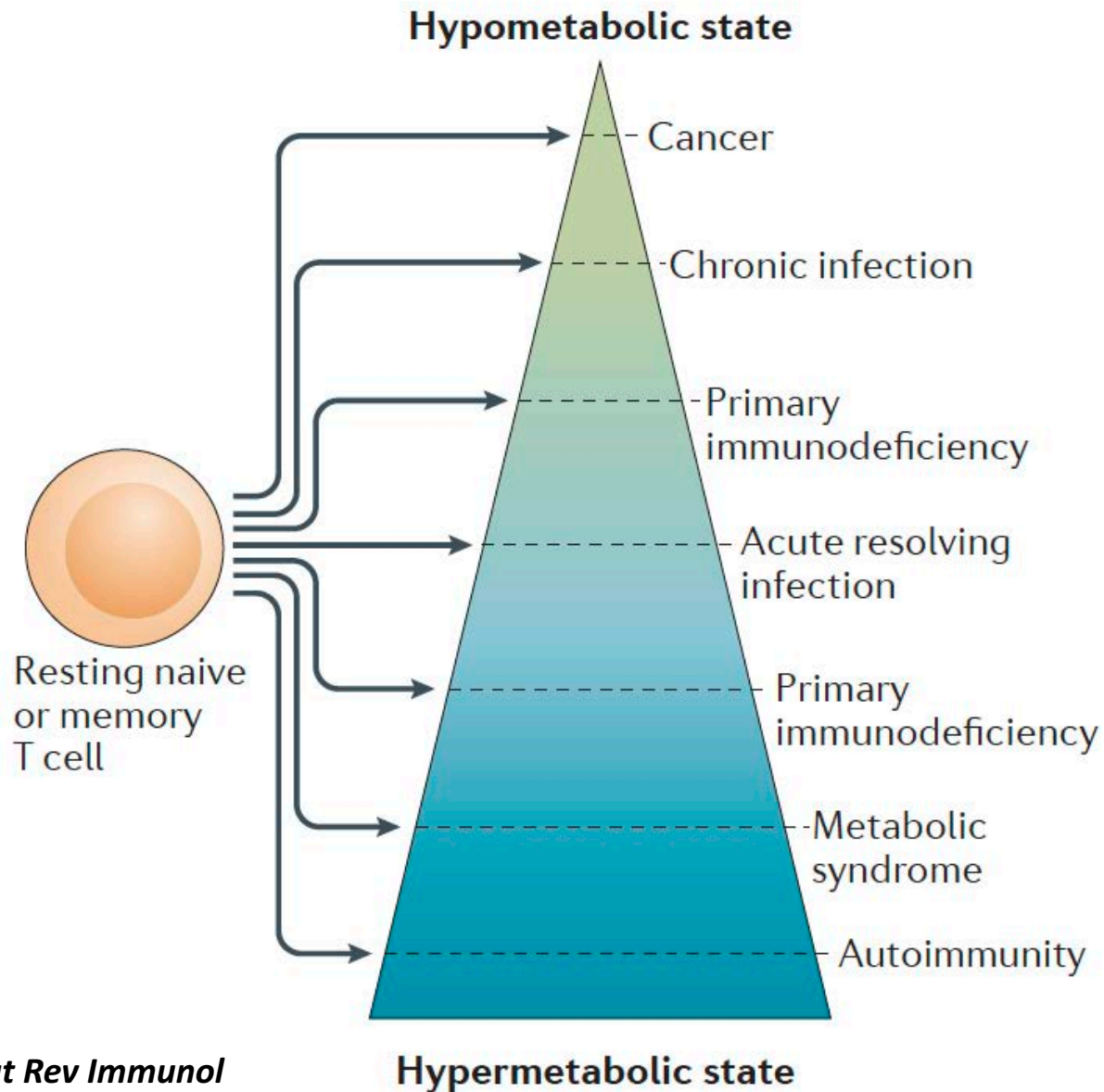


Orchestration of CD8 T Cell Differentiation by the PI3K/Akt Pathway

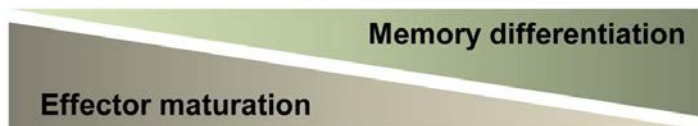
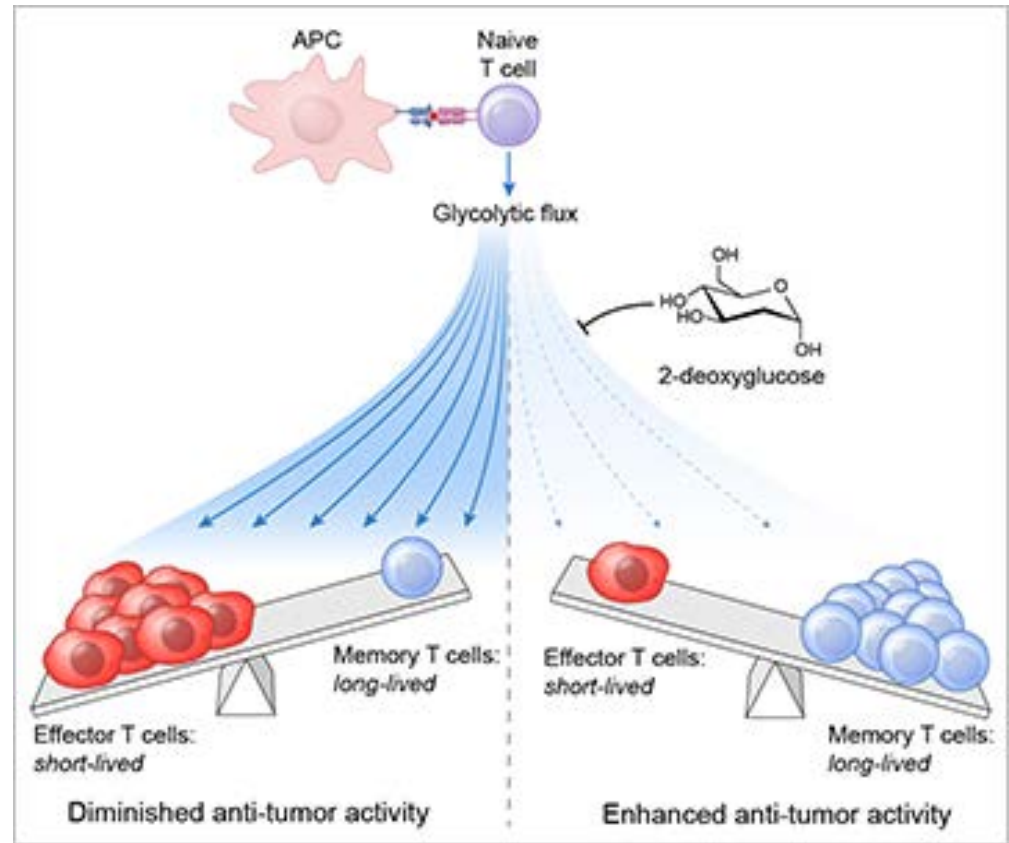
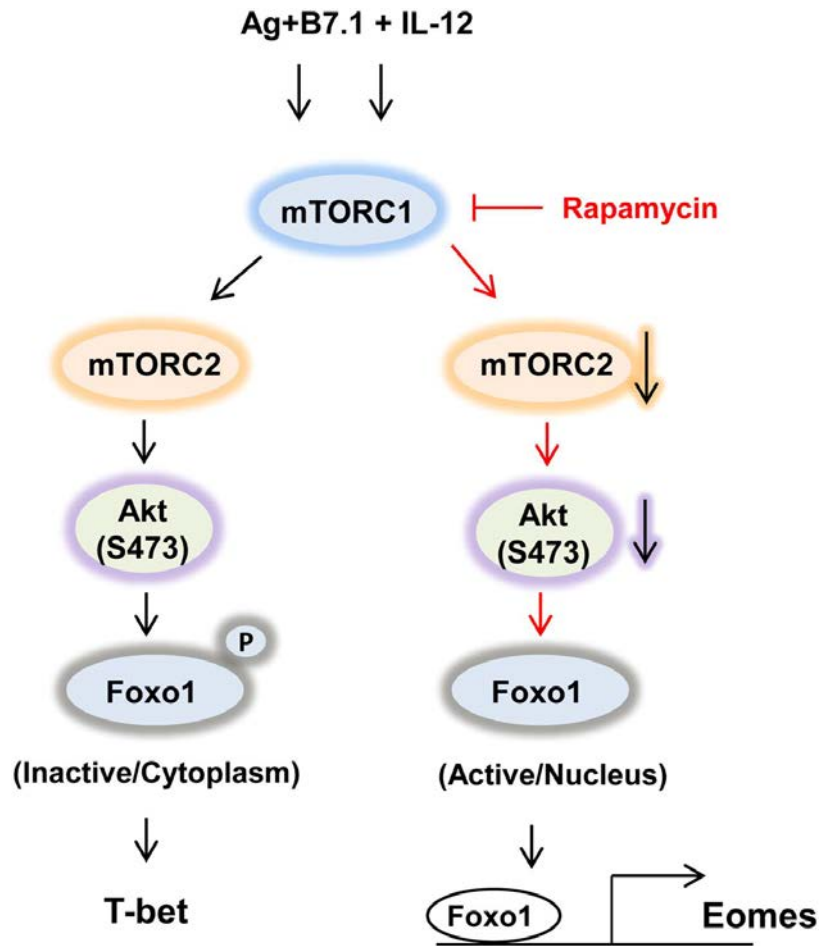


Why do we need a graded CTL response?

A Graded View of T Cell Metabolic States in Health and Disease



Altering the Differentiation of CD8+ T Cells Can Influence Their Function

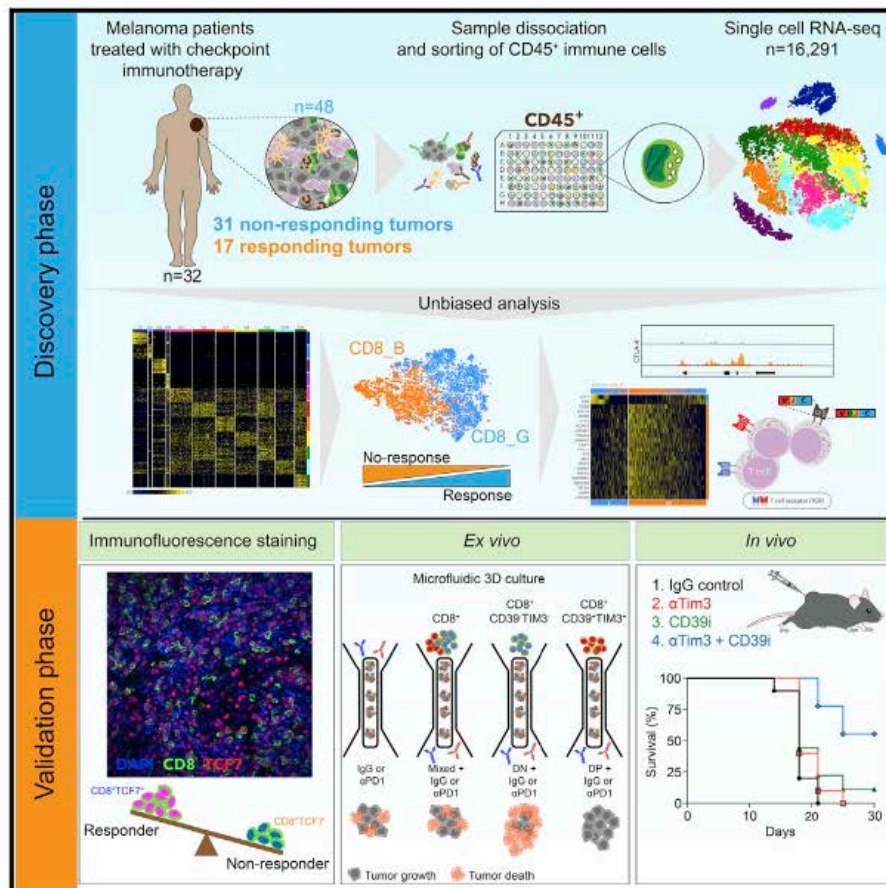


Rao et al. 2012 *Immunity* 36: 374

Sukumar et al. 2013 *JCI* 123: 4479

Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma

Graphical Abstract



Authors

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In Brief

Single-cell analysis of immune cells from melanoma patients treated with immune checkpoint therapy uncovers a **TCF7⁺** memory-like state in the cytotoxic T cell population and demonstrates its association with a positive outcome.

Three Signals are Required to Activate Naïve T Cells

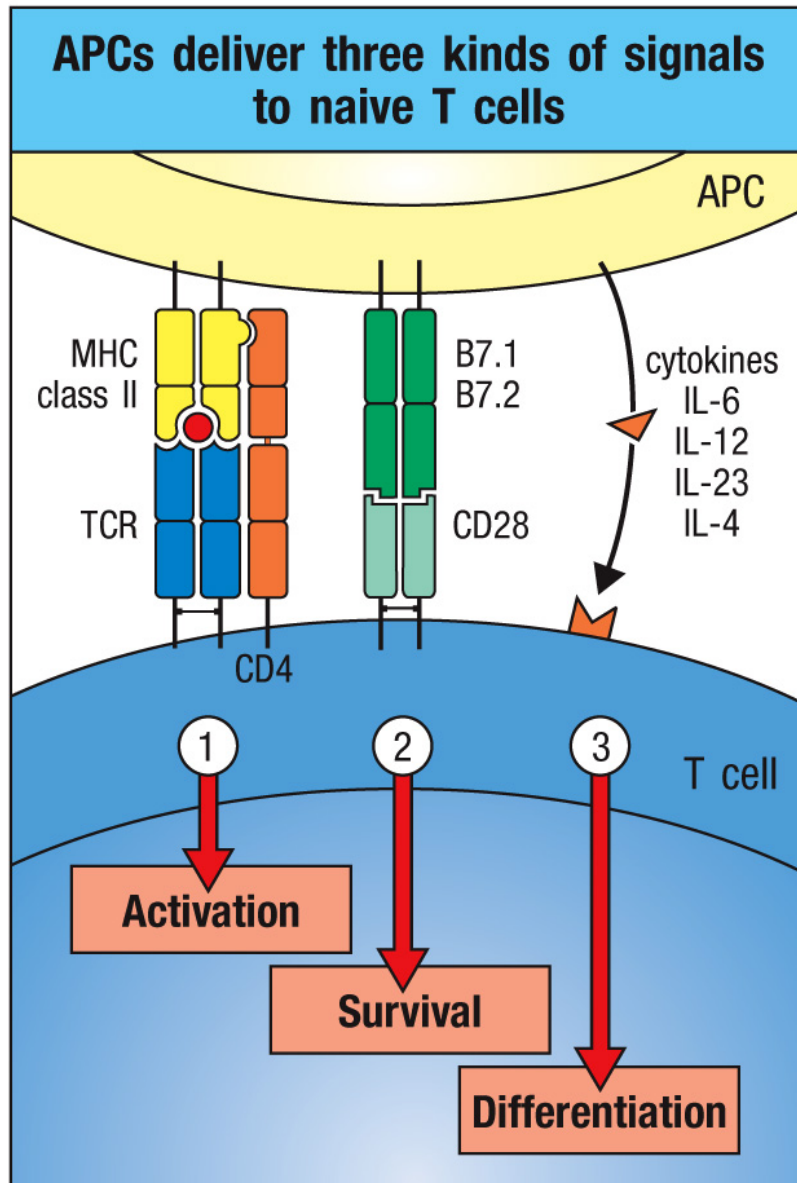


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CD28 Co-stimulation Induces IL-2 and IL-2R α (CD25) Expression on Activated T Cells

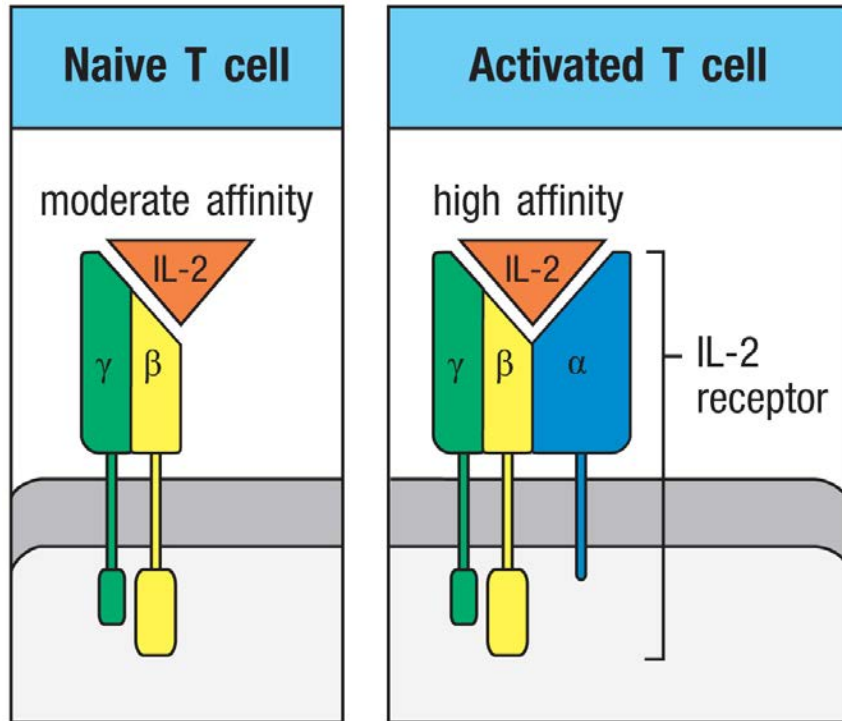


Figure 9.23 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Note: IL-2 at priming also programs secondary expansion of memory cells.

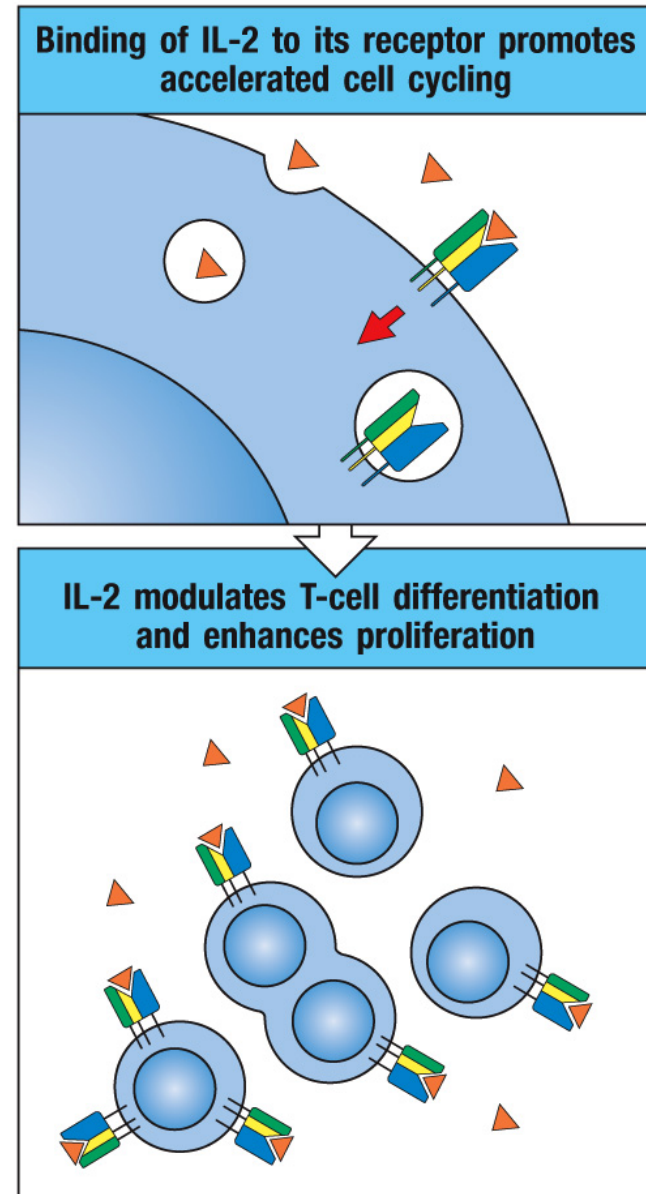
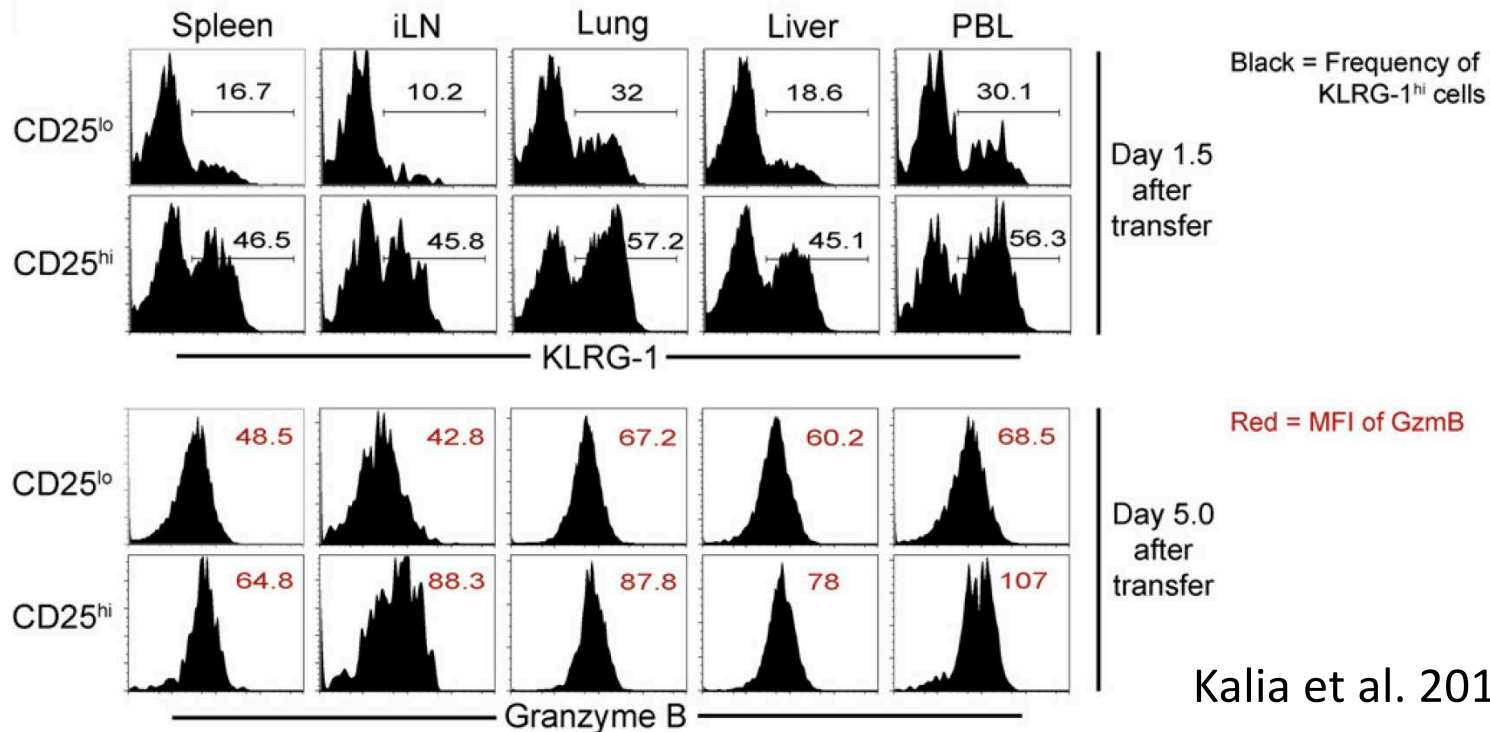
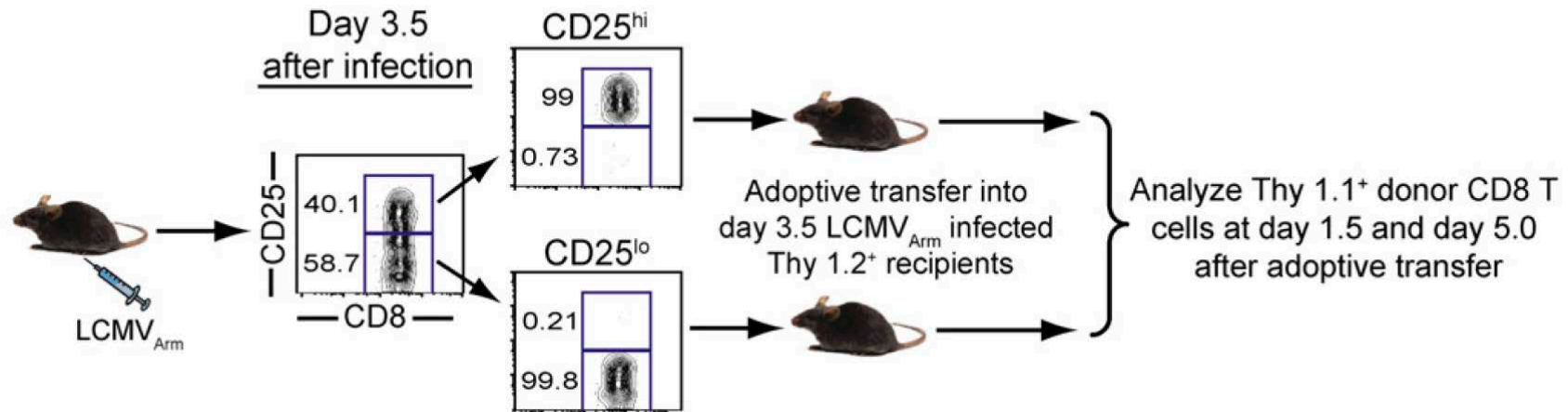
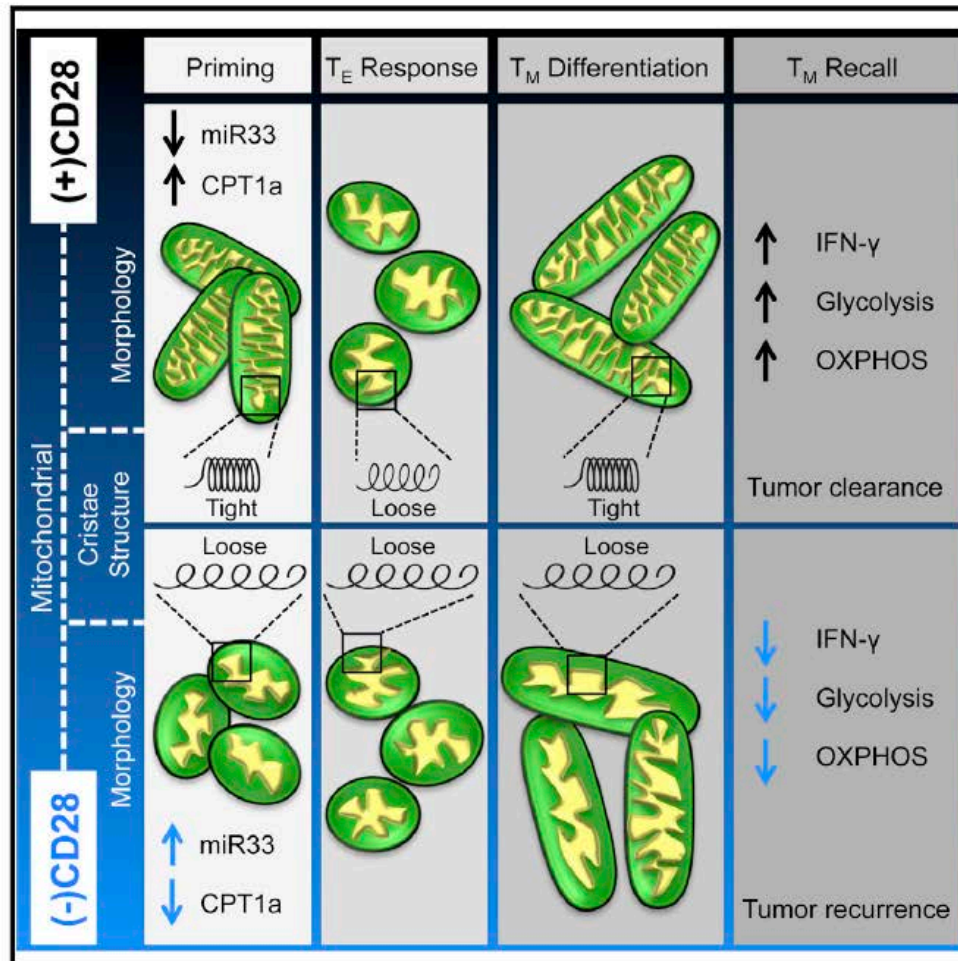


Figure 9.24 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Prolonged Interleukin-2R α Expression on Virus-Specific CD8⁺ T Cells Favors Terminal-Effector Differentiation In Vivo



Mitochondrial Priming by CD28



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In Brief

Costimulatory signals during the initial phase of T cell activation prime mitochondria with latent metabolic capacity essential for future T cell responses.

Highlights

- CD28 signals endow T cells with latent mitochondrial respiratory capacity
- CD28 regulates mitochondrial sphericity and cristae morphology in T cells
- CD28 costimulation restrains miR-33-dependent inhibition of Cpt1a expression
- CD28-mediated Cpt1a function supports generation of protective memory T cells

Activated T cells Express CD69 to Inhibit Their Egress from Lymph Node

- T cells that did not see their cognate antigen exit the LN by following sphingosine 1-phosphate (S1P) gradient via S1PR1.
- Activated T cells express CD69, which internalizes S1PR1, thus T cells cannot respond to S1P gradient and remain in LN.
- After T cell proliferation in LN, CD69 expression decreases and S1PR1 reappears.

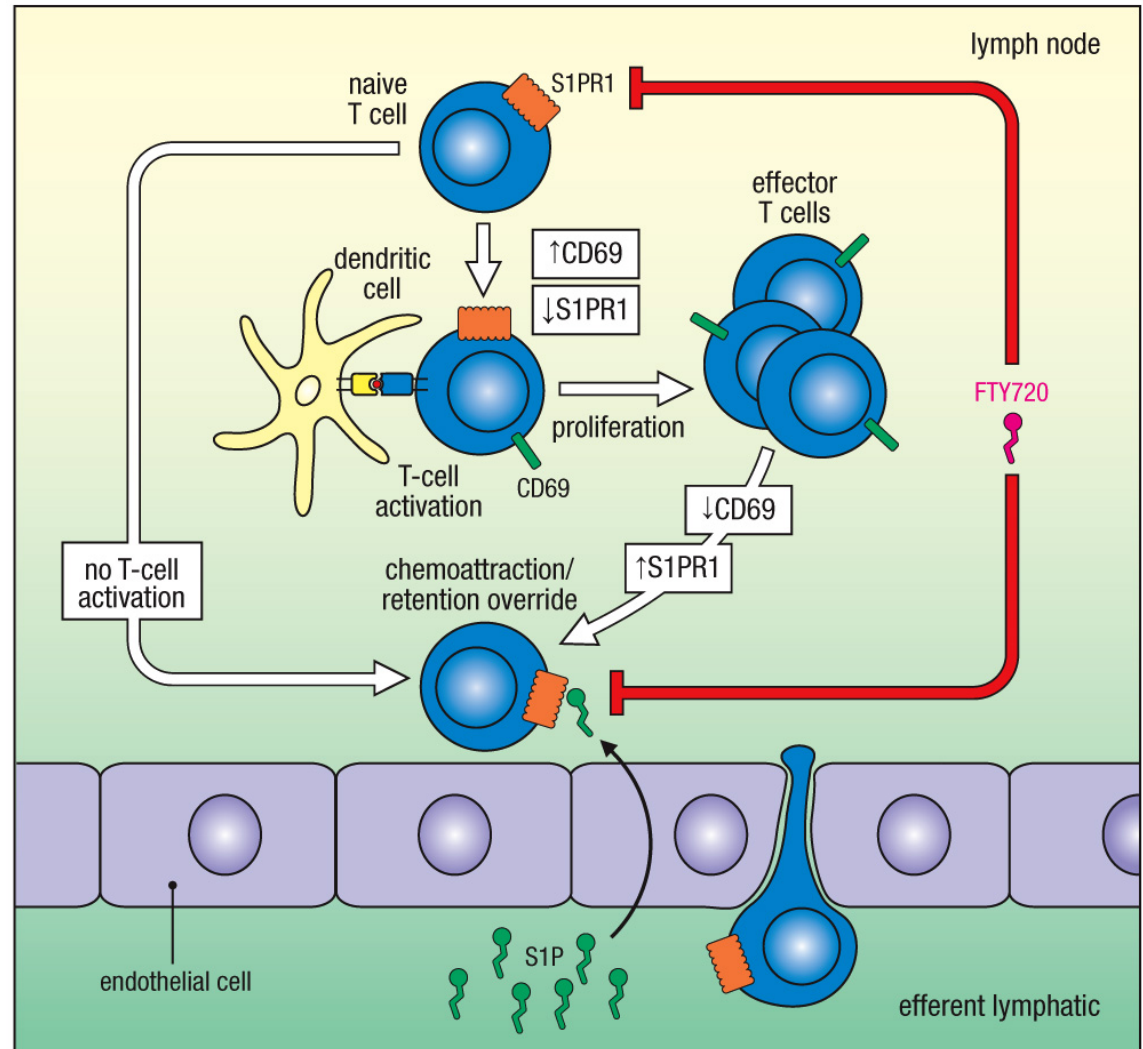


Figure 9.11 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

If Co-stimulation is Weak on DCs, CD4 T Cells Can “Help” CD8 T Cell Priming

1) CD4 T cells help further activate APC:

- B7 expressed by the DCs first activates the CD4 T cells to express IL-2 and CD40 ligand.
- CD40 ligand binds CD40 on the DC, delivering an additional signal that increases the expression of B7 and 4-1BBL by the DC.
- This provides additional co-stimulation to the naive CD8 T cell.

2) The IL-2 produced by activated CD4 T cells also acts to promote effector CD8 T-cell differentiation.

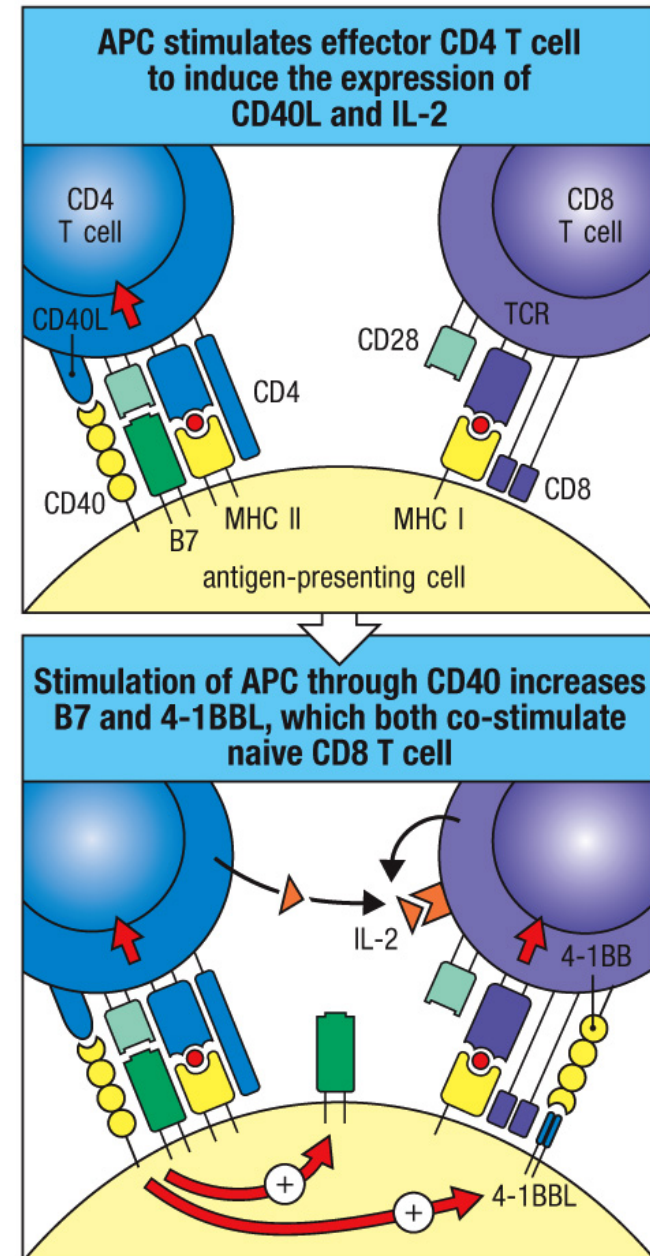


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Bystander CD8 T Cell Activation via IL-12 and IL-18

- Activated DCs that produce IL-12 and IL-18 can stimulate naïve CD8 T cells to produce IFN γ .
- CD8 produced IFN γ activates macrophages for the destruction of intracellular bacteria and can promote antiviral responses in other cells.

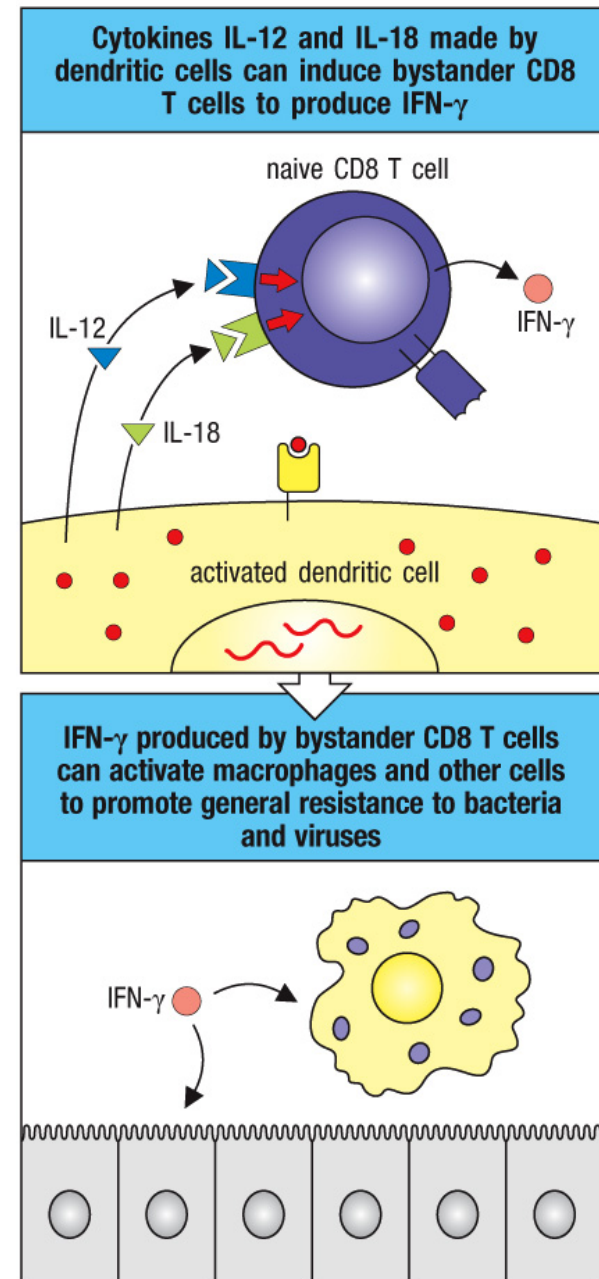
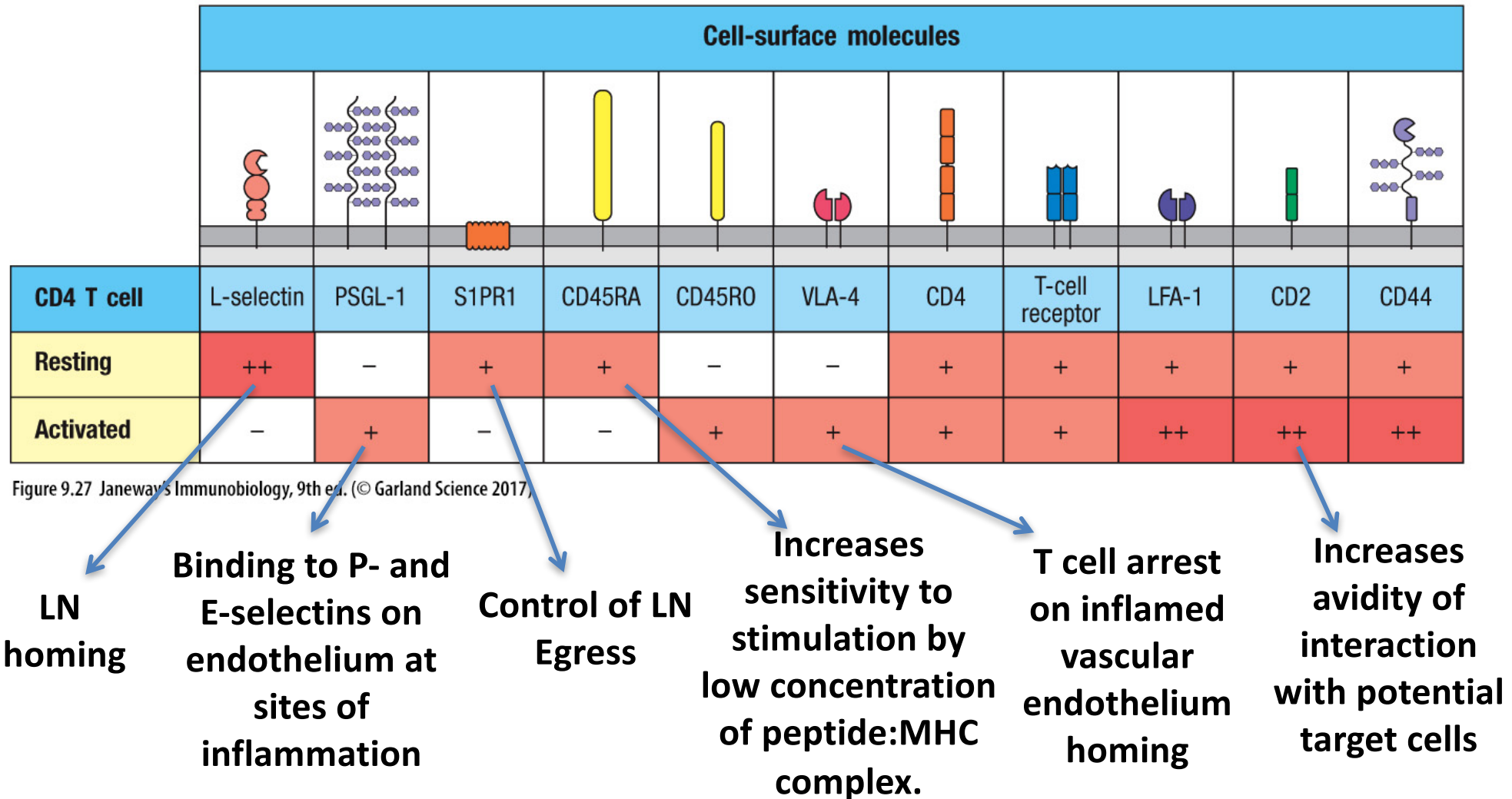


Figure 11.21 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

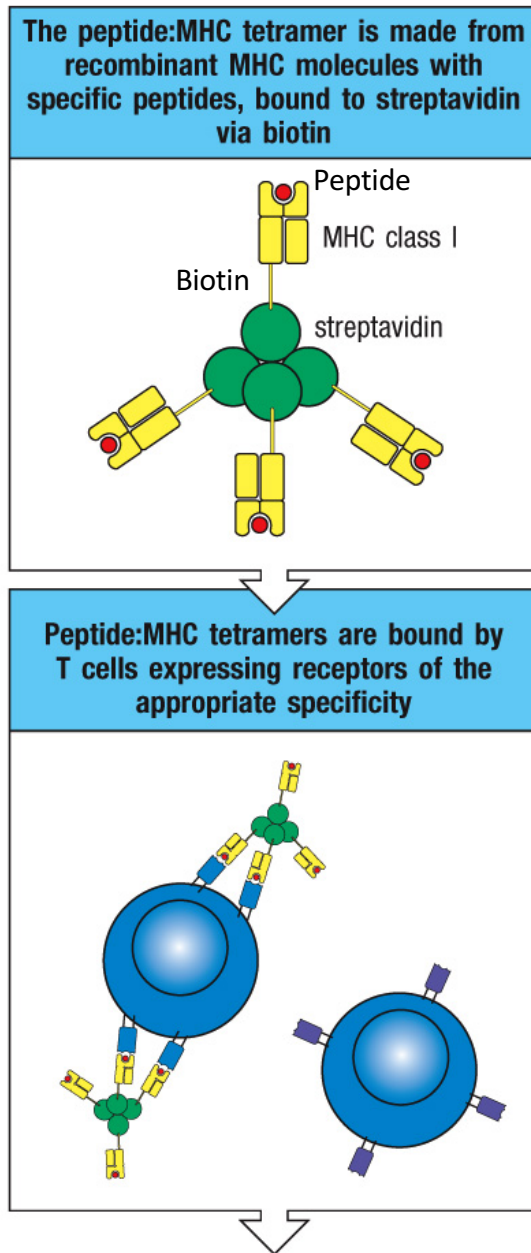
Activation of T Cells Changes the Expression of Several Cell-surface Molecules



Technical Side Notes:

- **Peptide:MHC Tetramers**
- **Antigen-specific TCR Transgenics**
- **Adoptive transfer of T cells**
- **CFSE labeling to monitor proliferation**
- **Monitoring T cell priming *in vivo*.**

Identification of T-cell Receptor Specificity Using Peptide:MHC Tetramers



Gated on CD3+ cells:

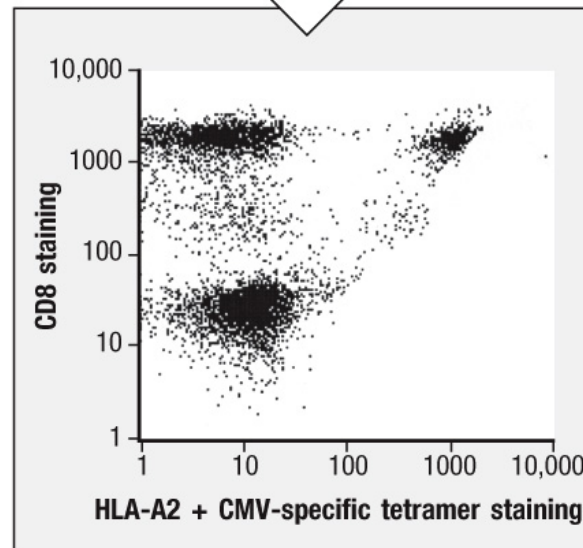
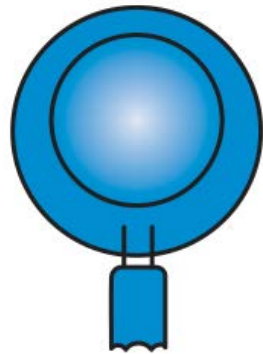


Figure A.30 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- Peptide:MHC tetramers are used to identify populations of antigen-specific T cells.
- Multimers of the peptide:MHC complex increase the avidity of the interaction with specific TCR.
- The streptavidin moiety is labeled with a fluorochrome to allow detection of those T cells capable of binding the peptide:MHC tetramer.

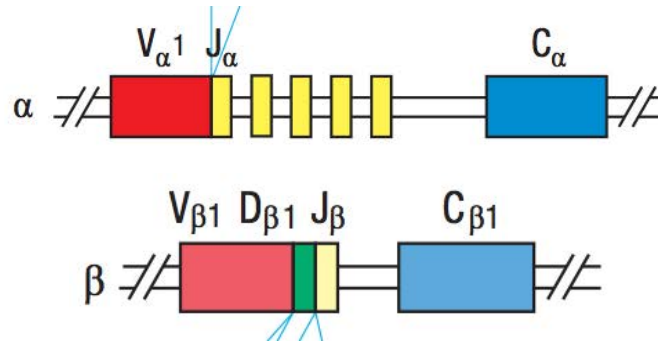
Antigen Specific TCR Transgenic Mice on Congenic Background

T cell clone with known TCR specificity and MHC restriction



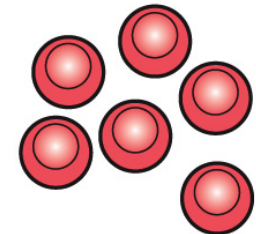
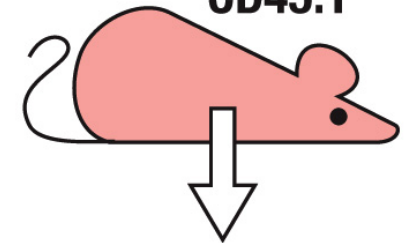
T-cell receptor

Generate rearranged α and β chain cDNA constructs



Inject into fertilized mouse ovum to create transgenic mice

Breed TCR transgenic mice to congenic and/or RAG^{-/-} background CD45.1



Create specific gene KO T cells with known Ag specificity.

100% congenically marked T cells with known specificity.

Adoptive Transfer of Congenically Marked Cells

- CD45.1 and CD45.2 are allelic variants of CD45, which is an abundant cell-surface receptor.
- These two alleles can be distinguished by specific antibodies (CD45.1 or CD45.2).
- Heterozygous mice are CD45.1.2, which is useful for co-adoptive transfers.

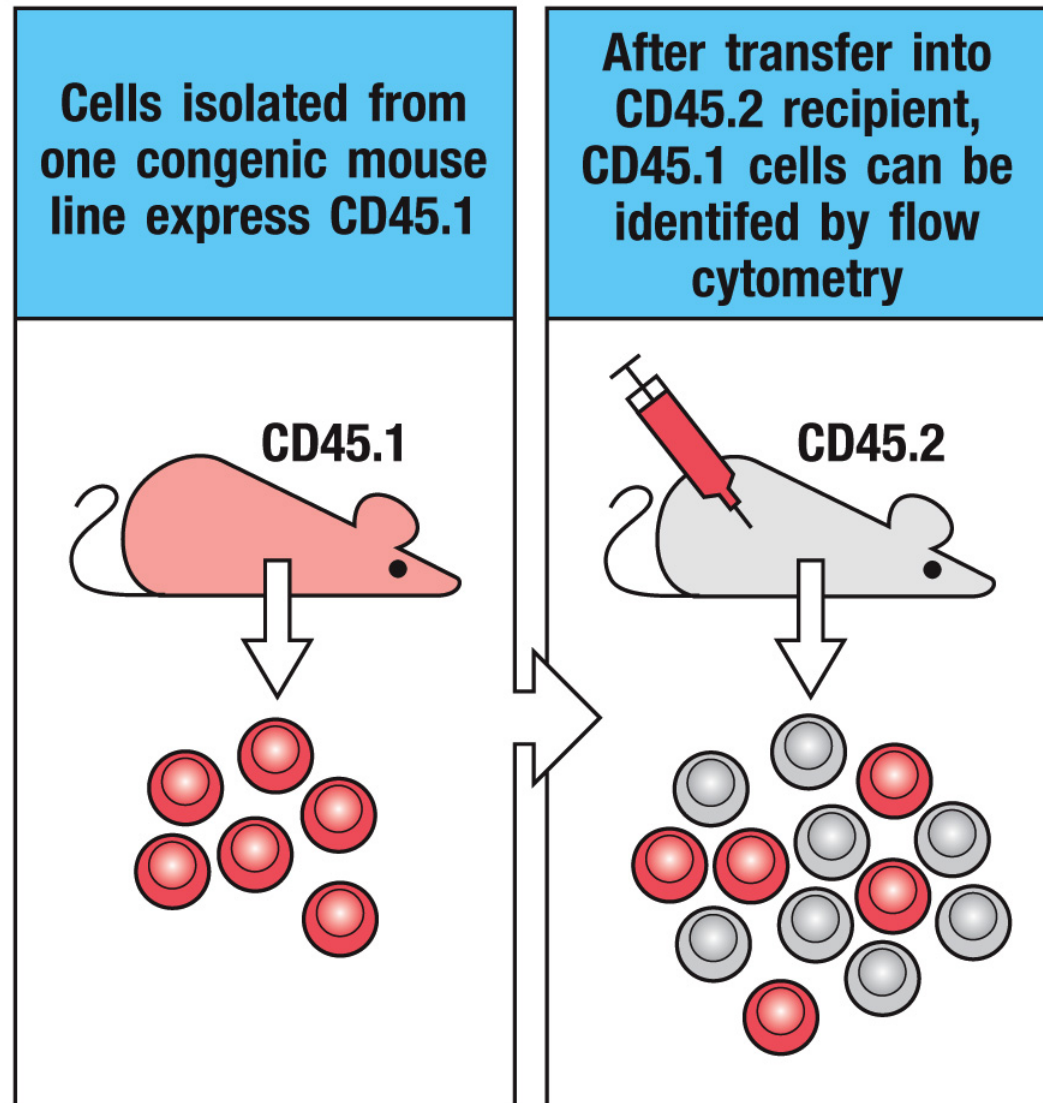


Figure A.41 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Flow Cytometric Assay for Cell Proliferation Based on CFSE Dilution

1) Incubate cells with a fluorescent dye such as carboxyfluorescein succinimidyl ester (CFSE).

2) Dye becomes covalently coupled to lysine residues on cellular proteins.

3) Each time the cell divides, each daughter cell inherits one-half of the CFSE-labeled proteins.

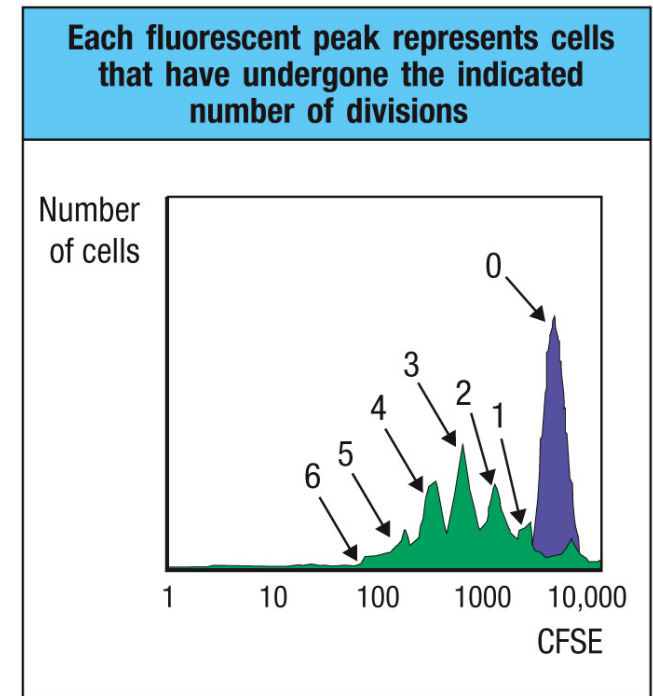
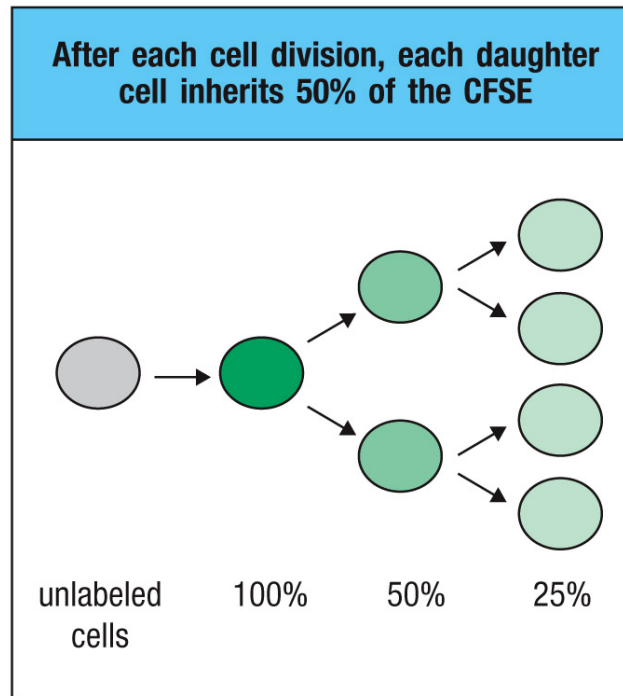


Figure A.33 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- Under optimal conditions, this assay is capable of detecting up to 7–8 cell divisions, after which CFSE fluorescence can no longer be measured.
- Bromodeoxyuridine (BrdU) or ki67 (antigen in nuclei of dividing cells) can be used to identify dividing cells.

II. Proliferation, Migration, CTL Activity

Proliferation, Differentiation, and Effector Function

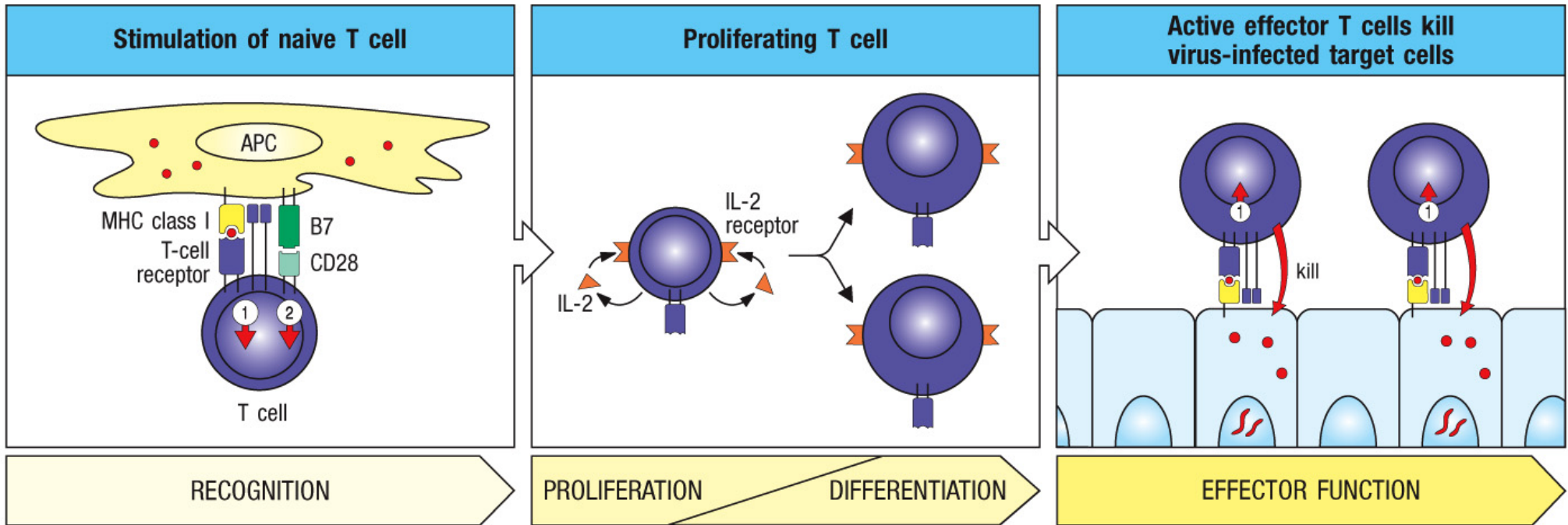


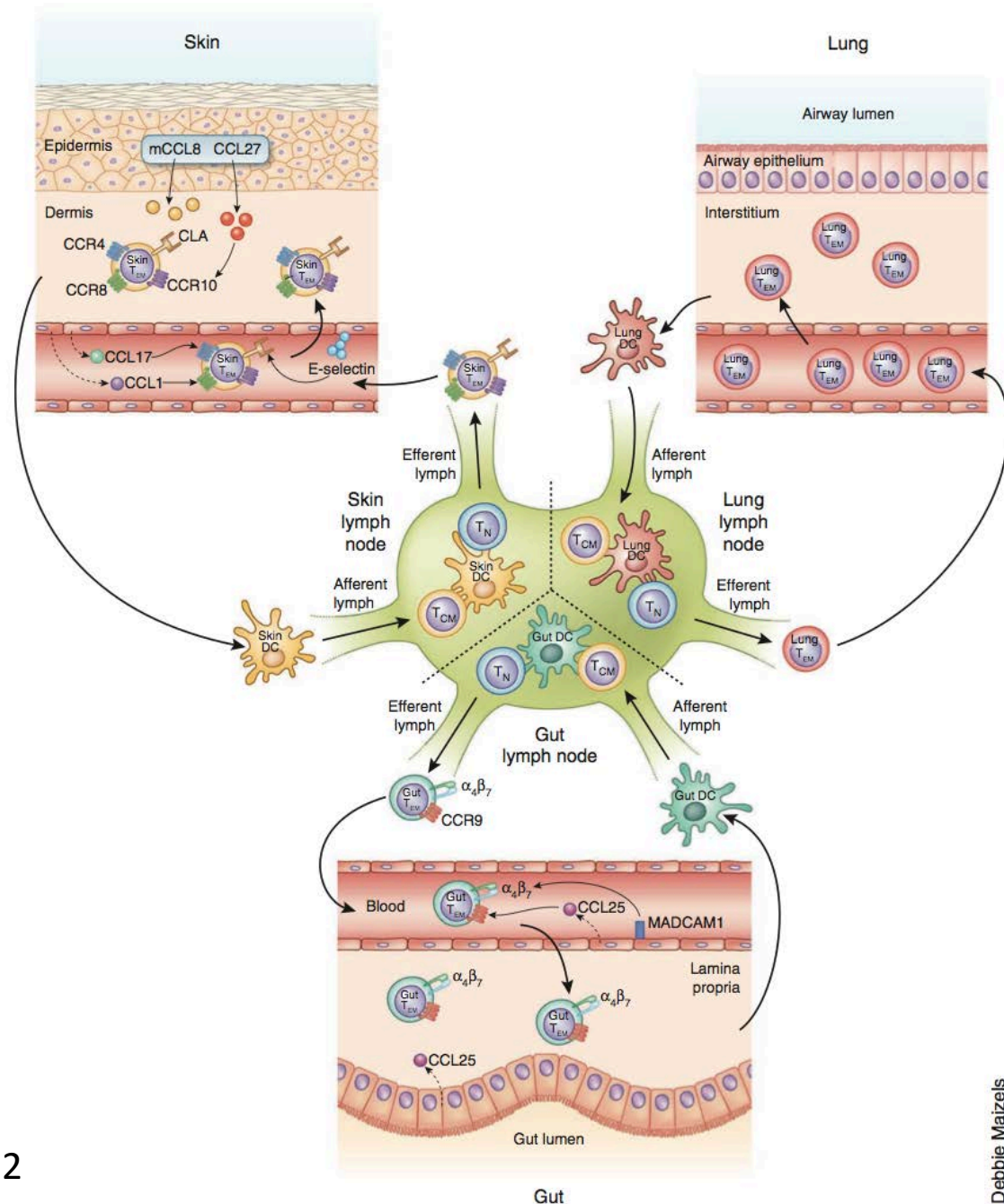
Figure 9.26 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- IL-2 signaling enhances clonal expansion and contributes to the differentiation of the T cells to effector cell status (and memory programming).
- Effector T cells leave the LN and migrate to site of infection.
- Any encounter that effector T cells have with specific antigen, triggers their effector actions without the need for co-stimulation.
- Thus, a CTLs can kill any virus-infected target cell, including those that do not express co-stimulatory molecules.

How do primed CTLs know where to go to exert their effector function?

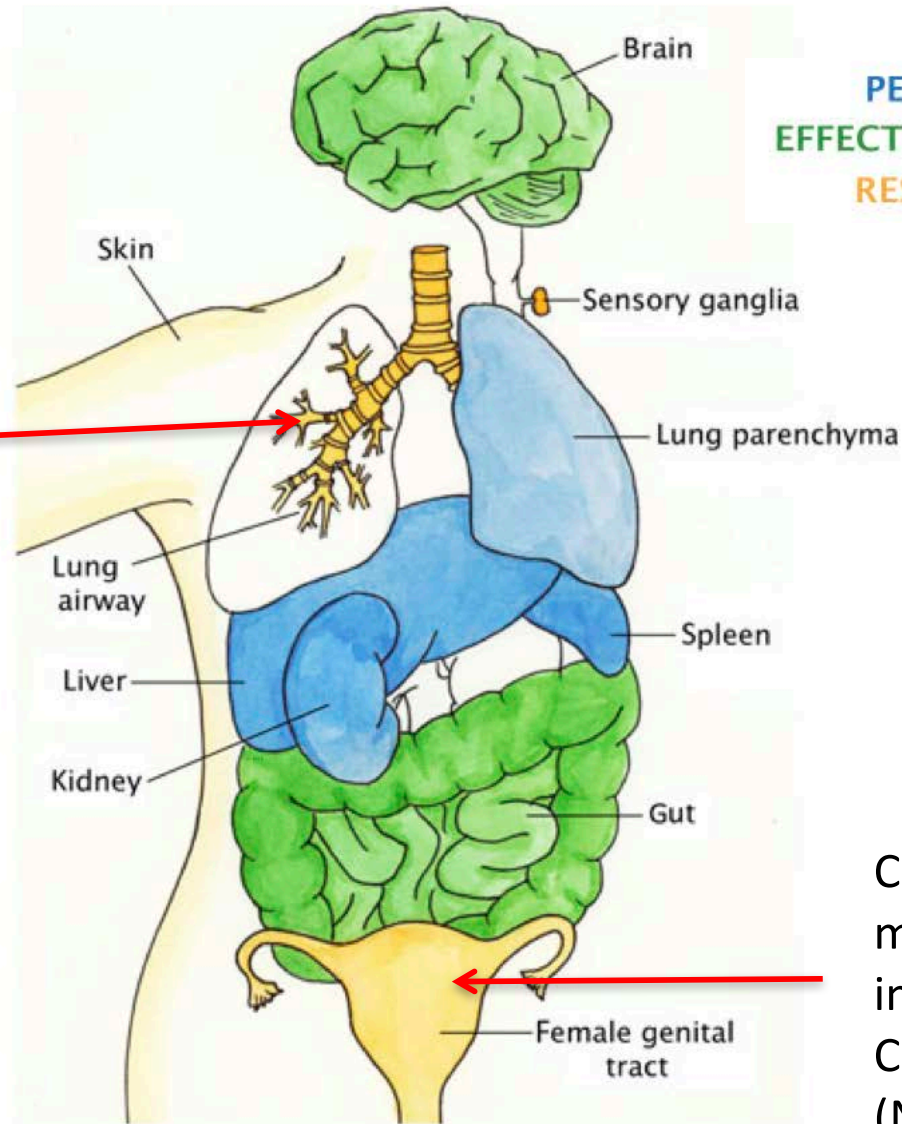
Antigen Sampling and Imprinting of the T cells for Migration Back to Site of Infection

Migratory DCs from various tissues can “imprint” T cells to express trafficking receptors that enable tissue-specific homing.



Restricted Effector T Cell Migration Into Some Tissues

Neutrophil trails guide influenza-specific CD8+ T cells in the airways.
(Lim et al. 2015 *Science*)



CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help.
(Nakanishi et al. 2009 *Nature*)

Interaction of CTLs with Their Infected Targets

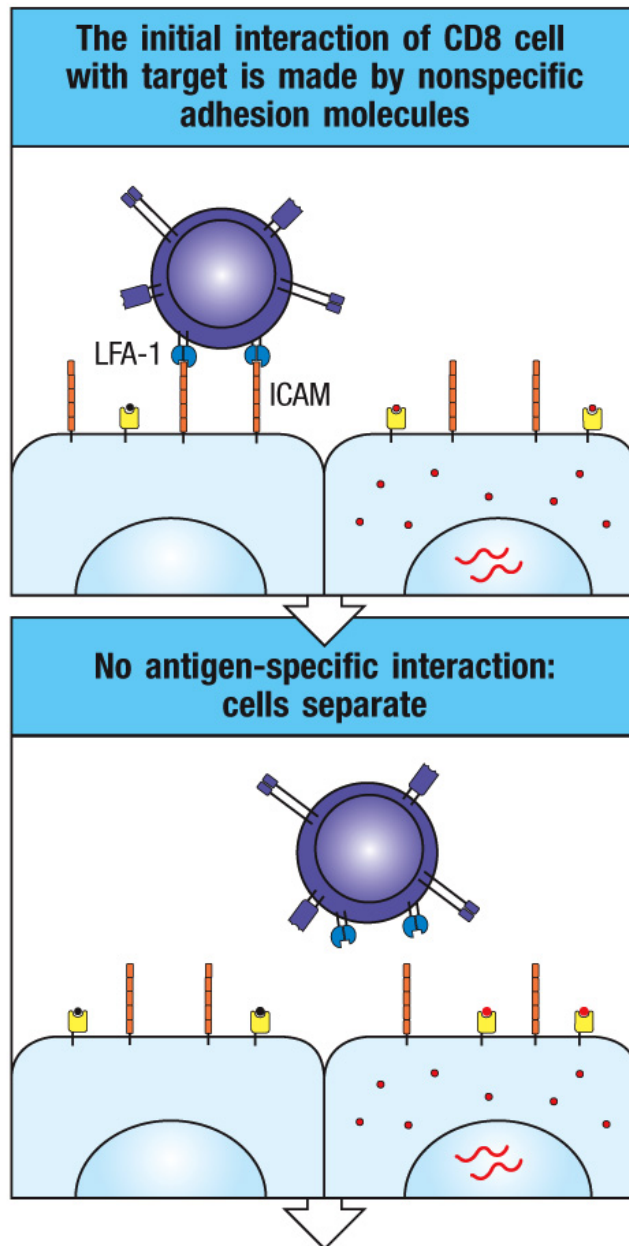


Figure 9.36 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

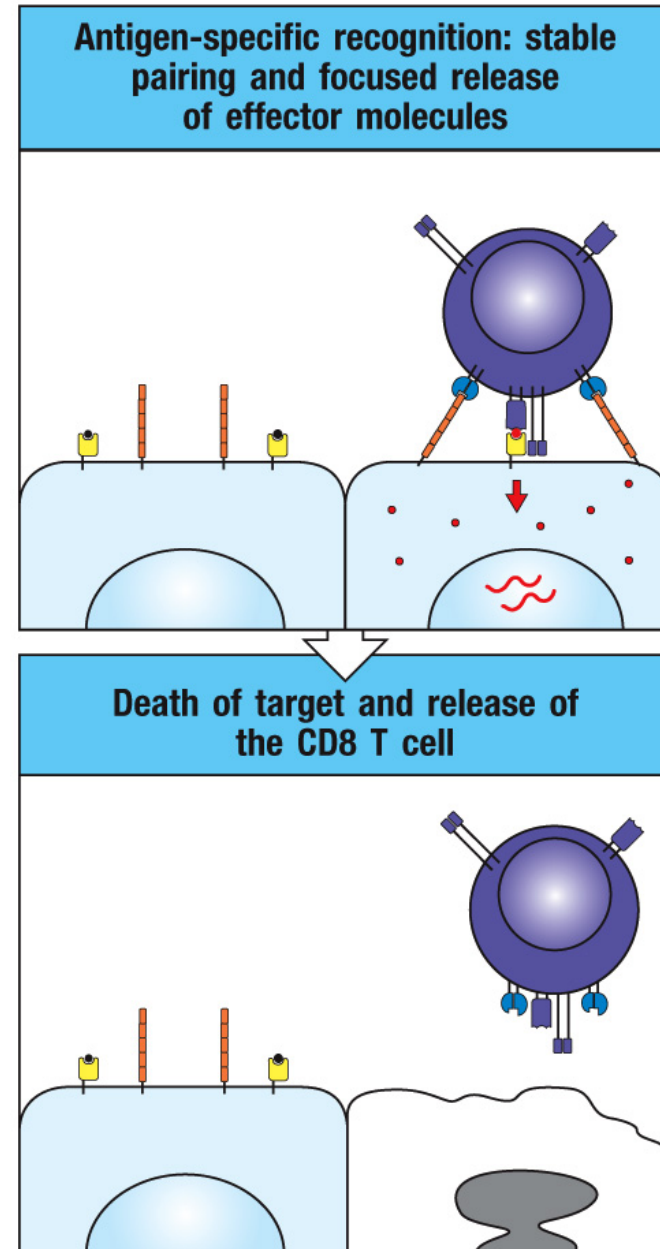


Figure 9.36 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Formation of Immunological Synapse

- Immunological synapse or the supramolecular activation complex (SMAC) is clustering of T-cell receptors and their associated co-receptors at the site of cell–cell contact.
- Central SMAC (cSMAC) contains most of the signaling proteins for T-cell activation.
- Peripheral (pSMAC) is the LFA-1 and the cytoskeletal protein talin, which connects LFA-1 to the actin cytoskeleton.

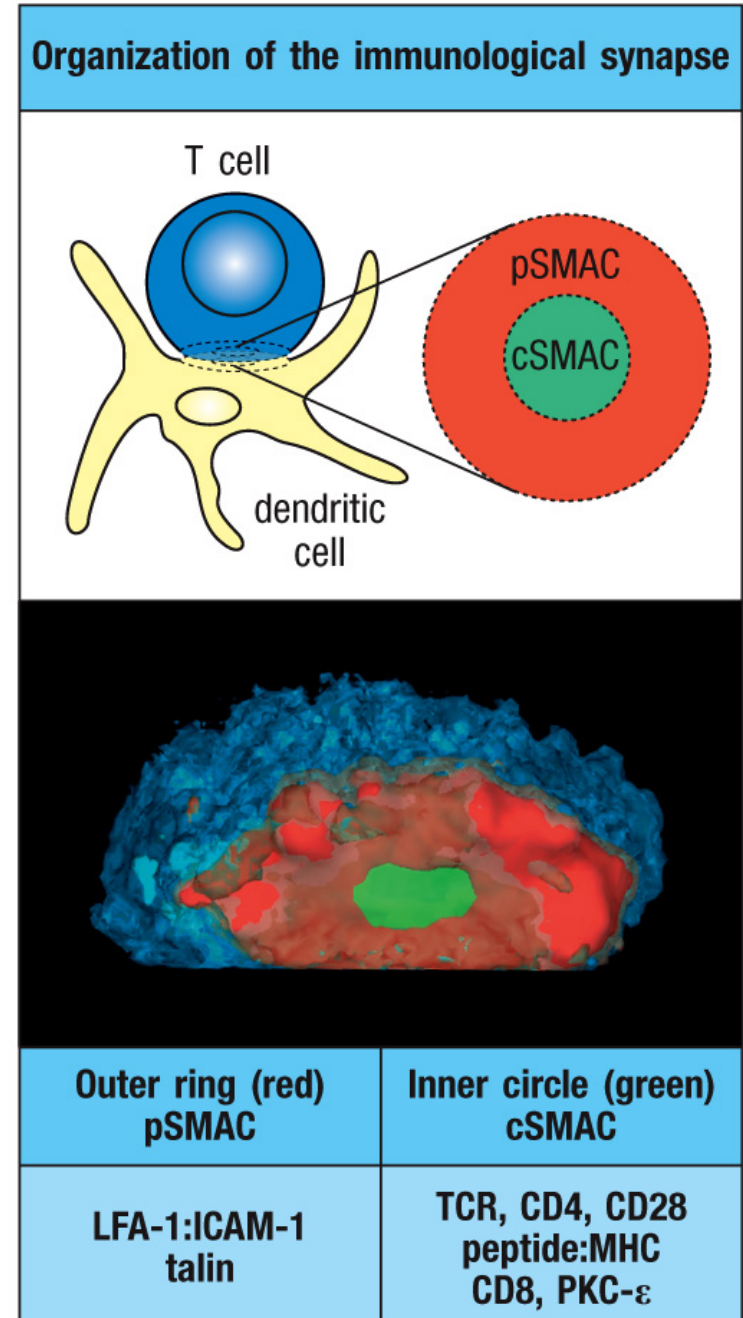


Figure 9.37 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Directed Release of Effector Molecules

T-cell receptor controls the delivery of effector signals in three ways:

- 1) it induces tight binding of effector cells to their target cells to create a narrow space in which effector molecules can be concentrated;
- 2) it focuses delivery of effector molecules at the site of contact by inducing a reorientation of the secretory apparatus of the effector cell;
- 3) it triggers the synthesis and/or release of the effector molecules.

Effector T-cell activity is thus highly selective for appropriate target cells, even though effector molecules themselves are not antigen-specific.

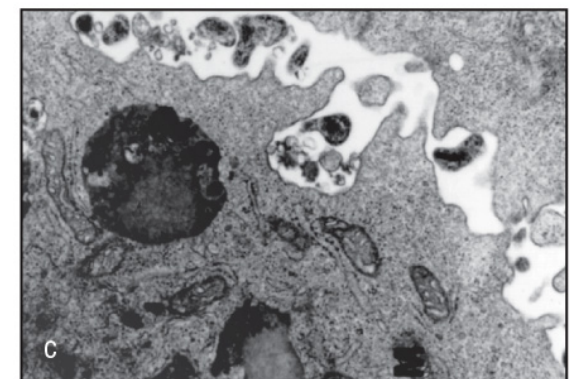
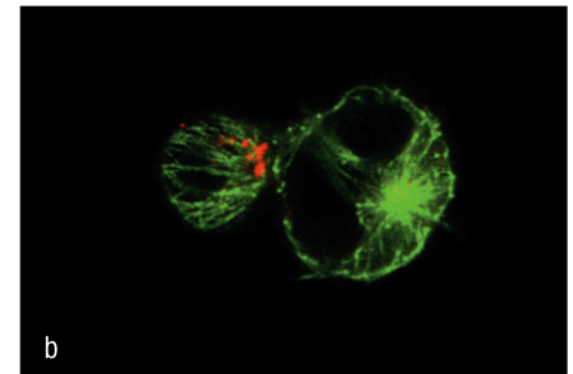
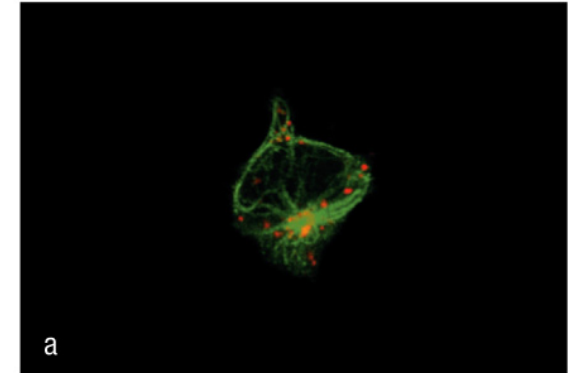
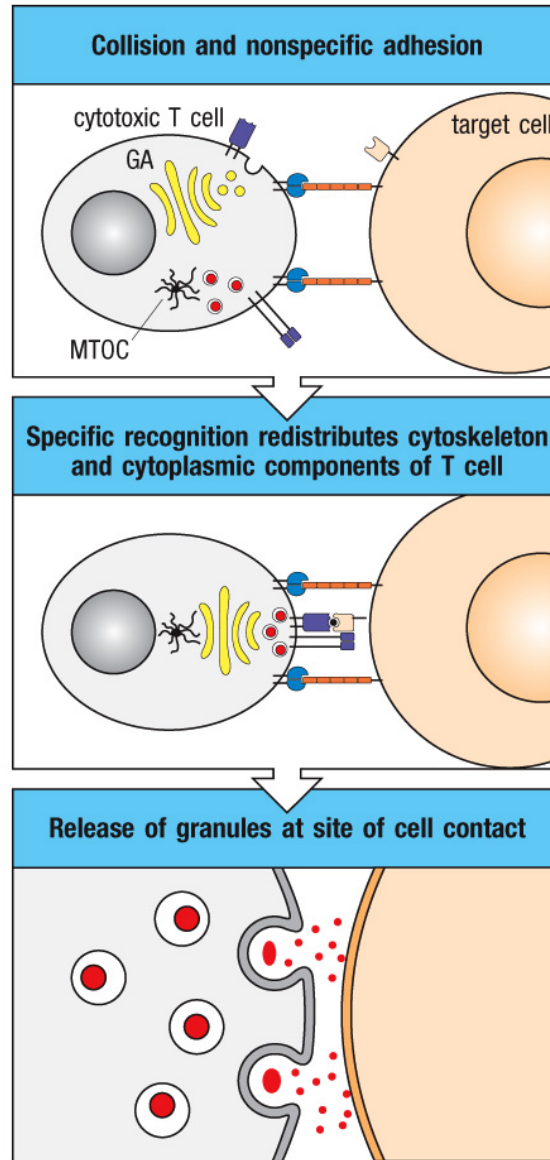


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Different Mechanisms of Killing by CTLs

CTLs release:

- Perforin – Aids in delivering contents of granules into the cytoplasm of target cell
- Granzymes – Serine proteases, which activate apoptosis once in the cytoplasm of the target cell
- Granulysin (not in mice) – Has antimicrobial actions and can induce apoptosis
- Effector cytokines to increase MHC-I; activate macrophages and induce NO production; activate NK cells and neutrophils; and enhance anti-viral activity.

CTLs carry:

- Fas ligand (CD178) – membrane-bound effector molecule, and when it binds to Fas (CD95) on a target cell, it activates apoptosis in the Fas-bearing cell.

CD8 T cells: peptide + MHC class I	
Cytotoxic (killer) T cells	
Cytotoxic effector molecules	Others
Perforin Granzymes Granulysin Fas ligand	IFN- γ LT- α TNF- α

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Extrinsic Pathway of Apoptosis (FasL/Fas)

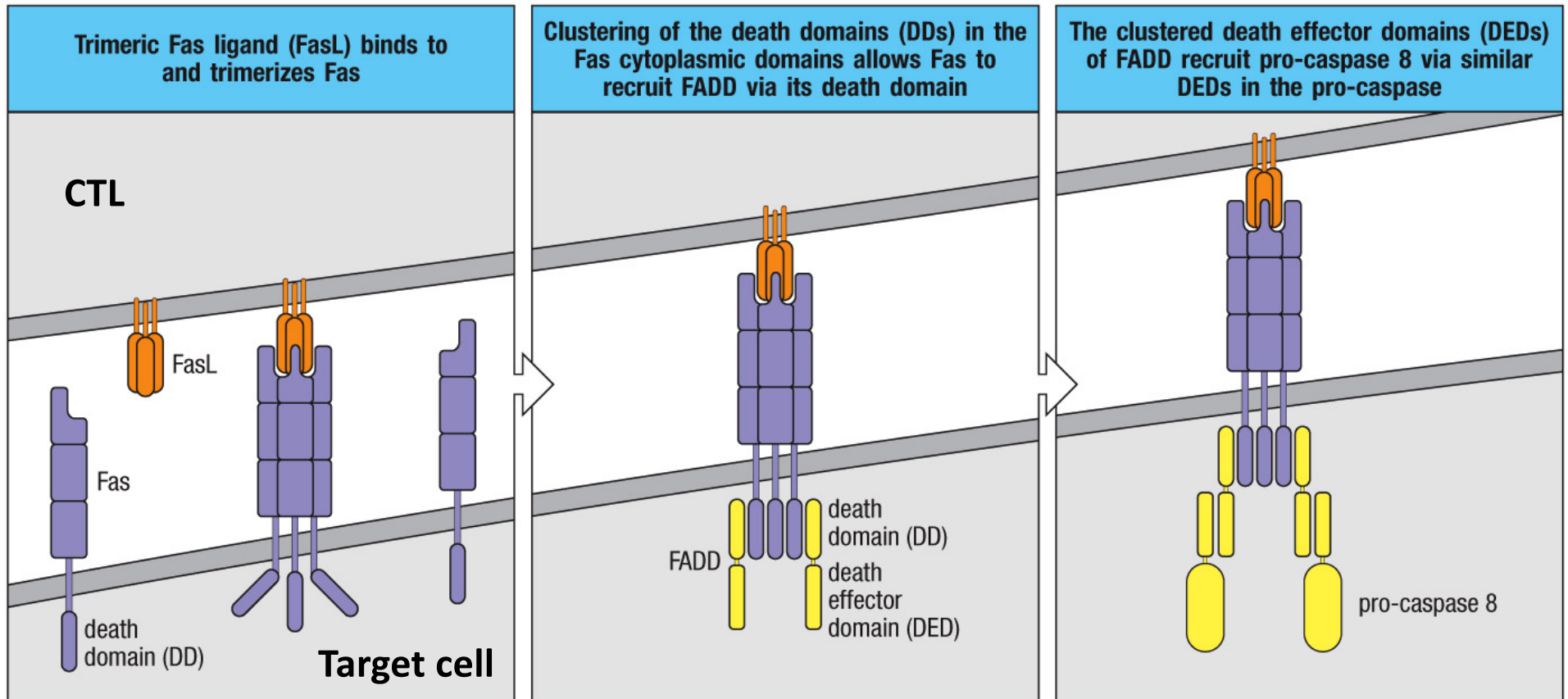


Figure 11.22 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Intrinsic Pathway of Apoptosis is Mediated by the Release of Cytochrome *c* from Mitochondria

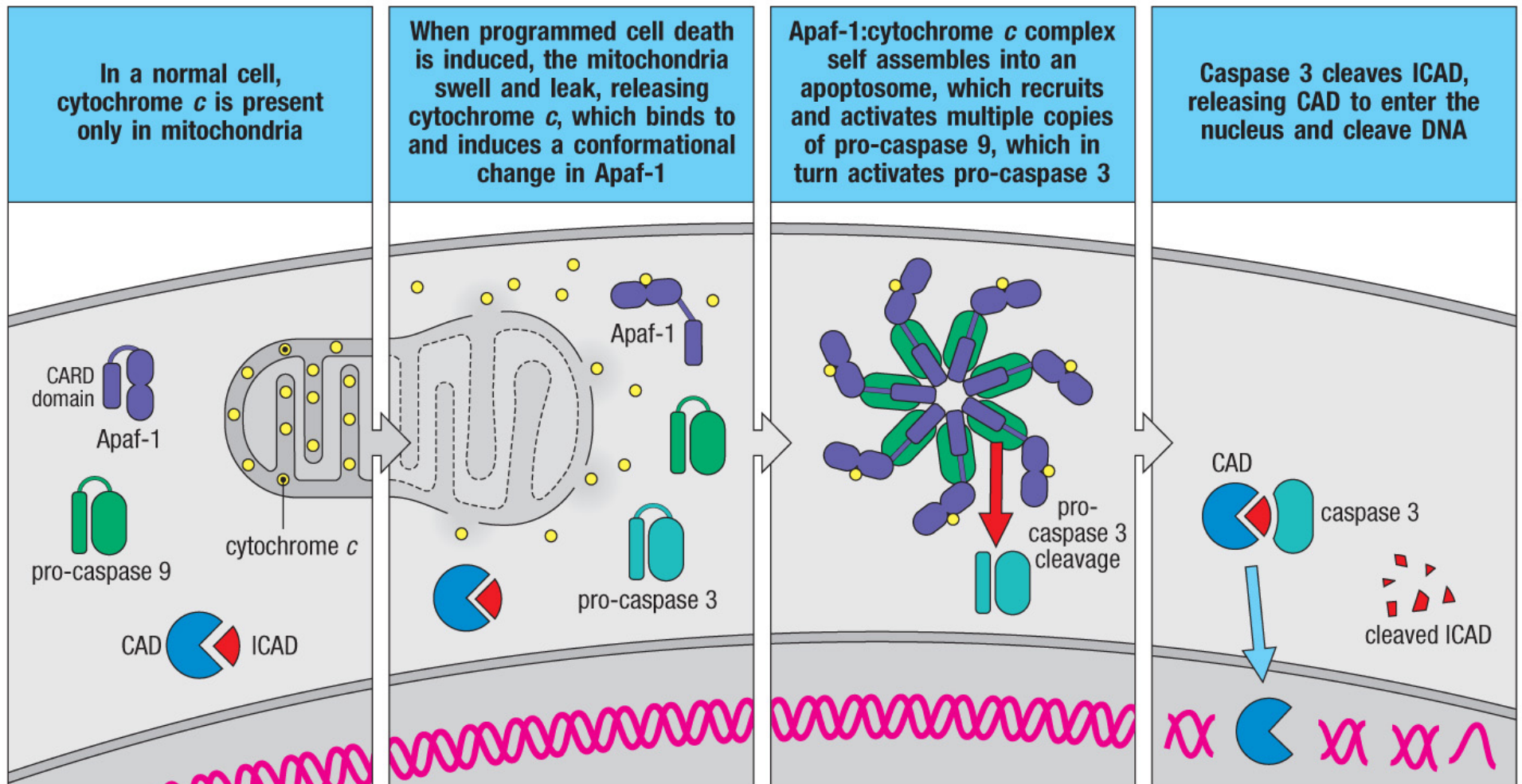


Figure 9.42 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Intrinsic Pathway of Apoptosis is Regulated by the Bcl-2 Family of Proteins

- Pro-apoptotic executioners bind to mitochondrial membranes and can directly cause cytochrome *c* release. (Form pores in the membranes?)
- Anti-apoptotic protectors bind to the mitochondrial membrane to block the release of cytochrome *c*. (Direct blocking the function of the pro-apoptotic family members?)
- Sentinels either block the activity of the anti-apoptotic proteins or stimulate the activity of the executioner pro-apoptotic proteins.

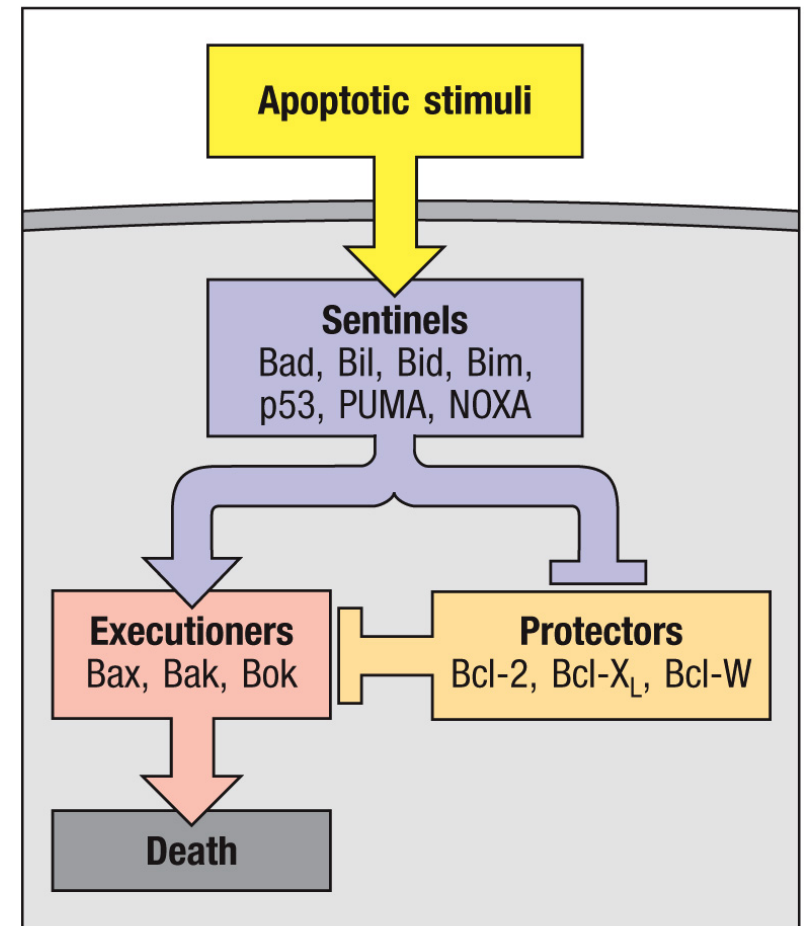


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Targeted Induction of Apoptosis by CTLs

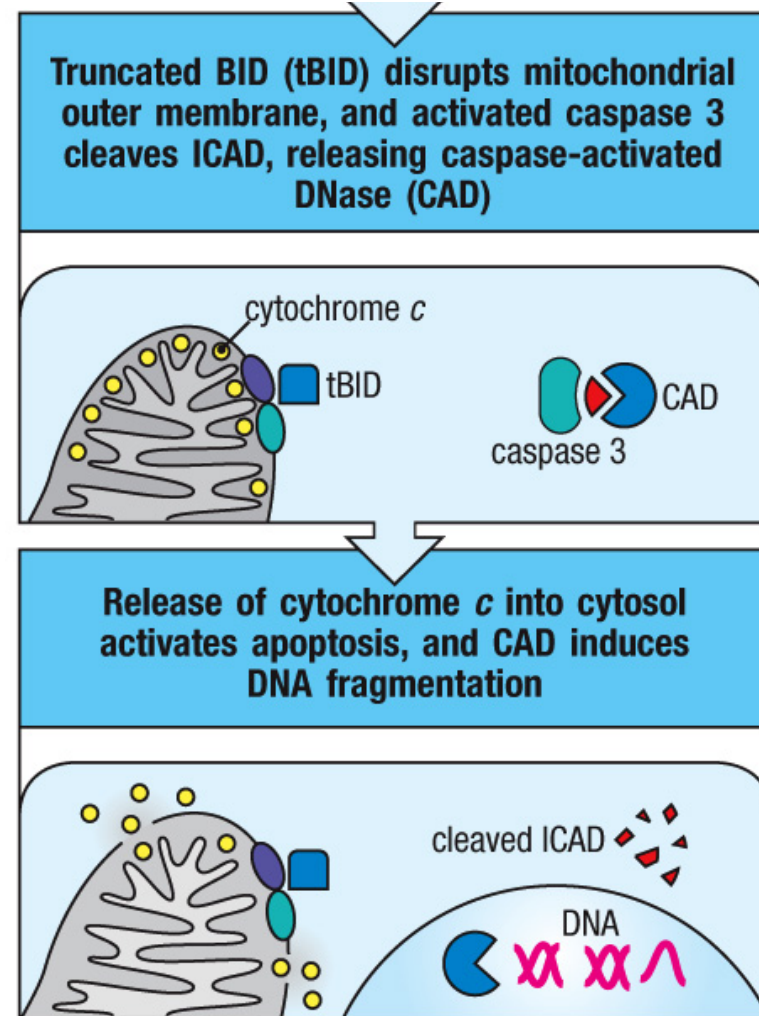
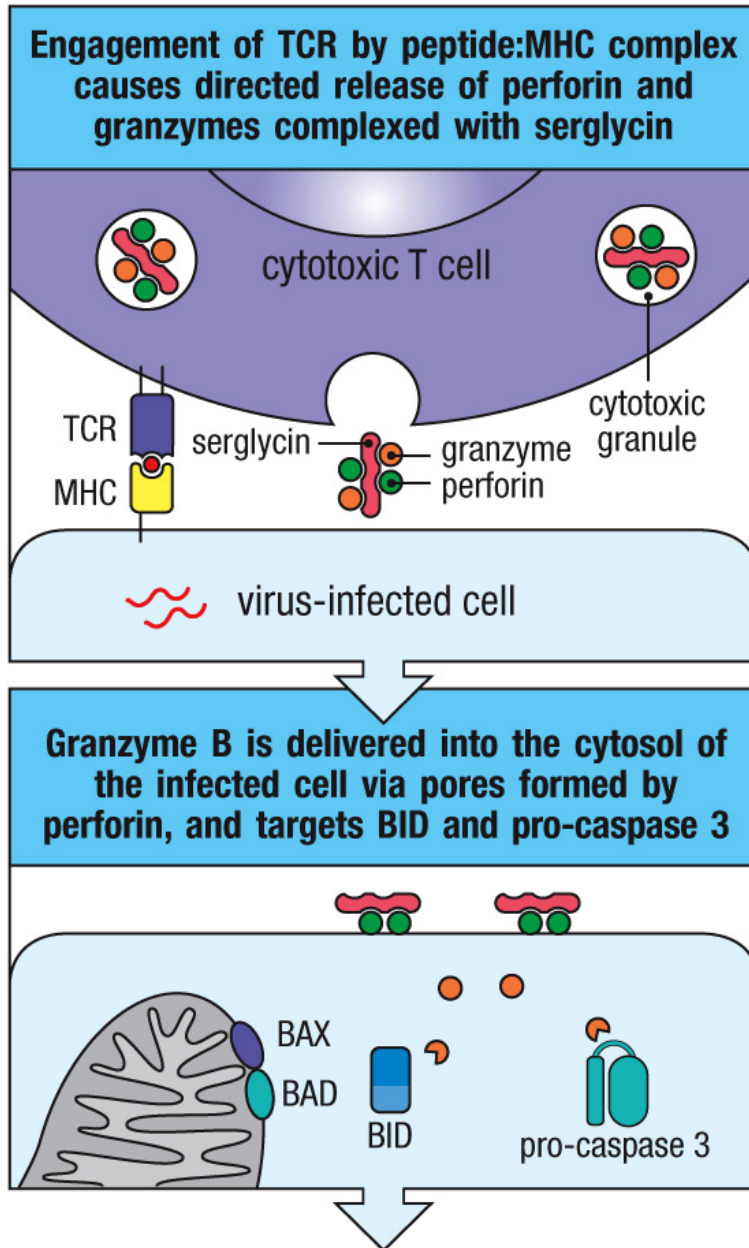


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Technical Side Notes:

- **Methods to measure apoptosis**
 - TUNEL
 - Annexin V
 - Ab detection of active Caspase 3/7
- **Measuring CTL activity *in vitro***
- **Measuring CTL activity *in vivo***

Detection of apoptotic cells with TUNEL Stain

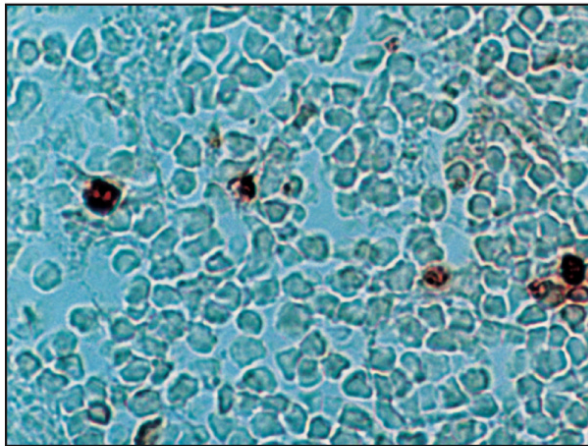
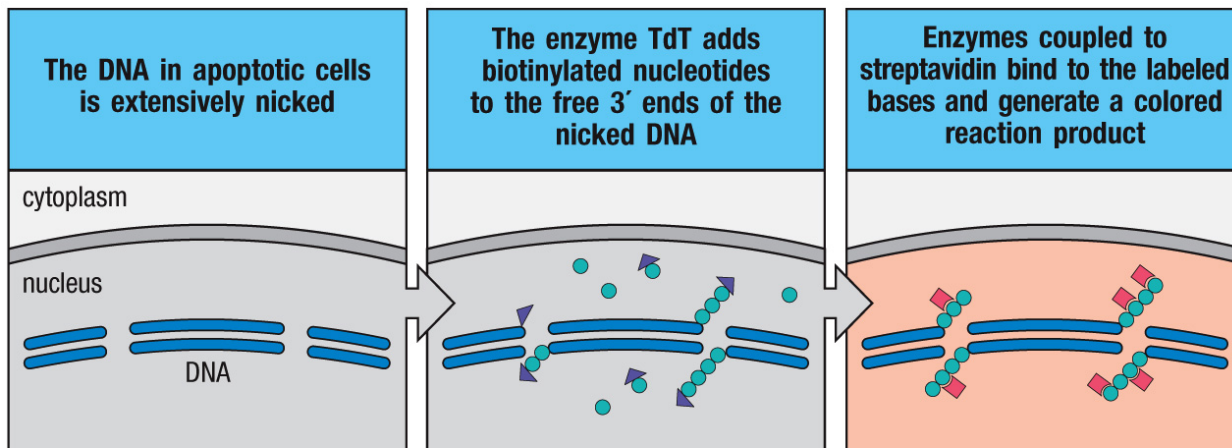


Figure A.35 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

TUNEL (TdT- dependent dUTP–biotin nick end labeling)

- The 3' ends of the DNA fragments generated in apoptotic cells are labeled with biotin-coupled uridine by using the enzyme terminal deoxynucleotidyl transferase (TdT).
- The biotin label is then detected with enzyme-tagged streptavidin, which binds to biotin.
- When the colorless substrate of the enzyme is added to a tissue section or cell culture, it produces a colored precipitate only in cells that have undergone apoptosis.

Detection of apoptotic cells with Annexin V

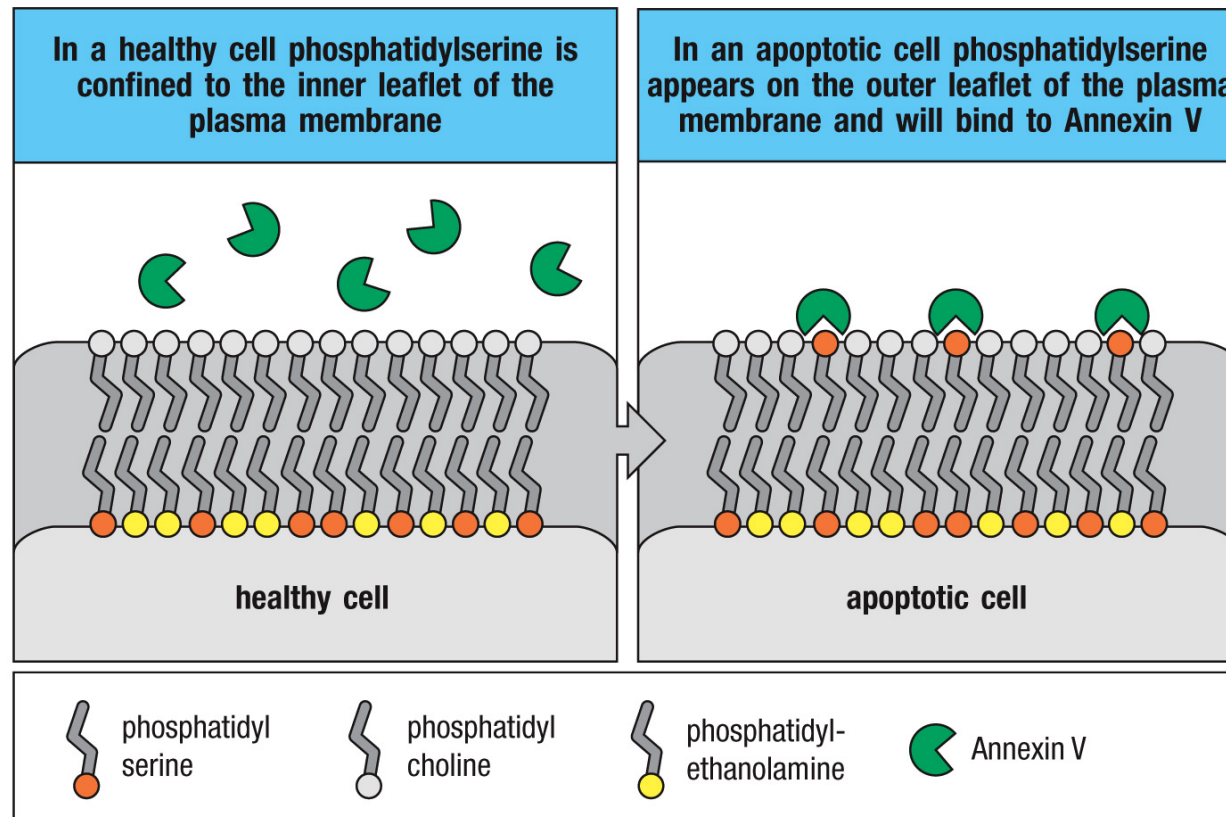


Figure A.36 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **When cells undergo apoptosis, the enzyme responsible for maintaining phosphatidylserine (PS) polarity, called flippase, is no longer active.**
- **As a result, PS's polar head groups become exposed on the extracellular face of the plasma membrane.**
- **Fluorescently labeled Annexin V can bind tightly to the exposed polar head.**
- **Annexin V staining is often combined with a viability dye such as propidium iodide (PI) or 7-aminoactinomycin D (7-AAD).**

Detection of Apoptotic Cells by Intracellular Staining for Active Caspases

- When cells are undergoing apoptosis, pro-caspase 3 is cleaved into two subunits that dimerize to form the active enzyme.
- Fluorescently labeled antibodies can be used to detect the active form of caspase 3 in fixed and permeabilized cells.

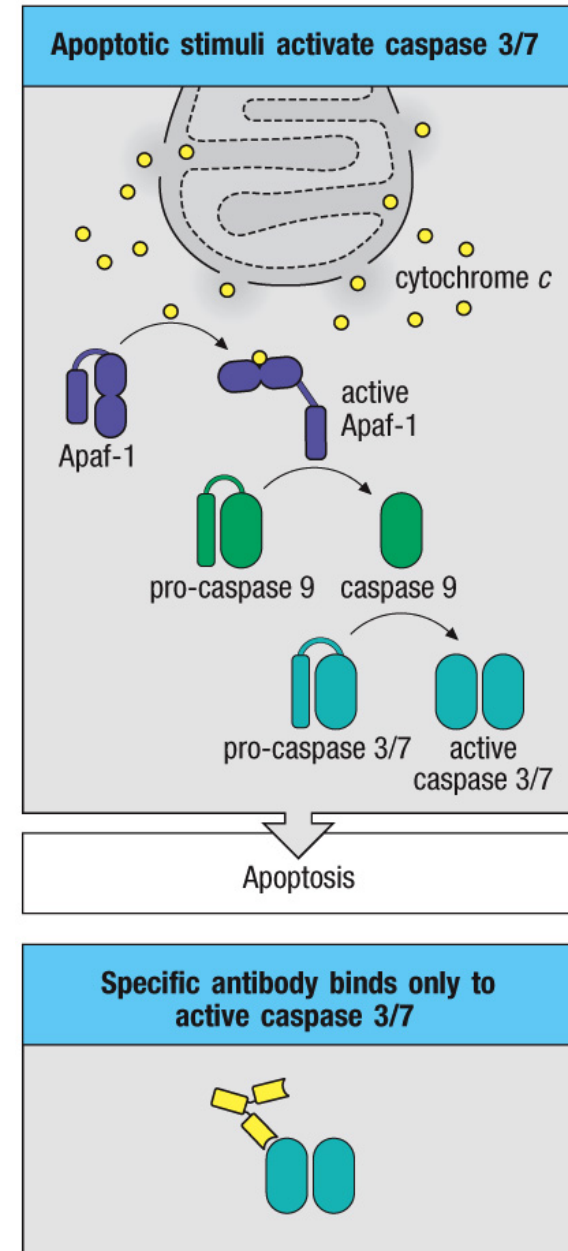


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In Vitro CTL Activity as Measured by Chromium Release From Labeled Target Cells

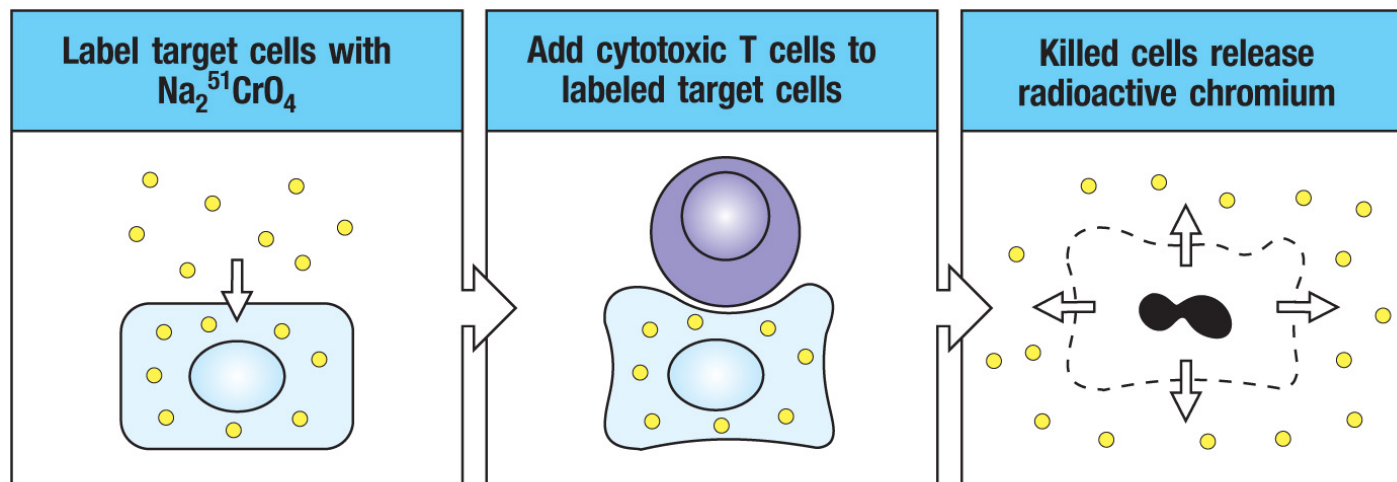


Figure A.38 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- Live cells will take up, but do not spontaneously release, radioactively labeled sodium chromate, $\text{Na}_2^{51}\text{CrO}_4$.
- When labeled cells are killed, the radioactive chromate is released and it can be measured in the supernatant.

In Vivo CTL Activity as Measured by Killing of CFSE Labeled Target Cells

- Target cells are incubated with MHC-I restricted antigenic peptide.
- These cells are incubated with a low concentration of CFSE.
- A control population of cells that is not given the antigenic peptide is incubated with a high concentration of CFSE.
- The two cell populations are mixed 1:1 and injected into experimental animals.
- Four hours later, spleen cells are analyzed by FACS.
- Specific target-cell lysis is calculated from the ratio of the two CFSE-labeled cell populations.

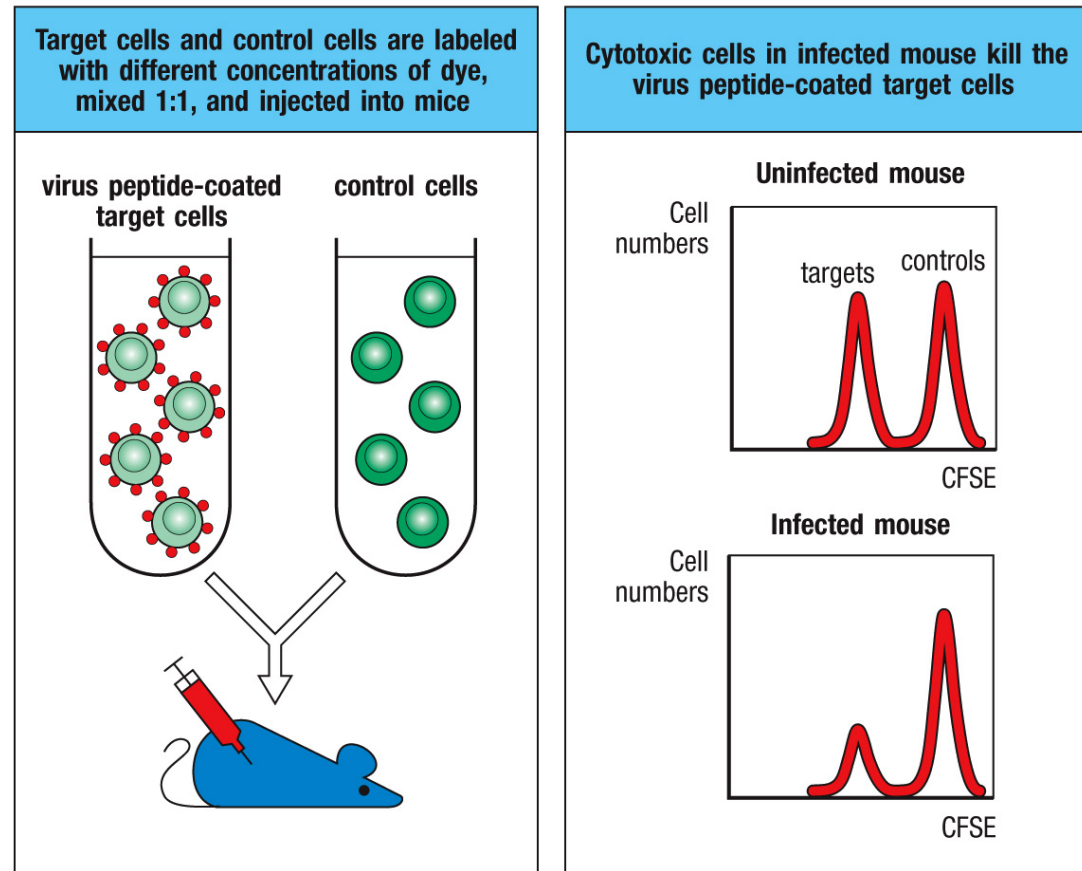
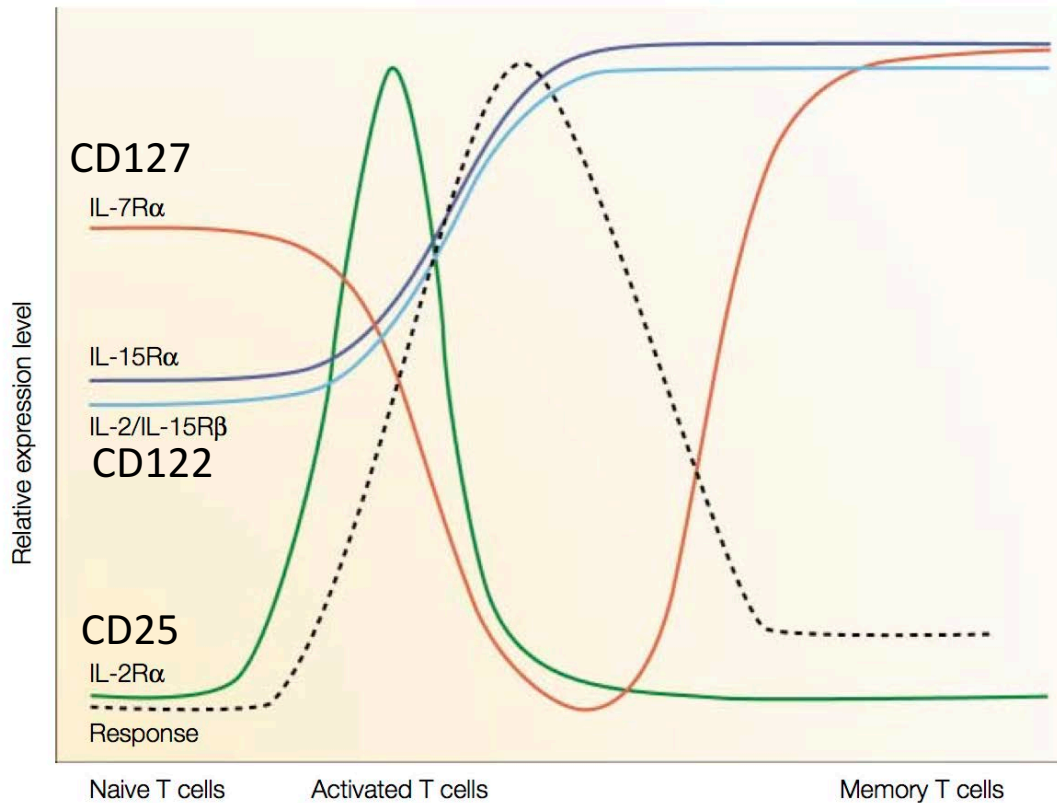


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III. Contraction and Memory Precursor Formation

Common γ c Cytokines Play an Important Role in Effector to Memory Transition



Schluns & Lefrancois 2003 *Nat Rev Immunol*

For mitogenic and anti-apoptotic signals:

- Naïve cells depend on IL-7
- Effector cells depend on IL-2
- Memory cells depend on IL-7 and IL-15

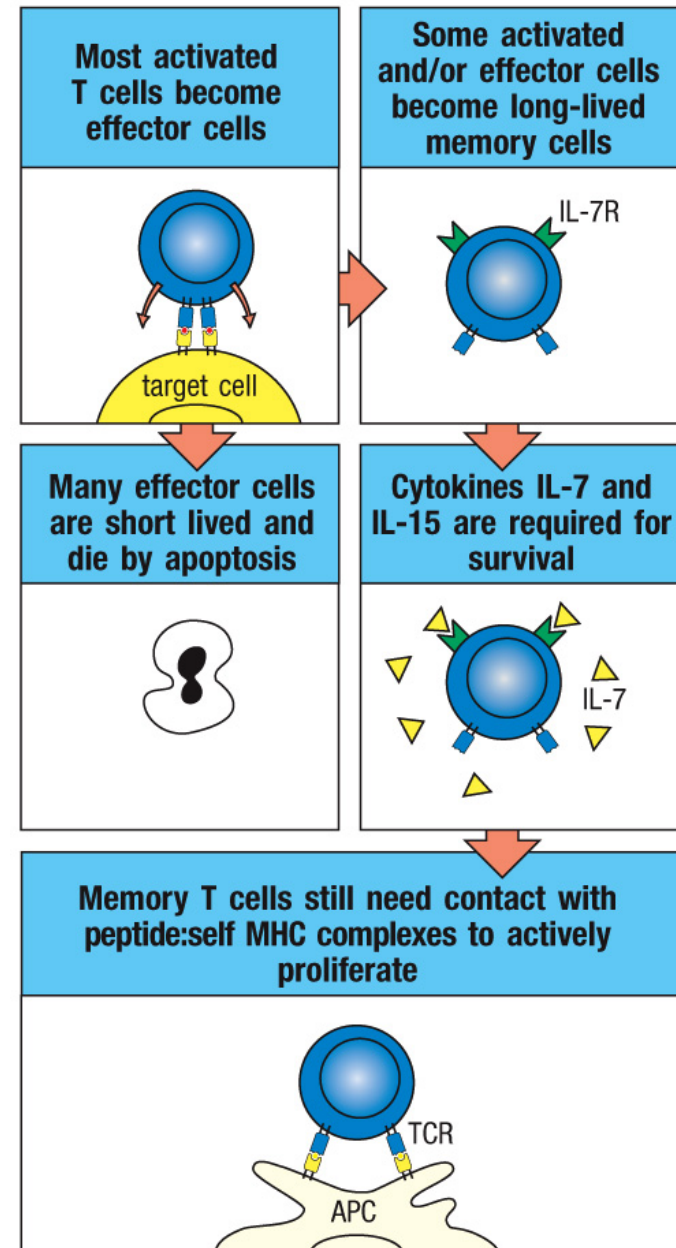


Figure 11.29 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Memory T Cells Arise from Effector T Cells That Maintain or Re-express IL-7R α

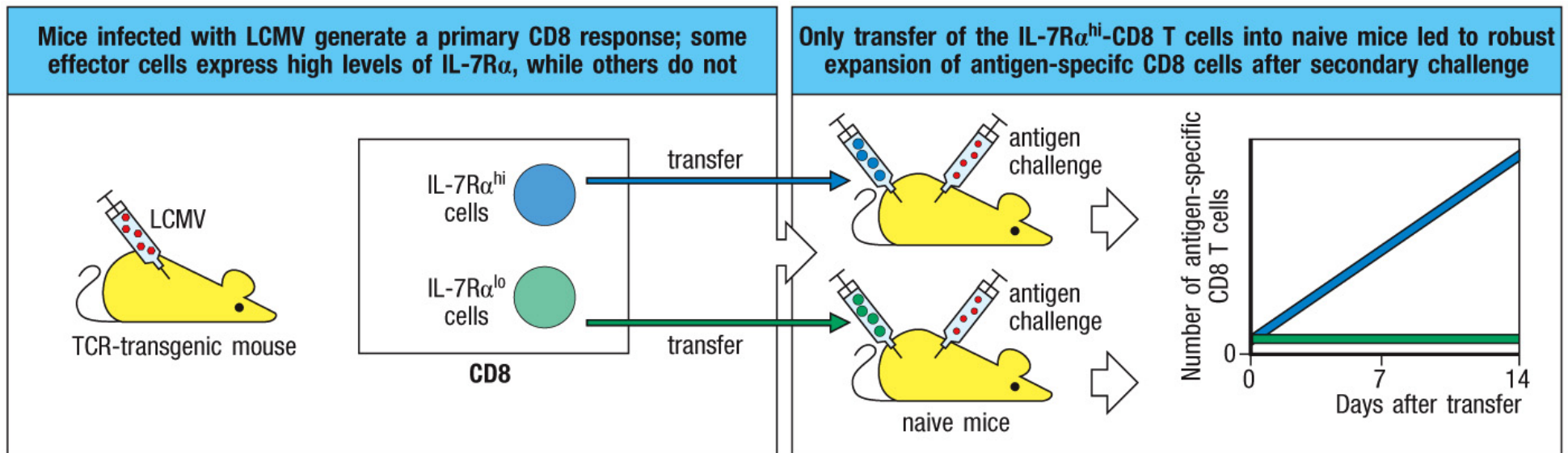
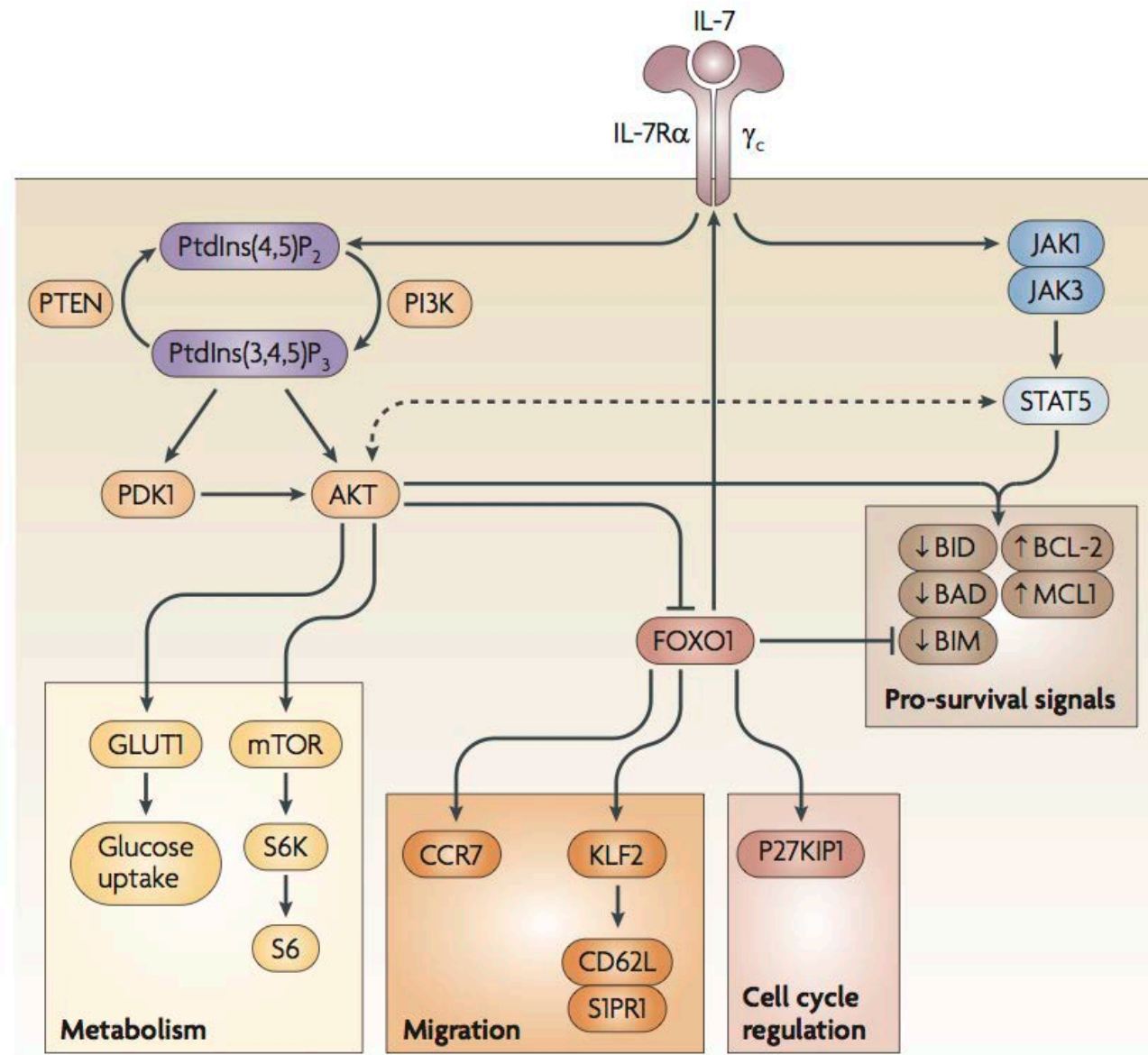


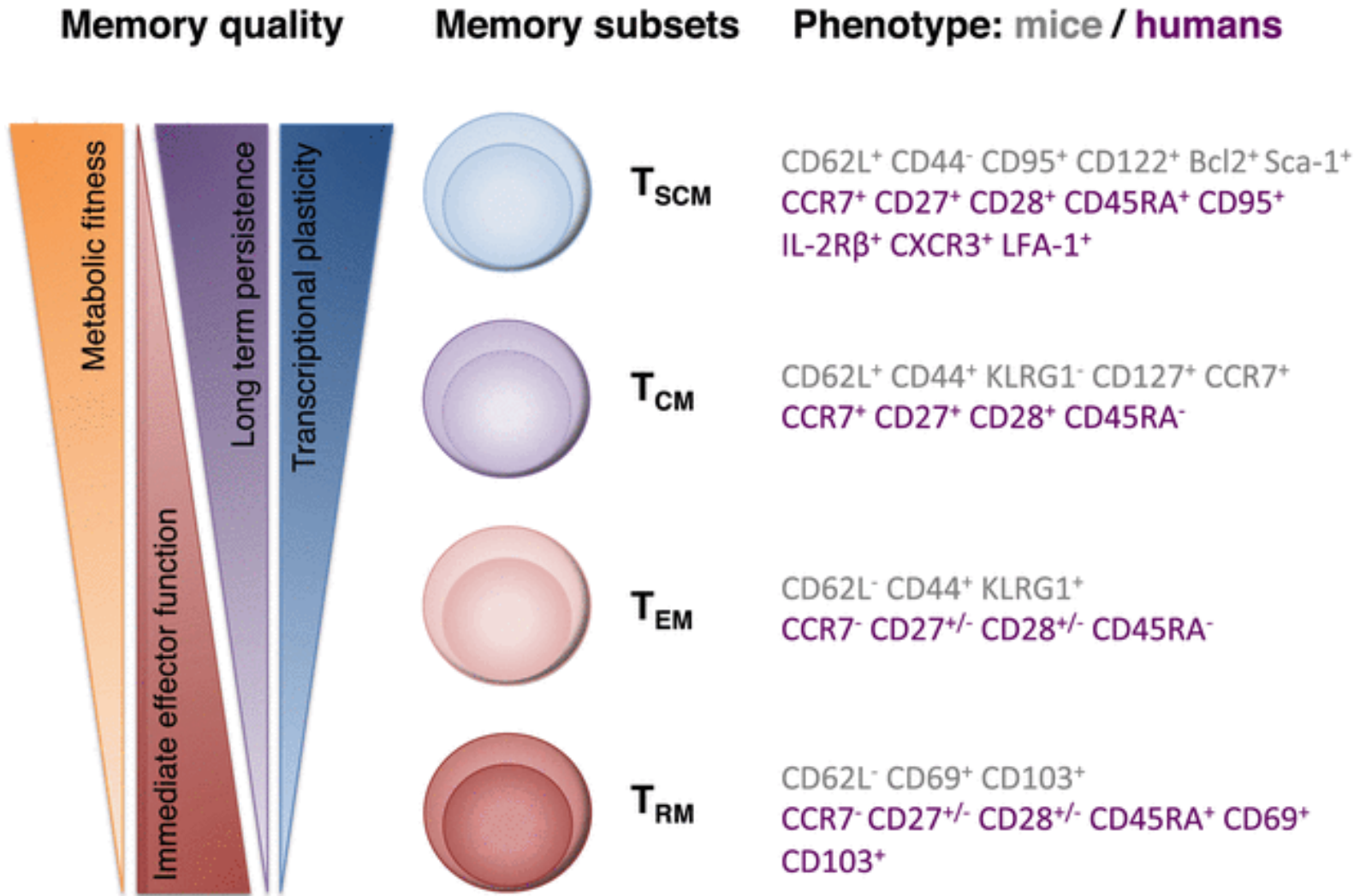
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IL-7R α ^{hi} Cells May Compete More Effectively for the Survival Signals Delivered by IL-7

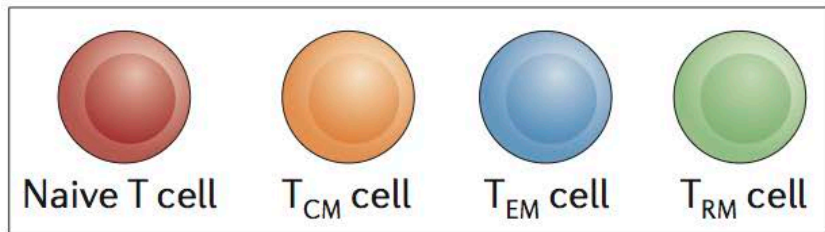
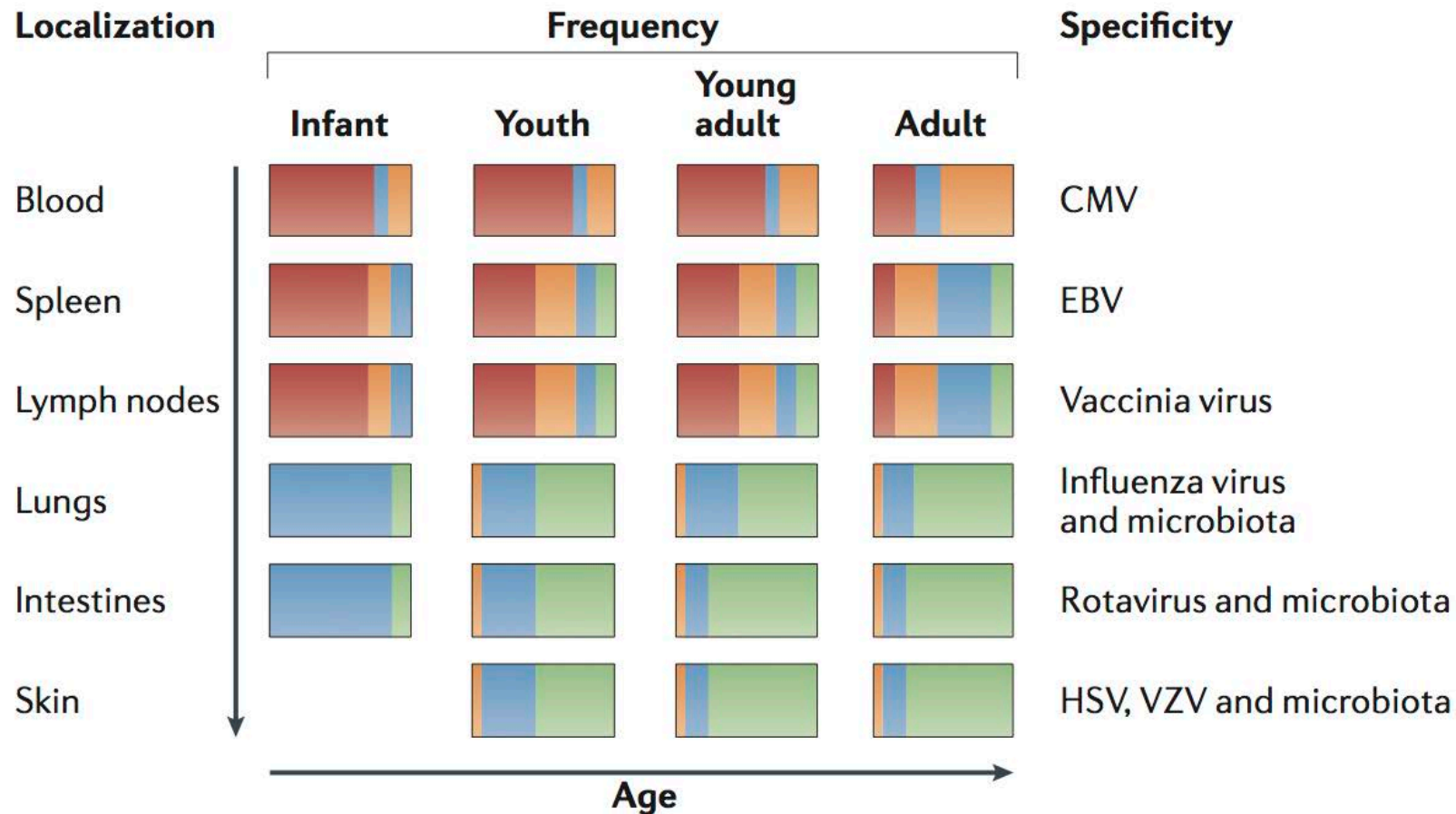


IV. Memory Cell Subsets and Compartmentalization

Mouse/Human CD8 Memory T Cell Subsets

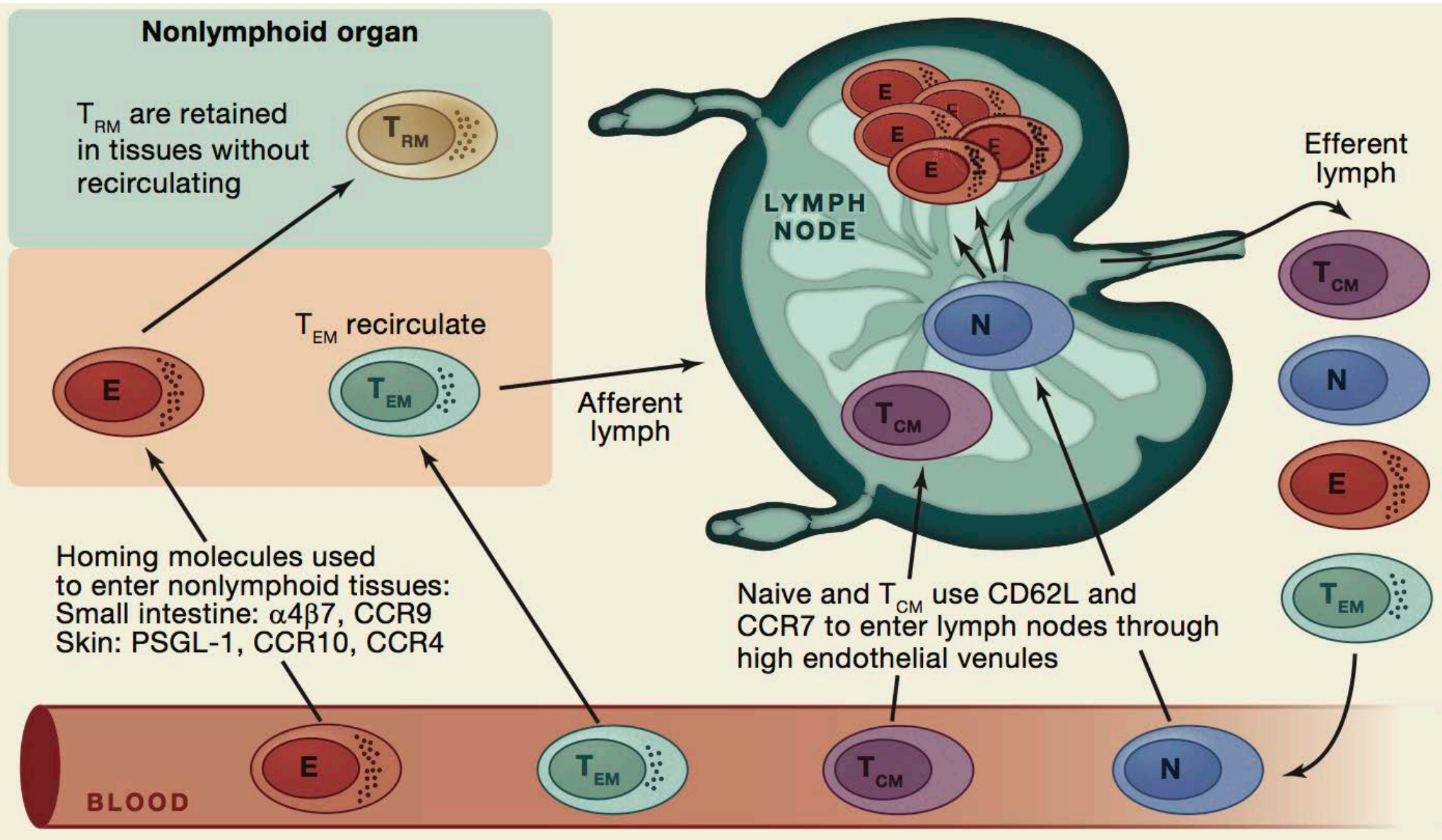


Compartmentalization of Memory T Cell Subsets in Space and Time



TRM cells may outnumber the recirculating T cells that migrate through the body.

TRM Cells are Derived From Effector T Cells



TRMs Reside in Tissues at the Site of Original Infection and Provide Protection Against Reinfection

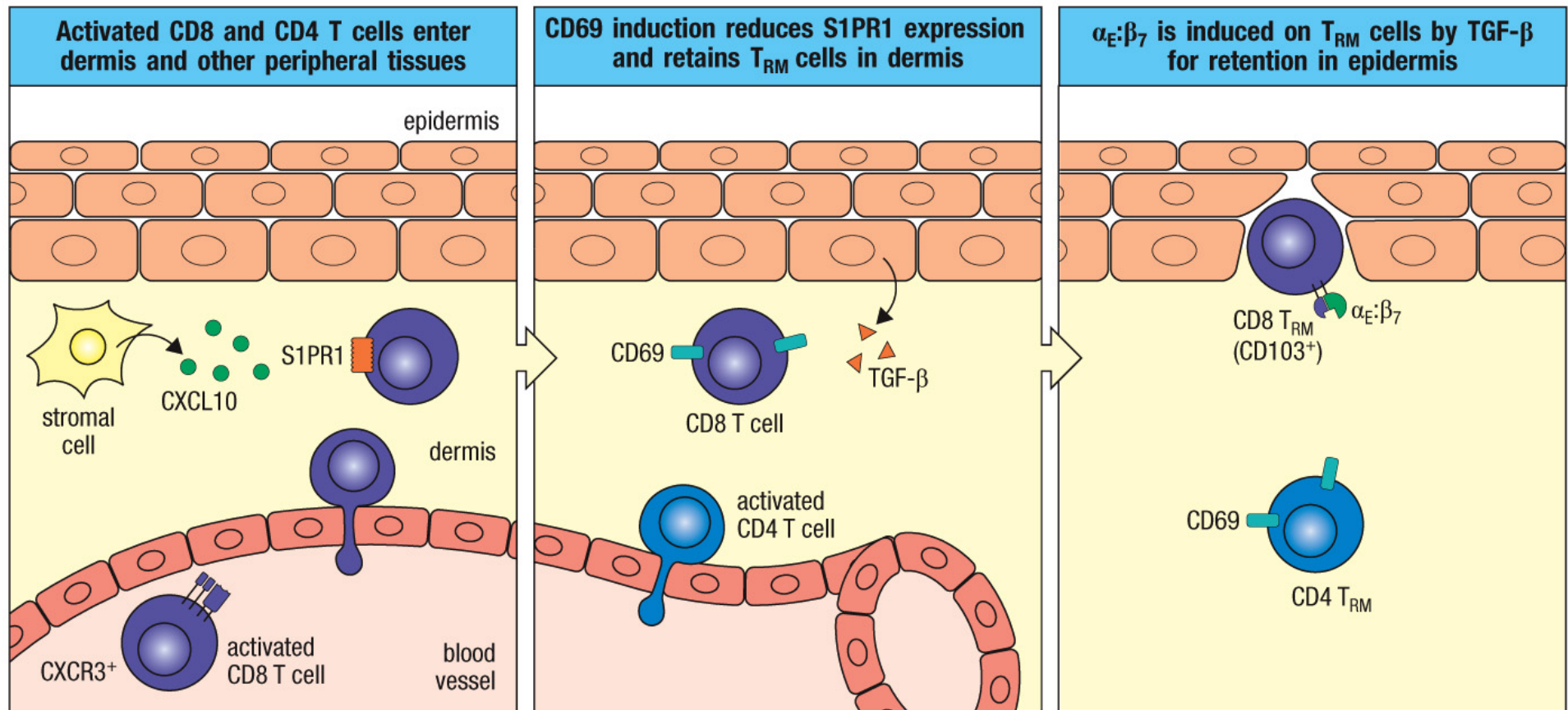
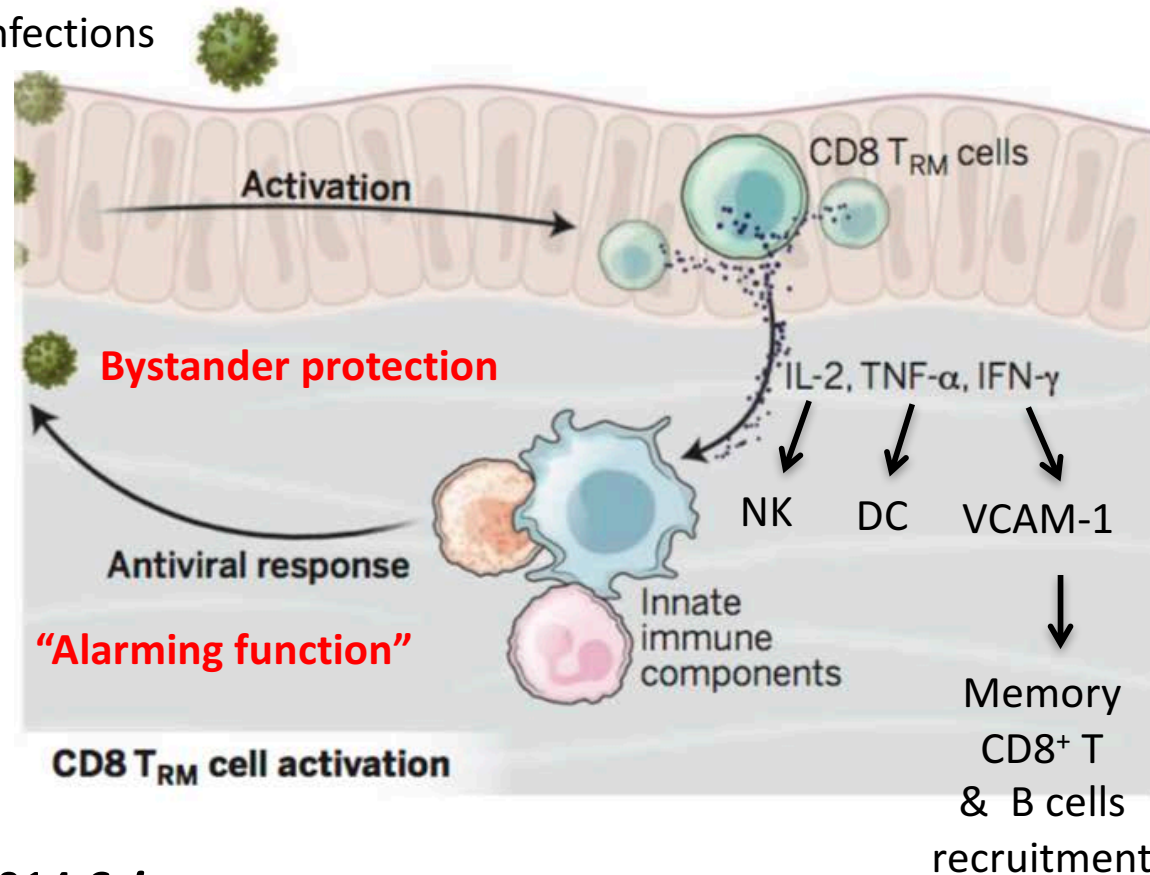


Figure 11.31 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

CD8 TRMs Trigger Protective Innate and Adaptive Immune Responses

Local viral infections



Schenkel et al. 2014 *Science*

“Tissue-resident memory T cells may be the immune cells of choice in designing strategies to stop pathogens before they establish a meaningful infection.”

Carbone and Gebhardt *Science* 2014

Hallmark of Immunological Memory

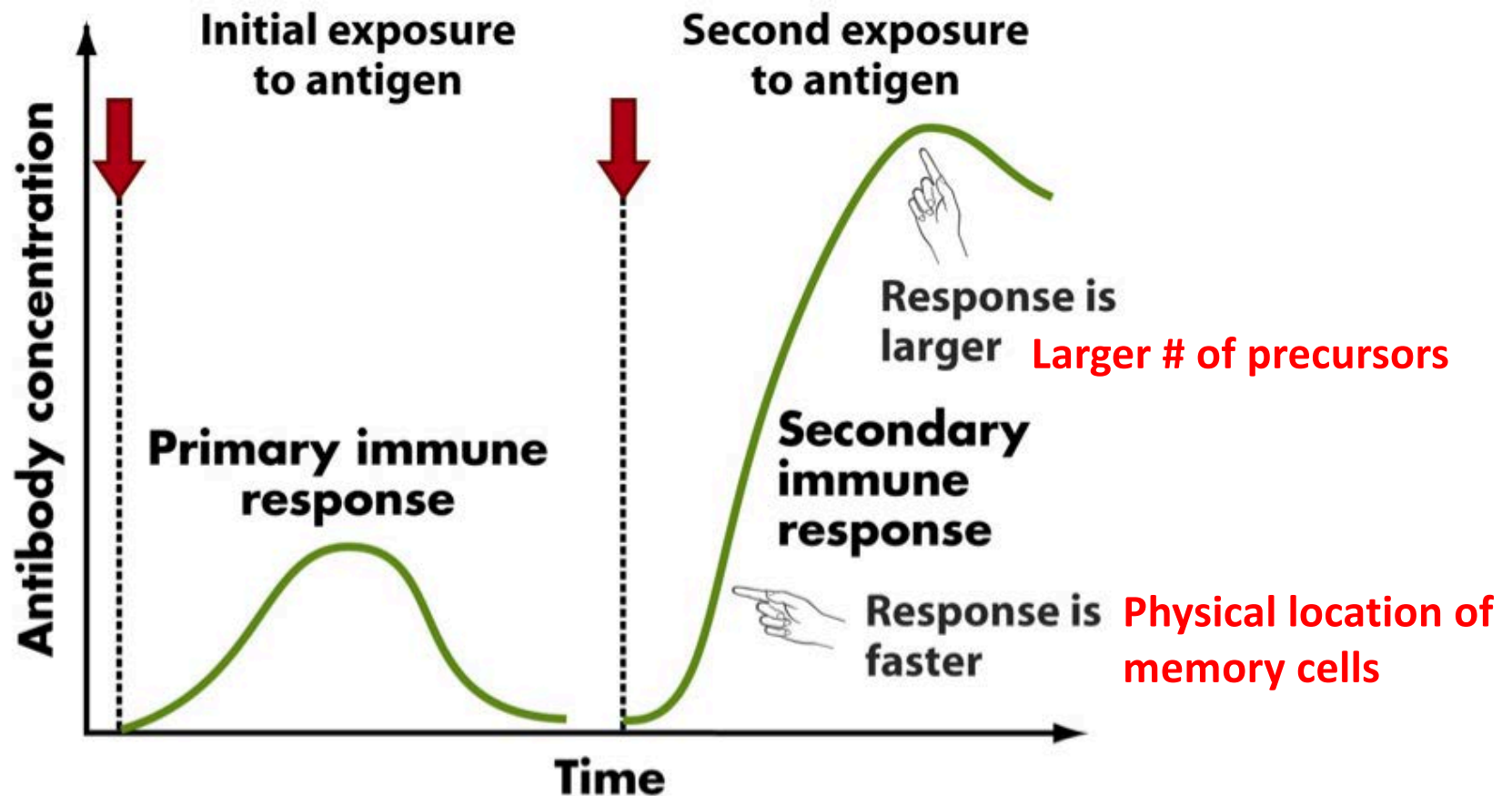


Figure 49-16 Biological Science, 2/e
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- Vaccination artificially induces protective immunity through generation of immunological memory.
- Many consider vaccination to be the most outstanding accomplishment of immunology in the field of medicine.

CD4 T Cells are Required for the Development of Functional CD8 Memory T Cells

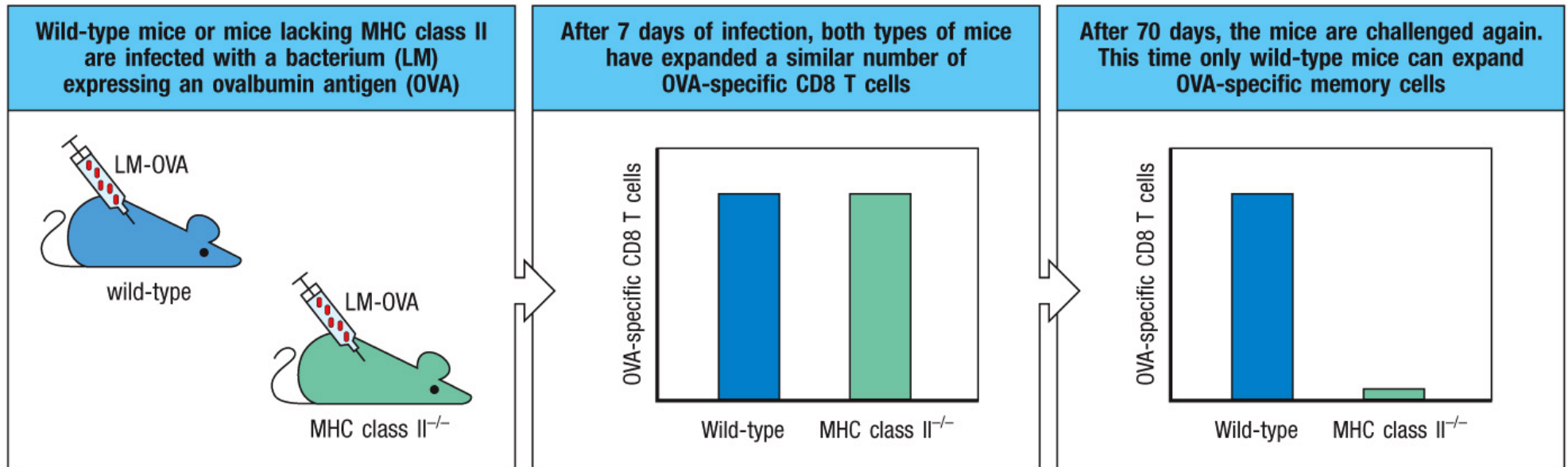


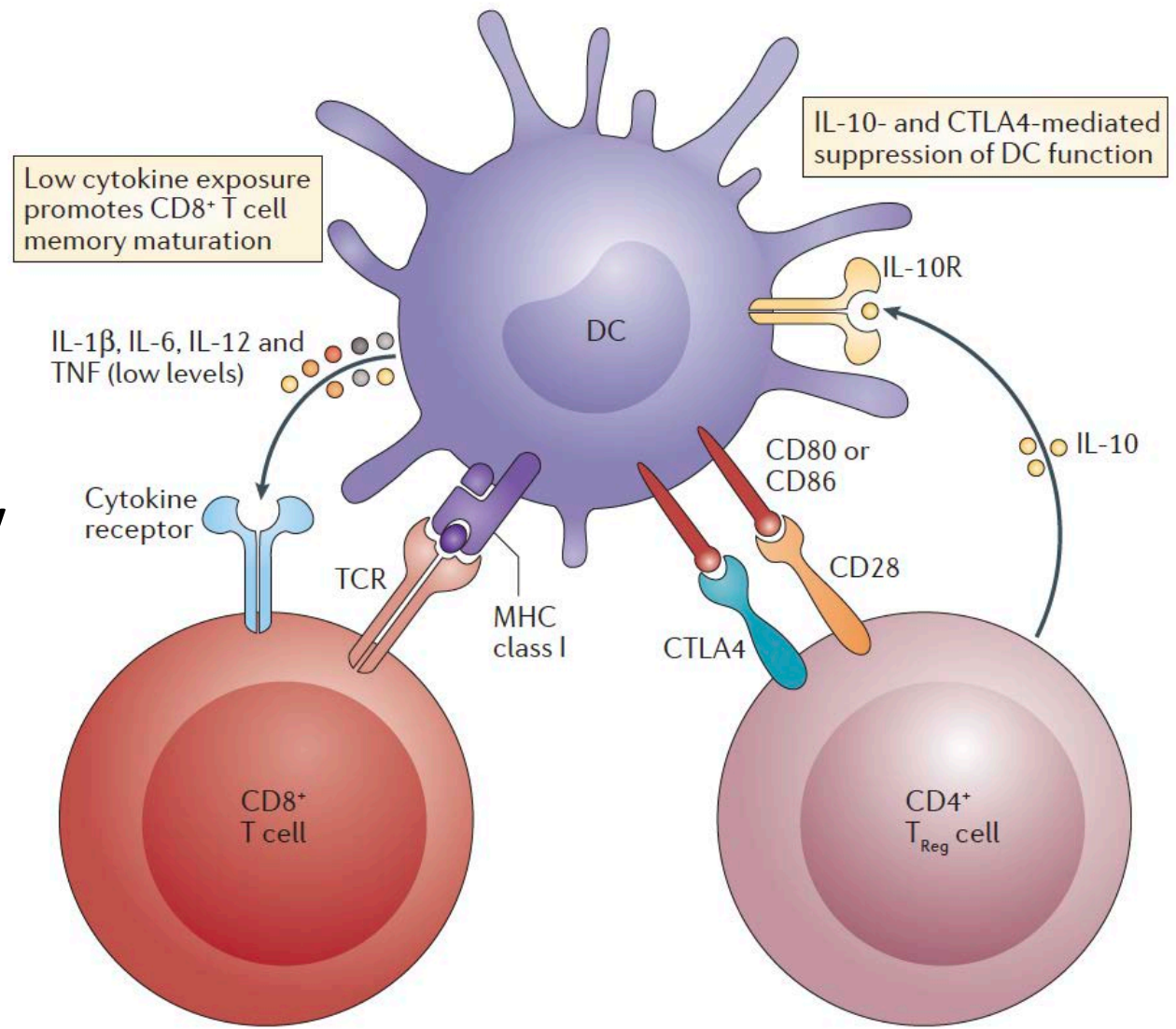
Figure 11.32 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **MHC-II^{-/-} mice fail to develop CD4 T cells.**
- **Expansion of effector CD8 T cells is not altered in MHC-II^{-/-} mice.**
- **Upon re-challenge Ag-specific memory cells fail to expand in MHC-II^{-/-} hosts.**

How do CD4 T cells help development of functional CD8 memory T cells?

IL-10 Produced by CD4⁺ Tregs Promotes the Maturation of Memory CD8⁺ T Cells

During the resolution phase, Treg cell-derived IL-10 acts to suppress the maturation state of DCs and limit their production of pro-inflammatory cytokines, which allow for the continued maturation of effector CD8 T cells into functional memory cells.



CD4 T Cells Promote the Maintenance of CD8 Memory Cells

- WT Mice are immunized with LCMV.
- Memory cells are transferred into either WT or MHC-II^{-/-} mice.
- Memory CD8 T cells decline in MHC-II^{-/-} mice.
- This has implications for conditions such as HIV/AIDS where CD4 T cell numbers are diminished.

What will it take to make a CTL-based vaccine for HIV?

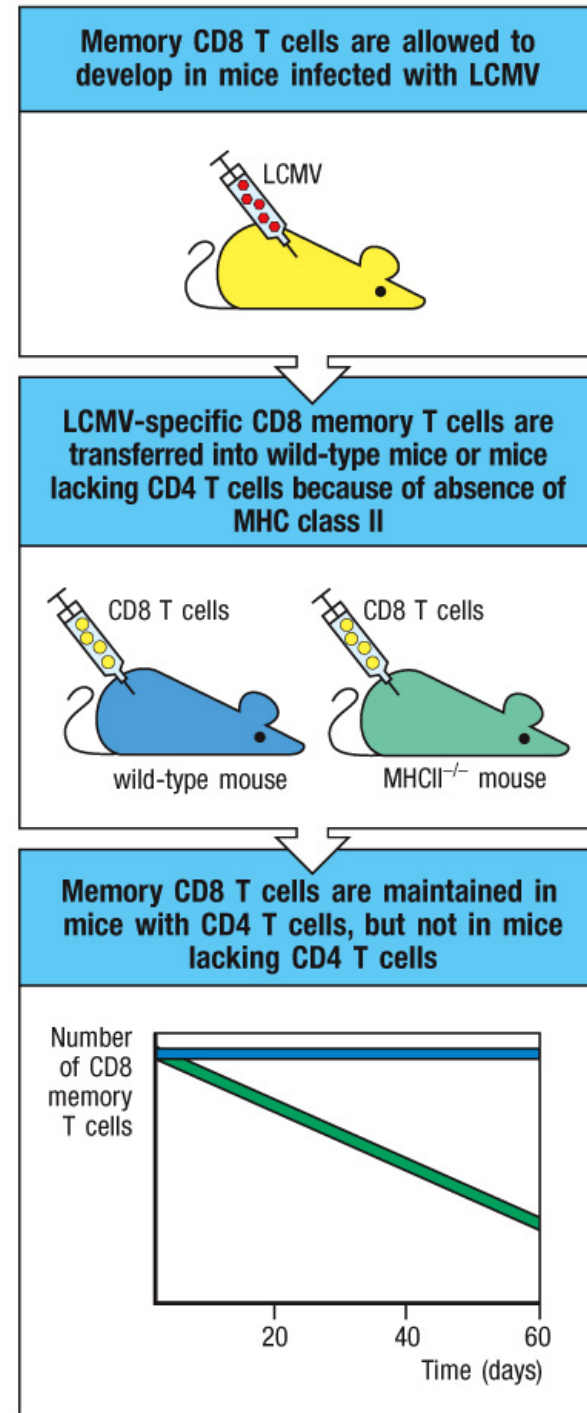


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