Adaptive Immunity:
Cellular mechanisms and signaling

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&
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Learning Objectives

- *To review the functional organization of the immune system*
- *To highlight the fundamentals of the adaptive immune response*
- *To integrate these into principles of cancer immunotherapy*

What is the immune system?

- A network of organs, tissues, cells, and effector molecules that cooperate to protect the organism from *pathogenic infection*
- Able to *evolve* to match the ever-changing threats of the microbial world
- Immune-related diseases include autoimmunity, allergy, organ graft rejection, & metabolic disorders, among others.

Why is the immune system?

Key concepts:

The immune system exists to respond to that which is *both* ***foreign*** (i.e. non-self) & ***dangerous*** (i.e. capable of causing damage).

The immune system initially 'senses' the presence of an infectious pathogen and mounts a rapid response via its innate arm

A subsequent adaptive response 'learns' the distinguishing molecular features of a given invader and produces effector and memory cells specific for those.

Why is the immune system?

Key concepts:

The immune system can ‘remember’ the identity of a pathogen through its antigen-specific memory cells which mount *faster* & *stronger* recall responses

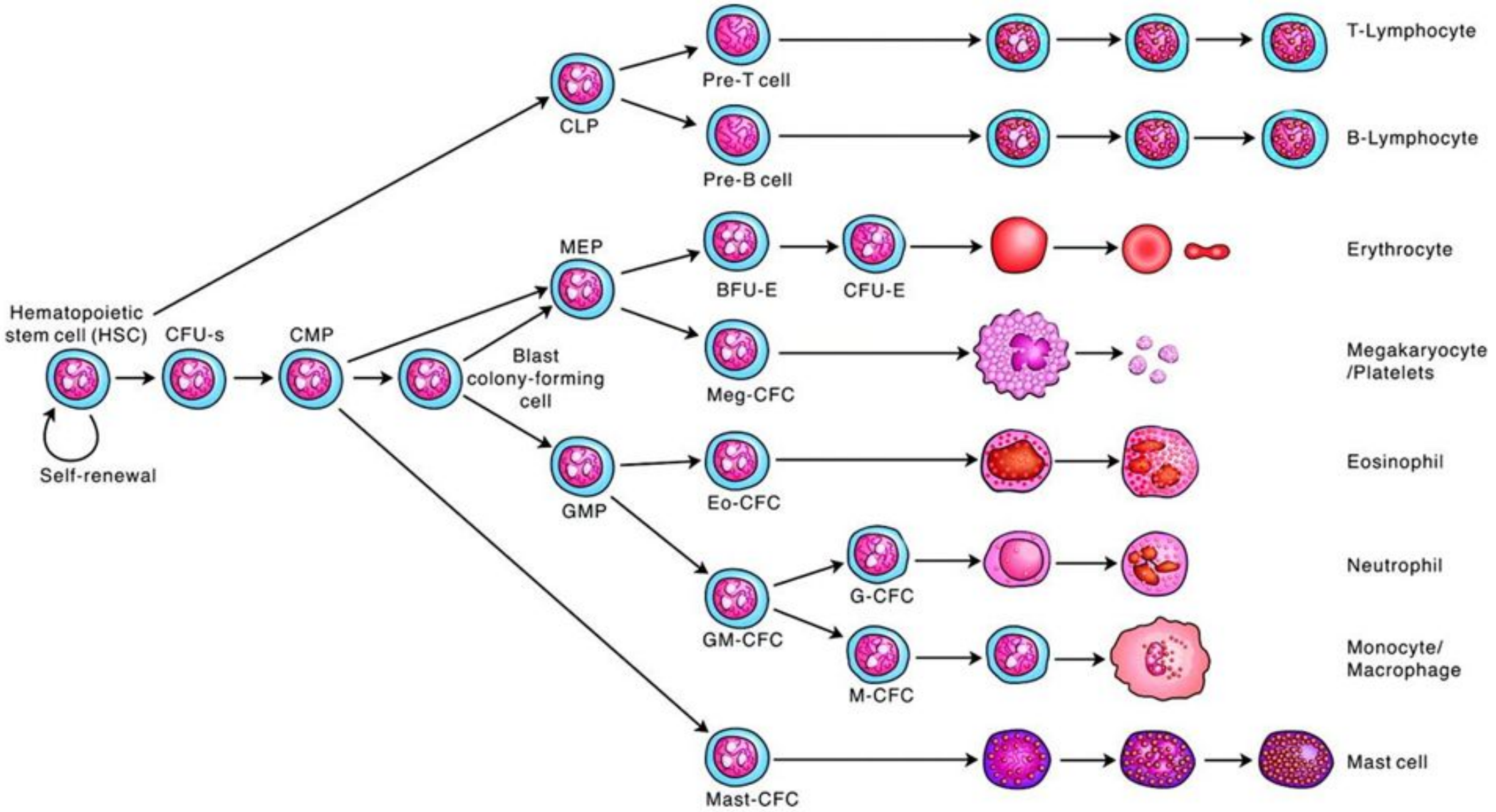
Optimal immune responses require coordination of both *sensing* and *learning* components

Where is the immune system?

Key concepts:

- Cellular and molecular components are located throughout the entire body
- Most immune cells are produced in bone marrow from hematopoetic stem cells
- Specialized organizing centers include spleen, lymph nodes, & thymus

Myelopoesis and Lymphopoeisis



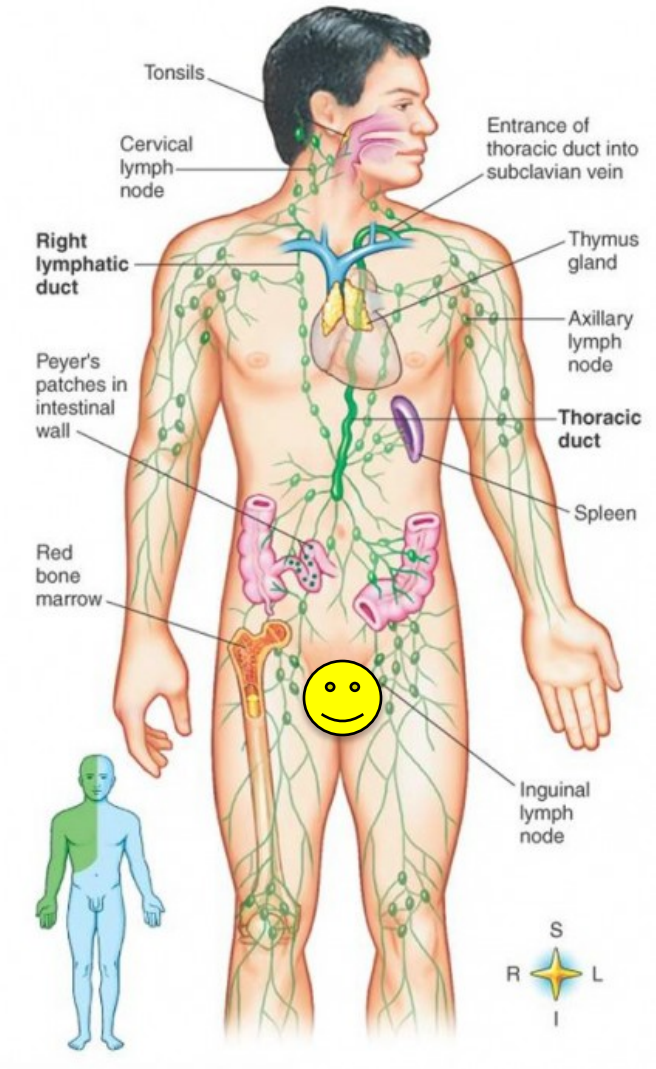
How is the immune system organized?

<u>3 components</u>

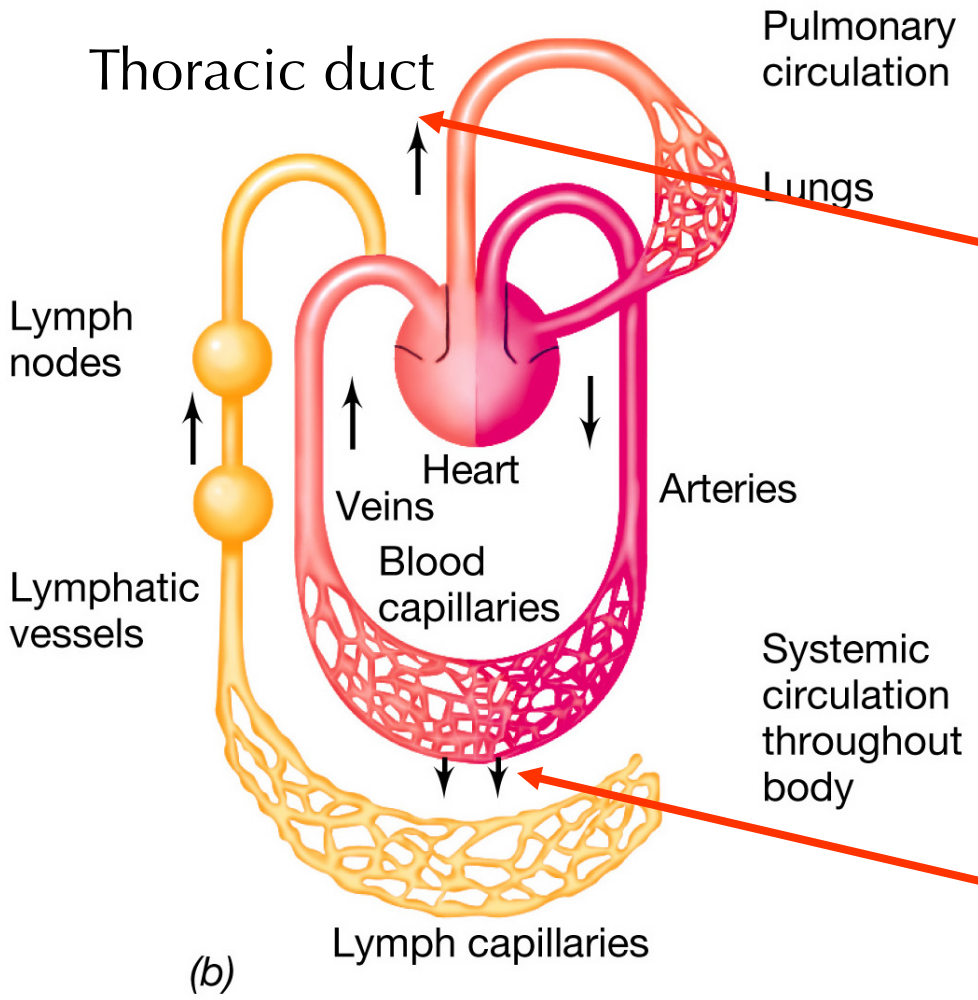
Lymphatic vessels

Fluid (lymph)

1°& 2° Lymphoid organs



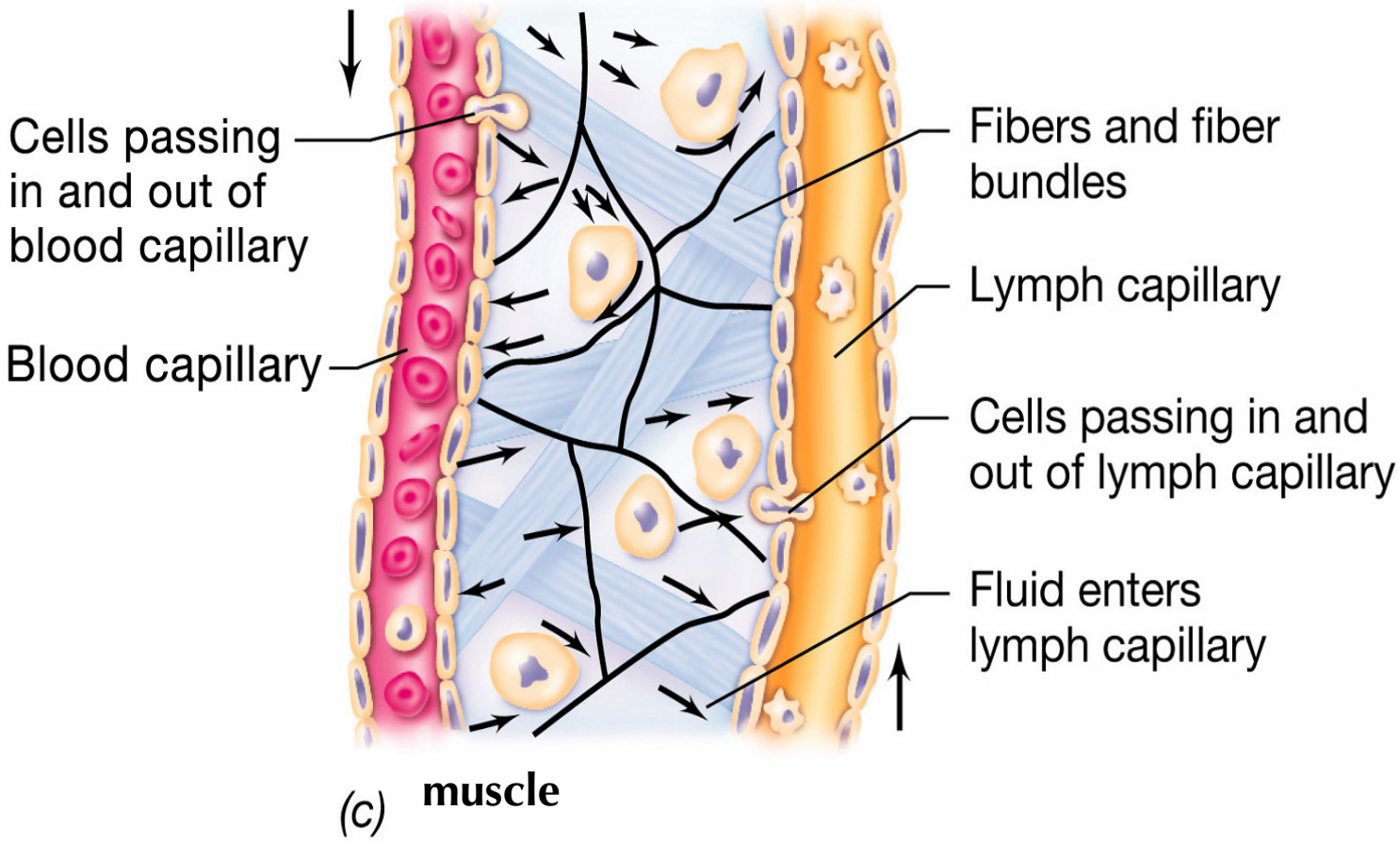
Overview of blood and lymph system and how leukocytes travel from one system to another



Lymph carrying antibodies and immune cells collect in thoracic duct where the lymph empties back into the blood circulatory system

Site of exchange between the blood and lymph systems

Immune cells travel back and forth from the blood and lymph circulatory systems and interact with extra-vascular tissues in the process— diapedesis



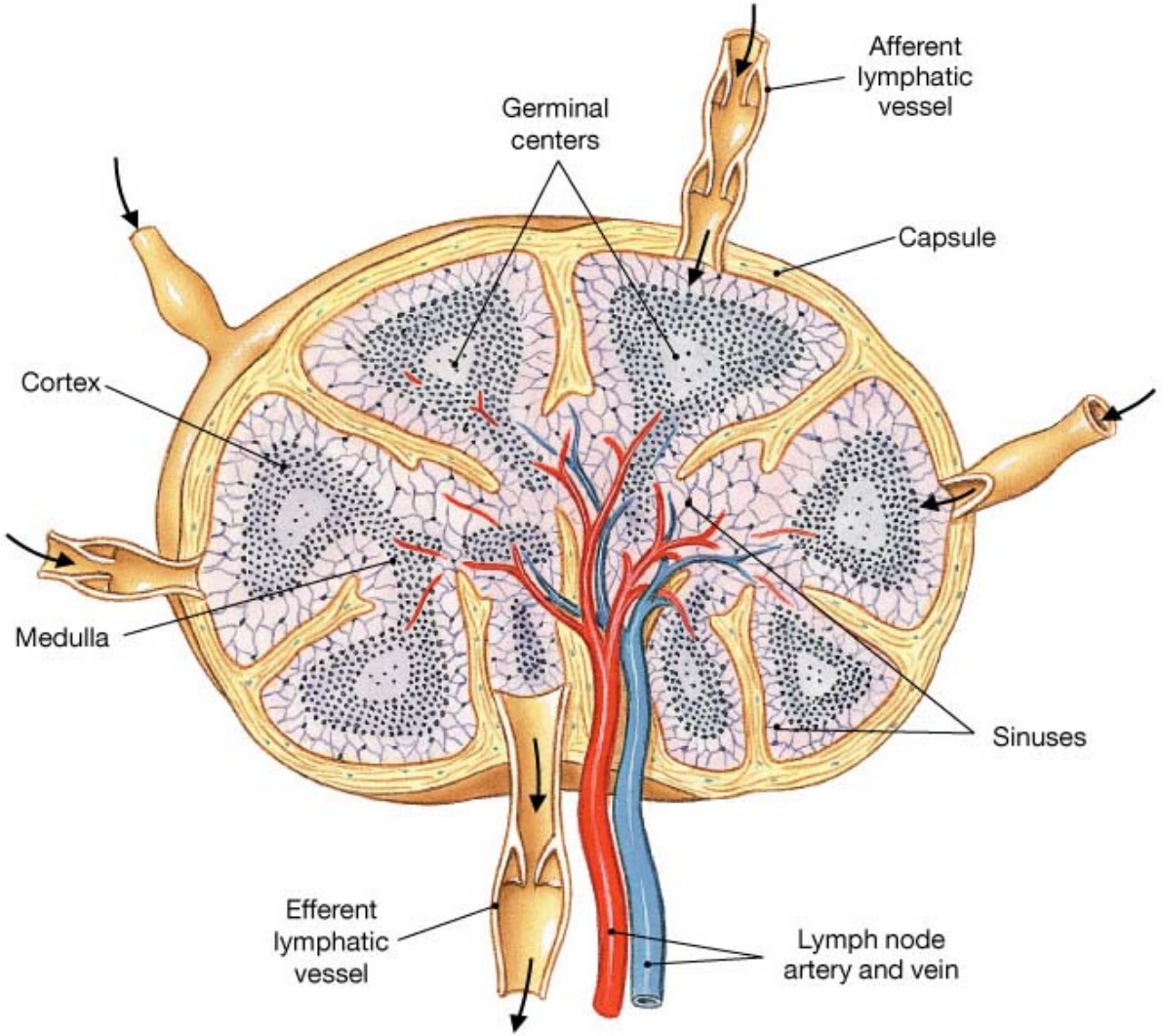
Types of Lymphocytes

- T cells (Thymus dependent)
  - 80% of circulating lymphocytes
  - Cytotoxic T cells
    - Directly attack foreign cells or body cells infected by viruses (cell-mediated immunity)
  - Helper T cells
    - Stimulate activities of both B and T cells
  - Regulatory T cells
    - Inhibit both T and B cells

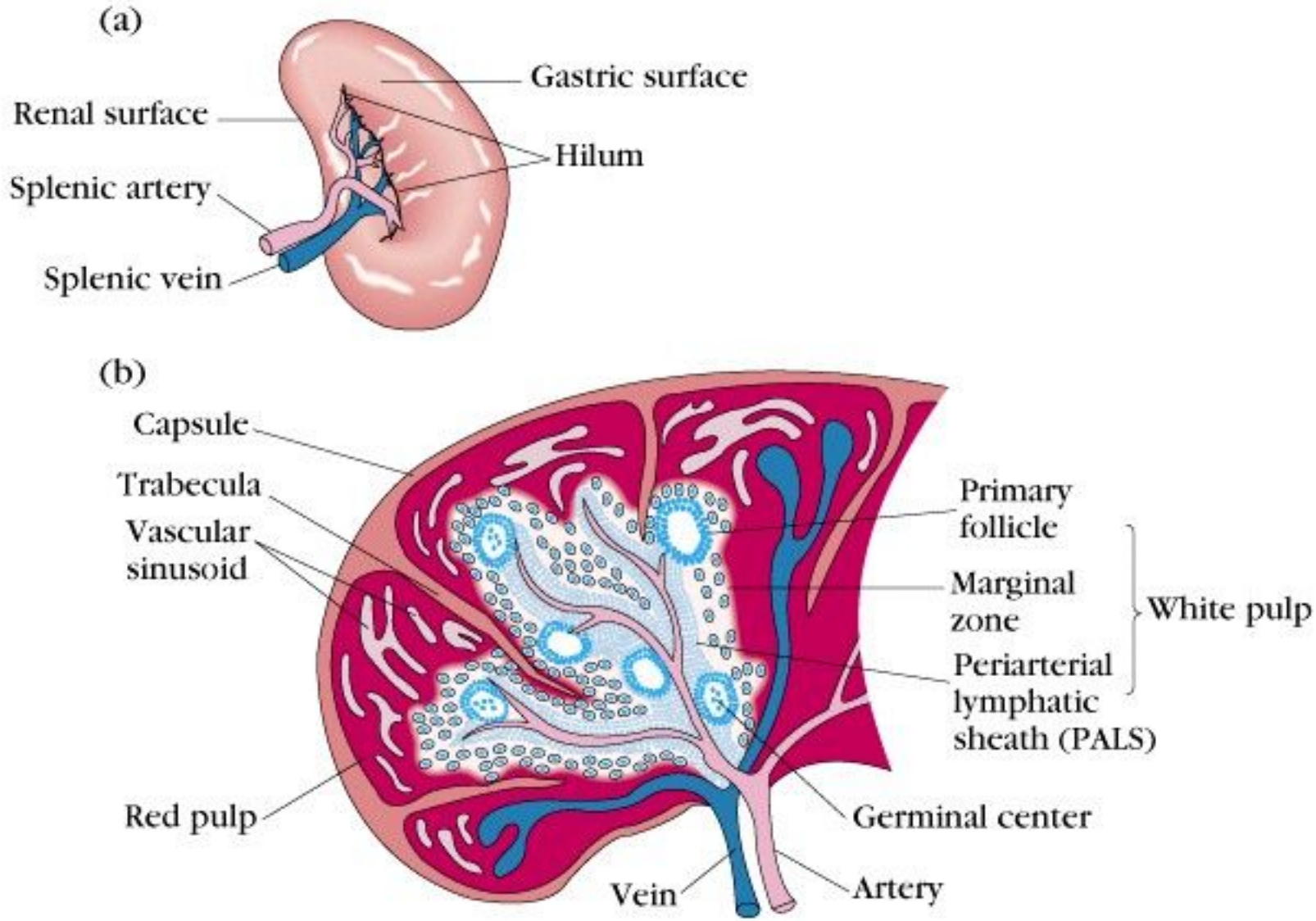
Types of Lymphocytes

- B cells (Bone-marrow derived)
  - 10-15% circulating lymphocytes
  - Plasma cells
    - Responsible for production and secretion of antibodies (immunoglobulins)
  - Responsible for antibody-mediated immunity
- NK cells (Natural Killer)
  - 5-10%
  - Attack foreign cells, normal cells infected with viruses, and cancer cells
  - Immunological surveillance

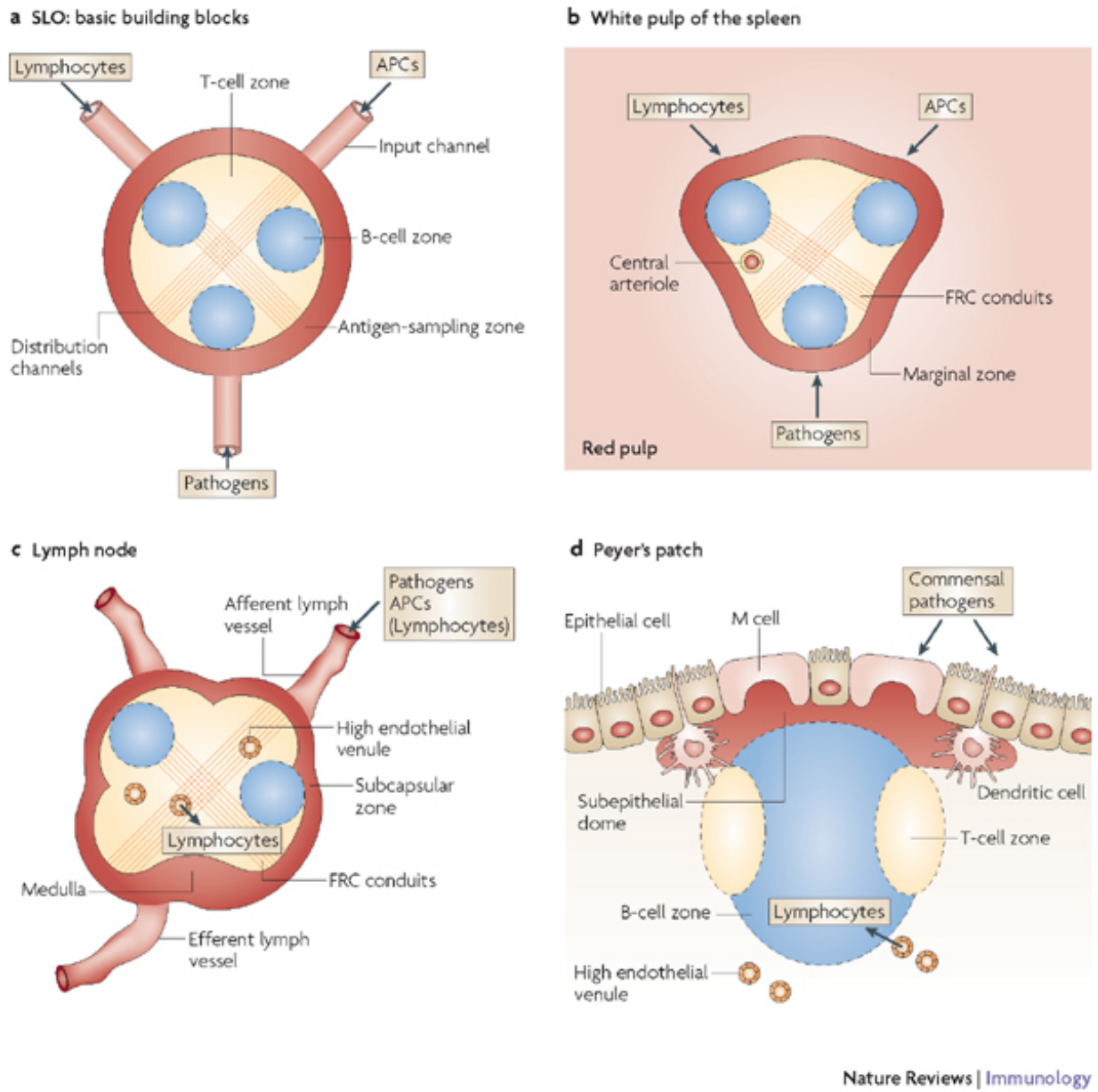
Lymphoid Organs: Lymph Nodes



Lymphoid Organs: Spleen



B and T cells are primed in distinct compartments of secondary lymphoid organs



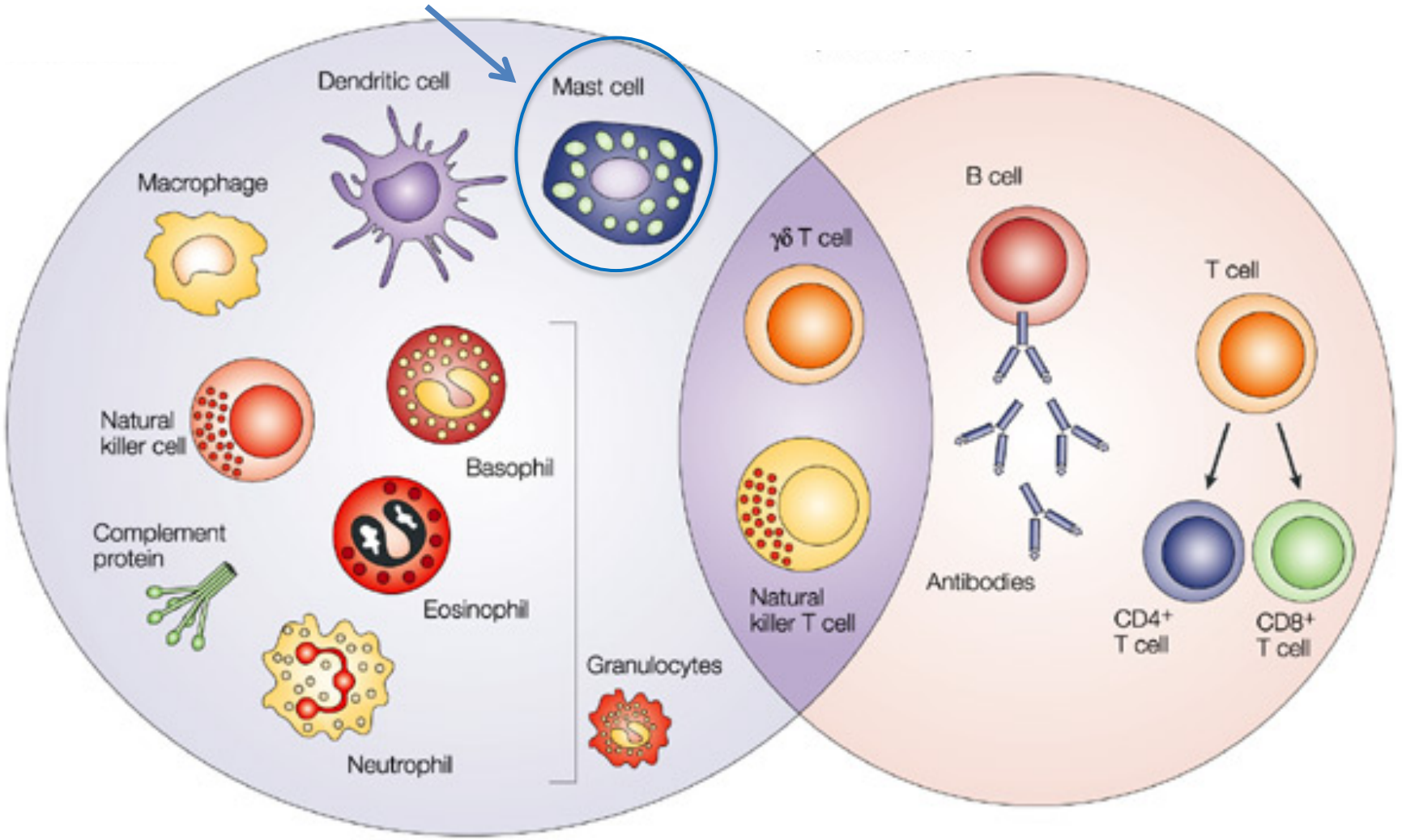
How is the immune system organized?

Two main arms: Innate and Adaptive:

- The innate system is always "on" and ready to mount an immediate early response by sensing molecules common to entire classes of microbes
- The adaptive system takes days to respond, but can resolve infections and confer immune memory by sensing molecules specific to a particular invader

Innate & Adaptive Immunity

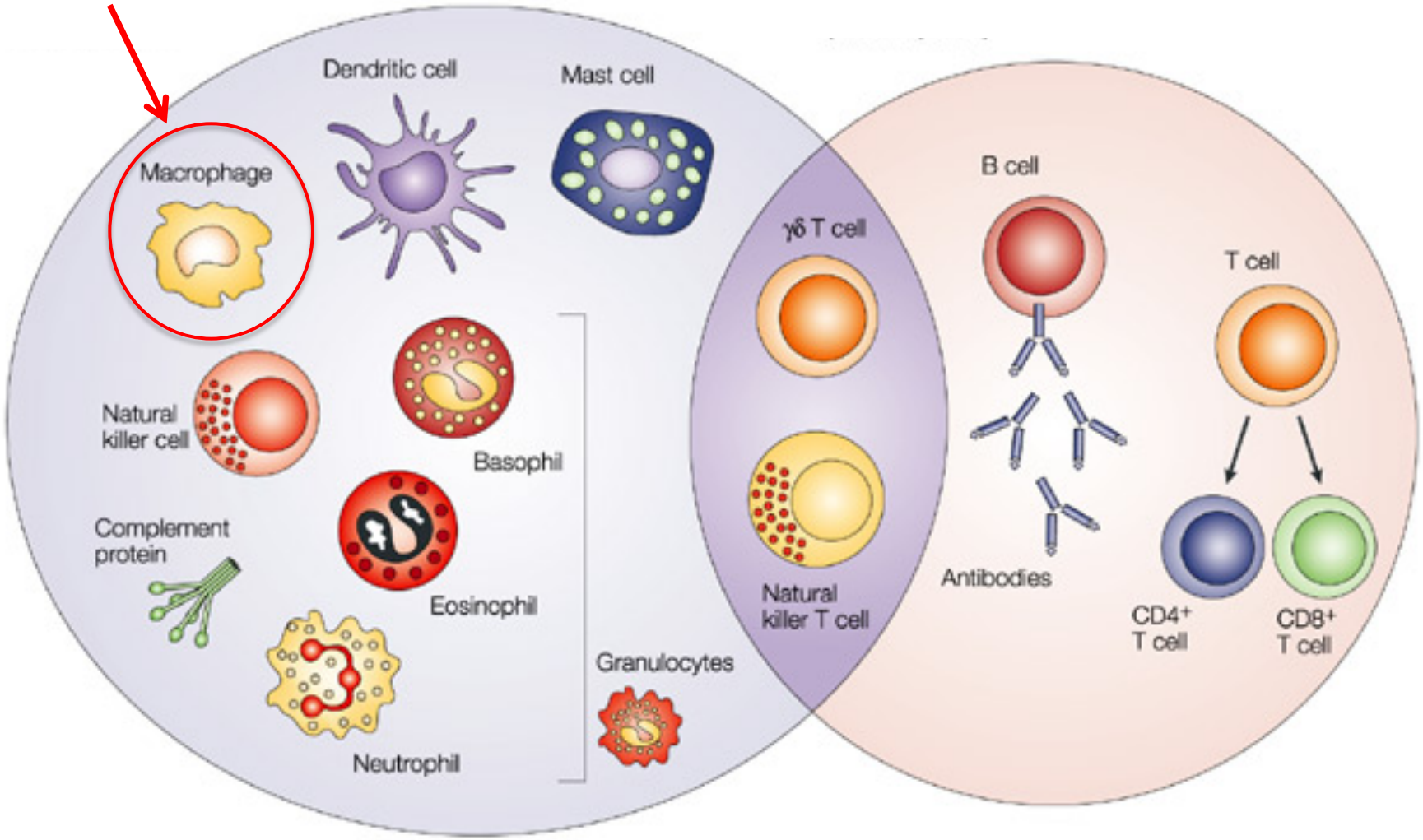
Mast Cells: Part of a cellular "early-warning" system, produces histamine and inflammatory mediators in response to direct damage, allergens, or inflammation



G. Dranoff, Cytokines in cancer pathogenesis and cancer therapy , Nature Reviews Cancer 4:11-122

Innate & Adaptive Immunity

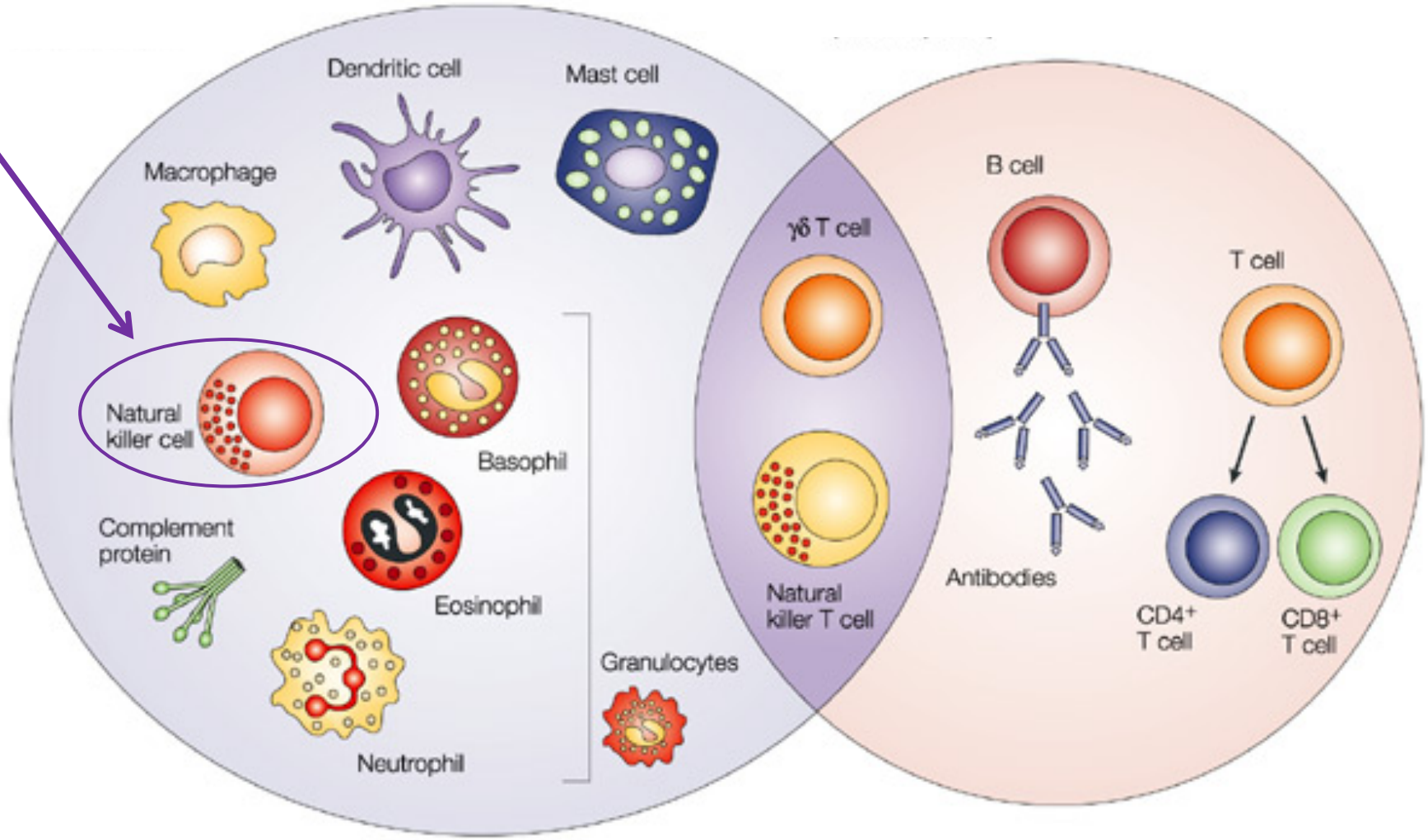
Macrophage: Most frequent and active phagocyte ("Big Eater"), ingests and destroys fluid-phase and particulate antigens
Found in every tissue in distinct phenotypic states



G. Dranoff, Cytokines in cancer pathogenesis and cancer therapy , Nature Reviews Cancer 4:11-122

Innate & Adaptive Immunity

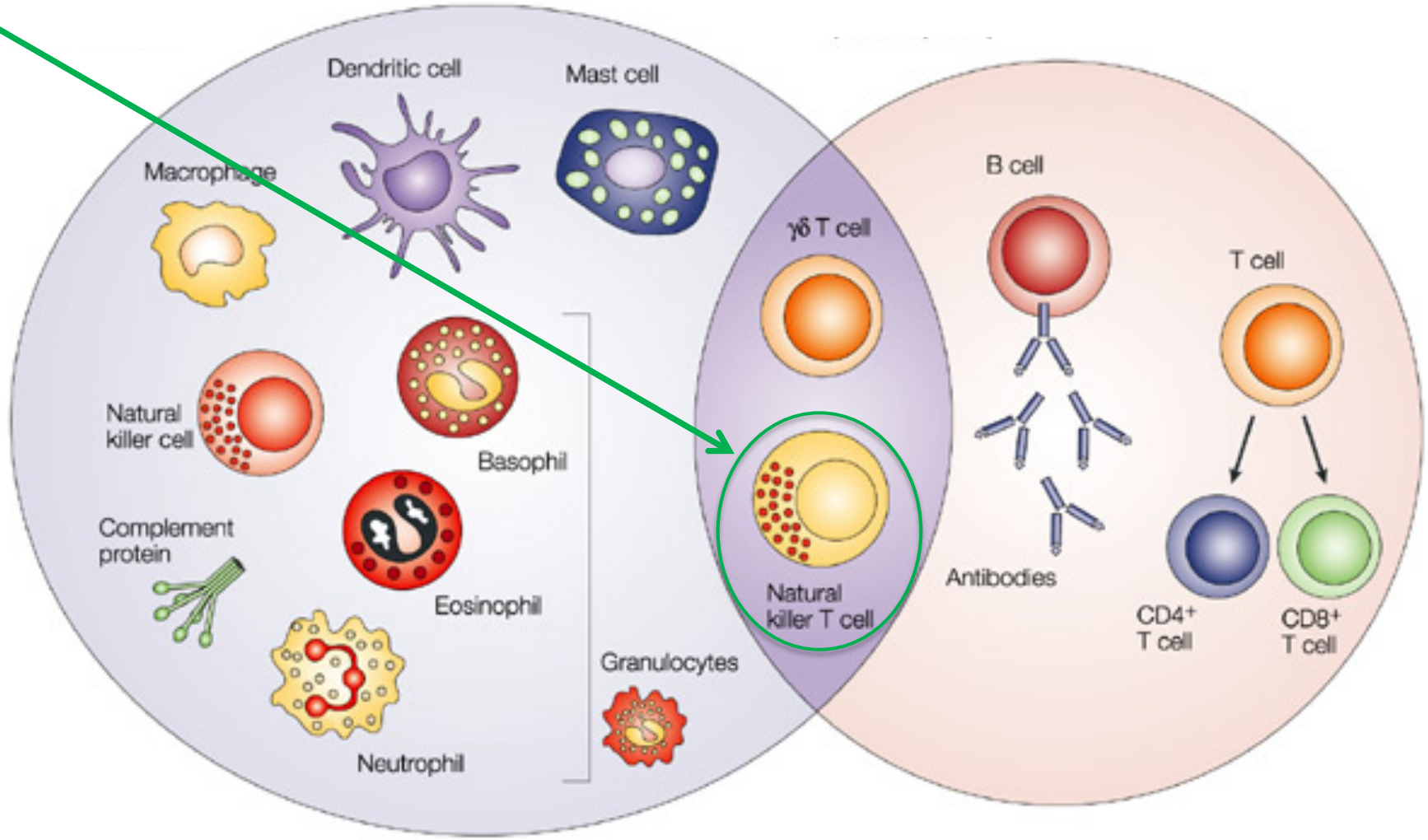
NK Cell: Cytotoxic against cells lacking MHC molecules (“missing self”) or expressing markers of cellular stress



G. Dranoff, Cytokines in cancer pathogenesis and cancer therapy , Nature Reviews Cancer 4:11-122

Innate & Adaptive Immunity

NKT Cell: Express invariant receptors specific for lipid antigens bound to non-classical CD1d MHC molecules



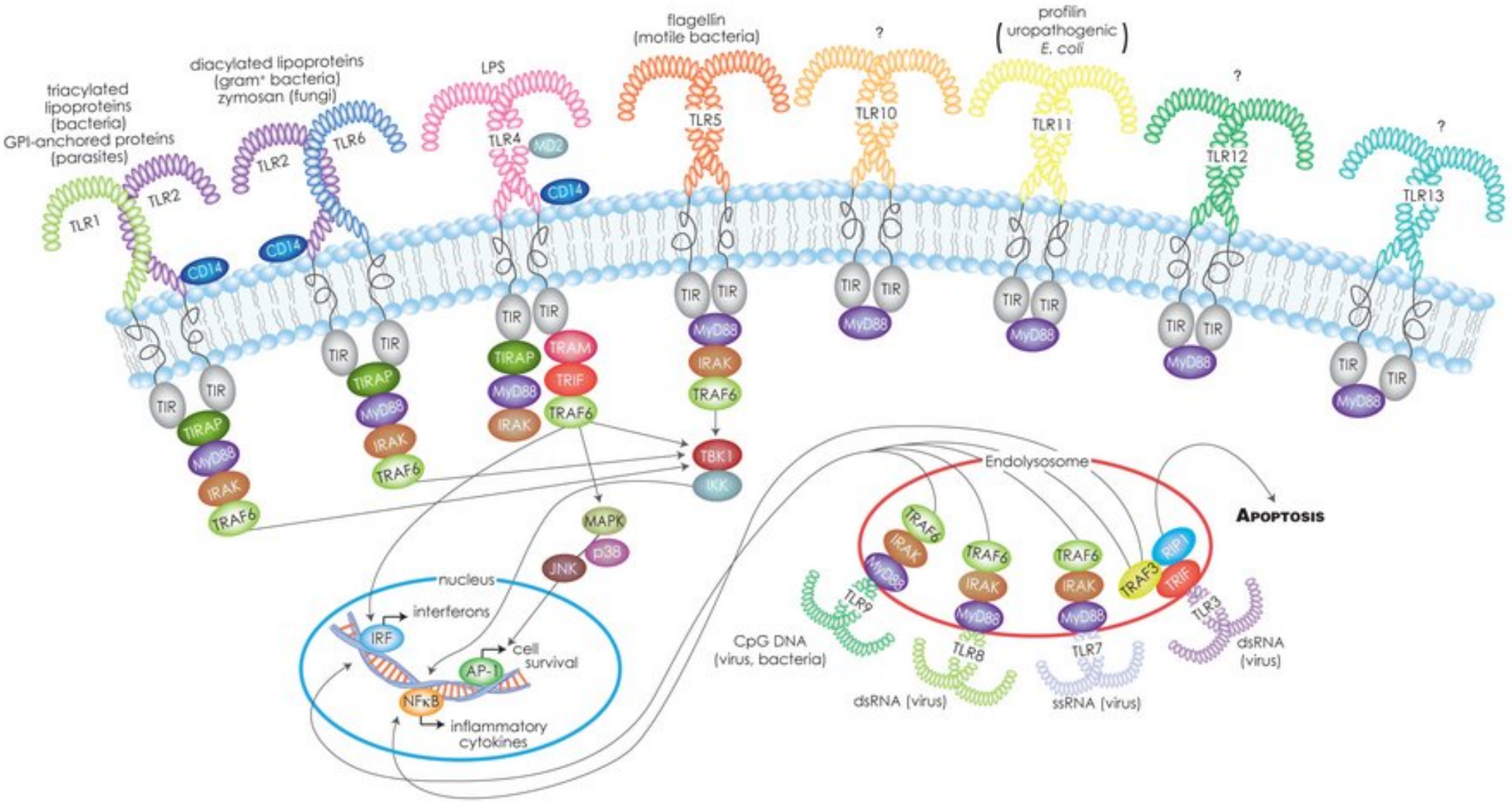
G. Dranoff, Cytokines in cancer pathogenesis and cancer therapy , Nature Reviews Cancer 4:11-122

Innate immune system

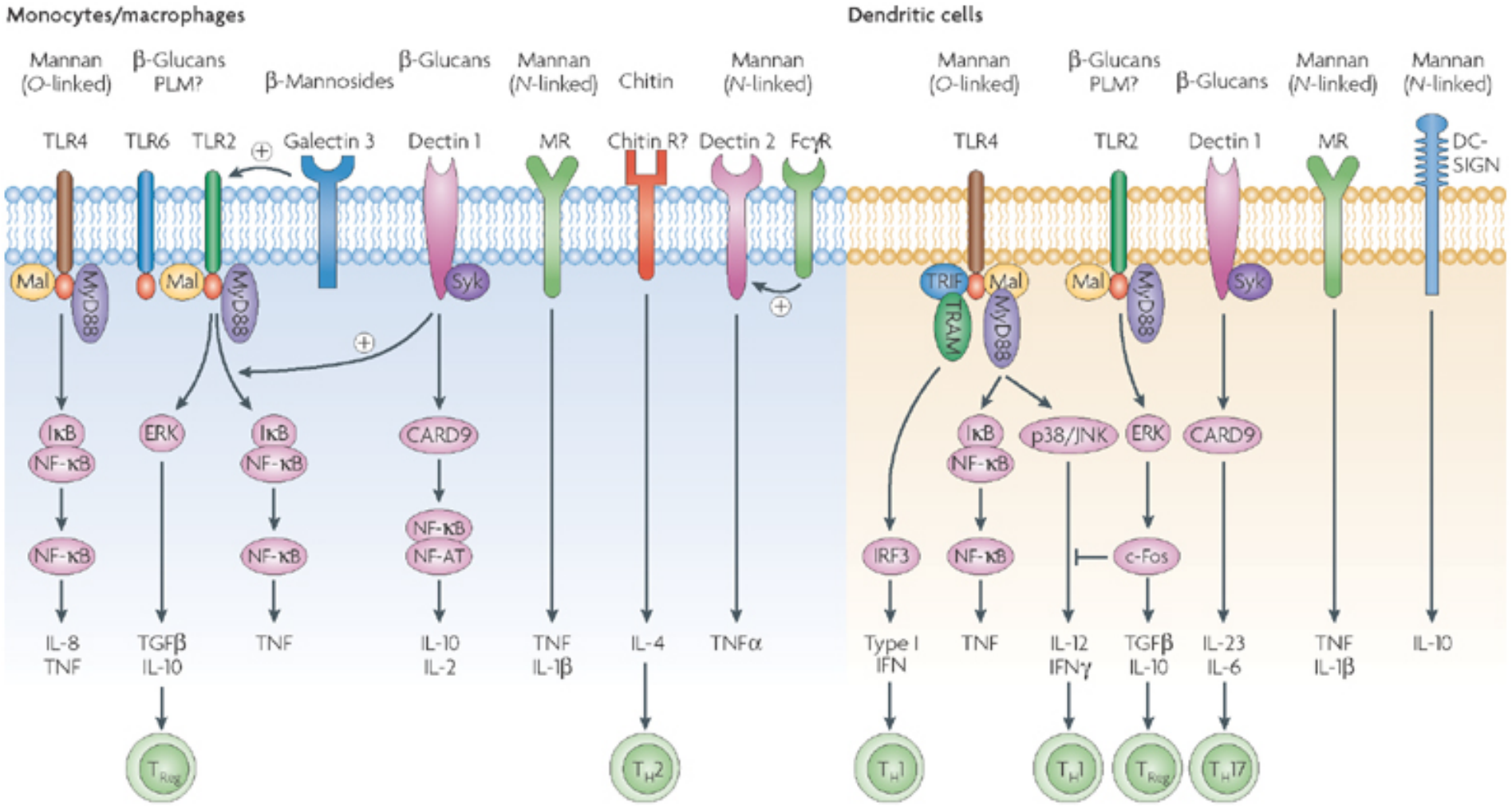
Notable features

- Response time minutes to hours
- Cells include macrophages, monocytes, neutrophils, dendritic cells, NK cells, mast cells, ILC
- Effector molecules include complement, cytokines, chemokines, among others
- Recognize molecular features common to classes of microbes (flagellin, LPS, dsRNA, etc) or ‘alarm’ cytokines

Microbial pathogens are ‘sensed’ by Toll-like receptors (TLRs)



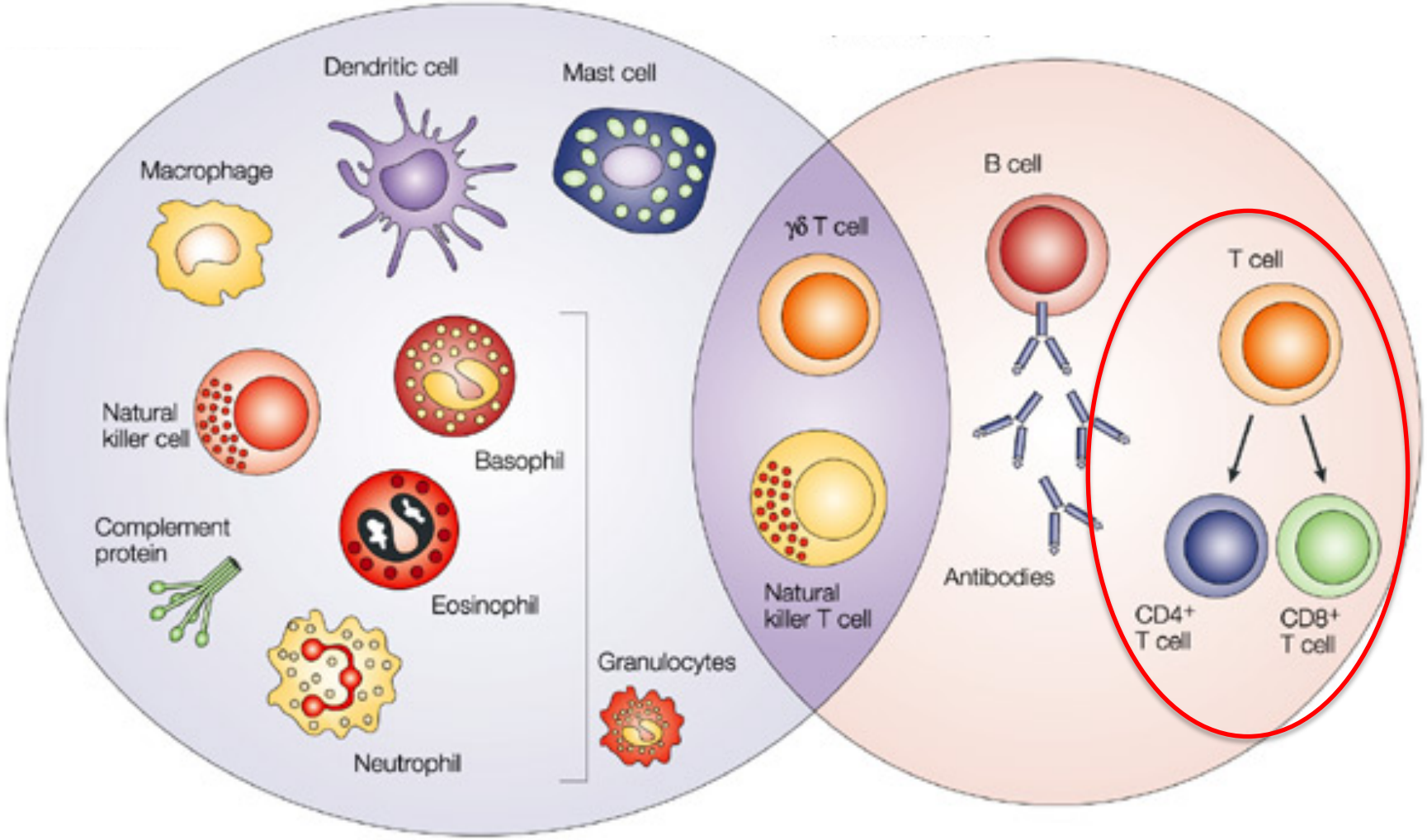
Multiple signaling/uptake receptors mediate Ag-sensing in Mϕ and DC



Innate & Adaptive Immunity

Innate arm
(fast and non-specific)

Adaptive arm
(slower but Ag-specific)



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Adaptive immune system

Notable features

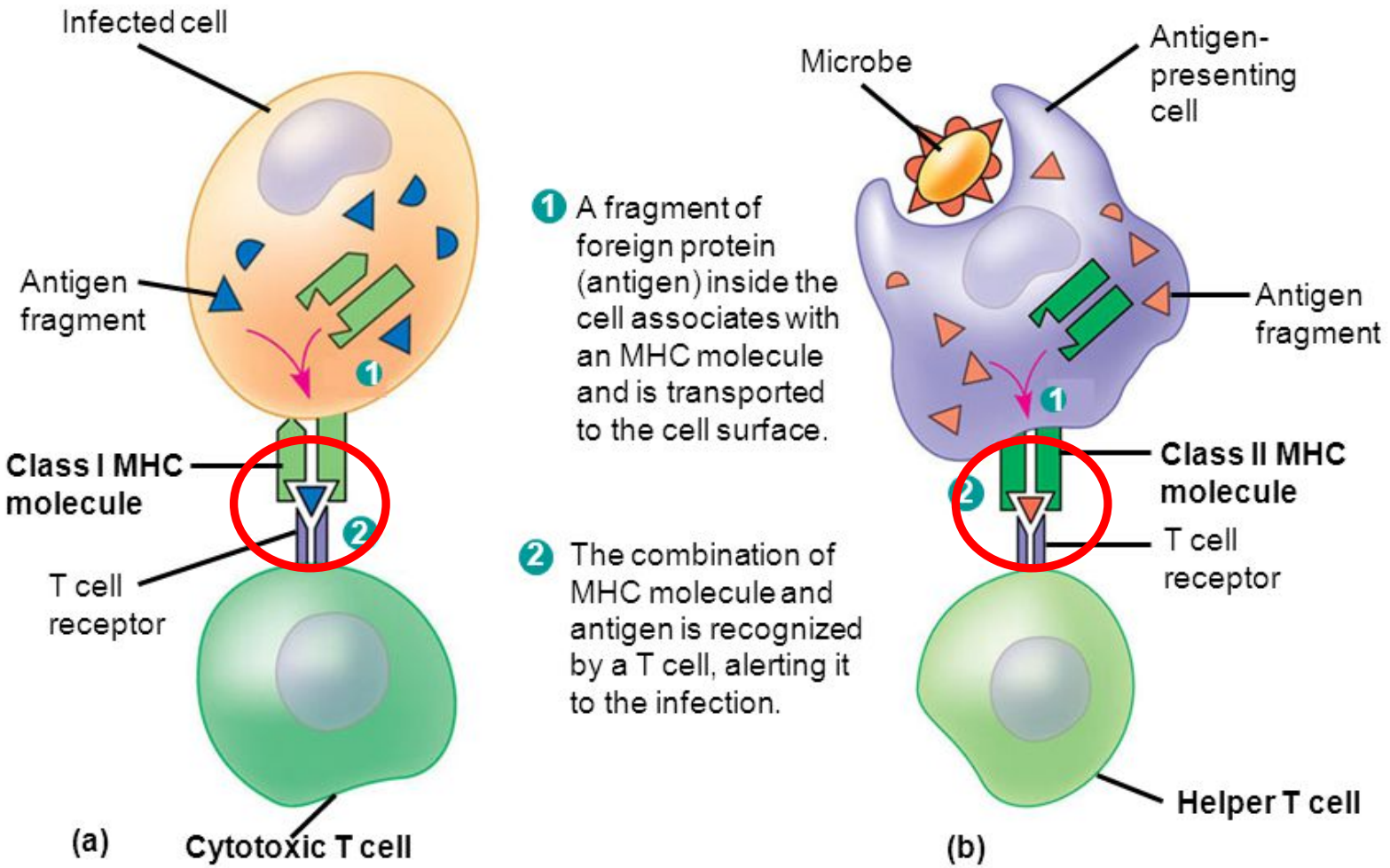
- Response time days to weeks
- Cells express clonally distinct Ag receptors:
  - $CD4^+$ *'helper'* T cells
  - $CD8^+$ *'killer'* T cells
  - $CD25^+$ $FoxP3^+$ *'regulatory' T cells*
  - B cells

Adaptive immune system

Notable features

- Effector molecules include inflammatory cytokines, chemokines, growth factors, *cytotoxic molecules and antibodies*
- Response of a single clone involves short-lived effector cells and long-lived memory cells
- Memory cells can undergo rapid expansion upon re-exposure to same Ag

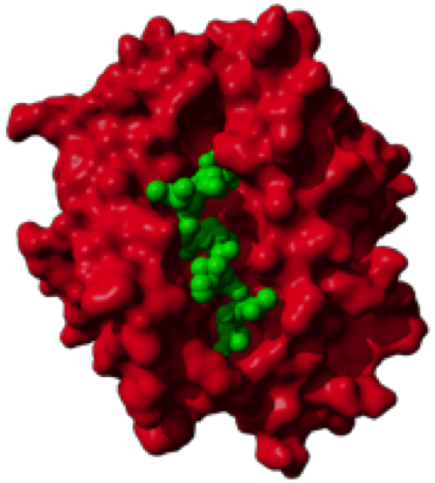
T cells recognize protein fragments bound to surface MHC molecules



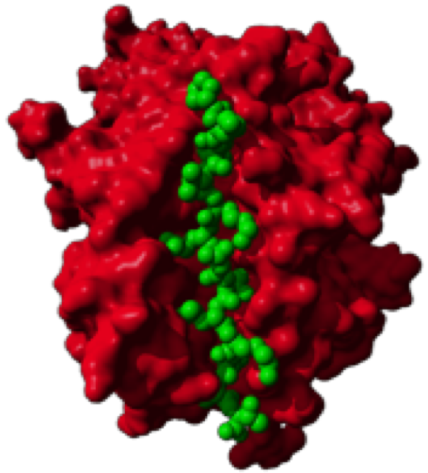
Structure of a MHC class I/peptide complex

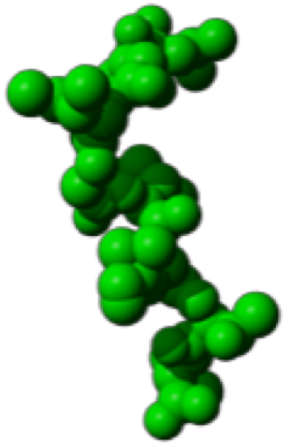
Class I
HLA-A2.1

Class II
HLA-DR1



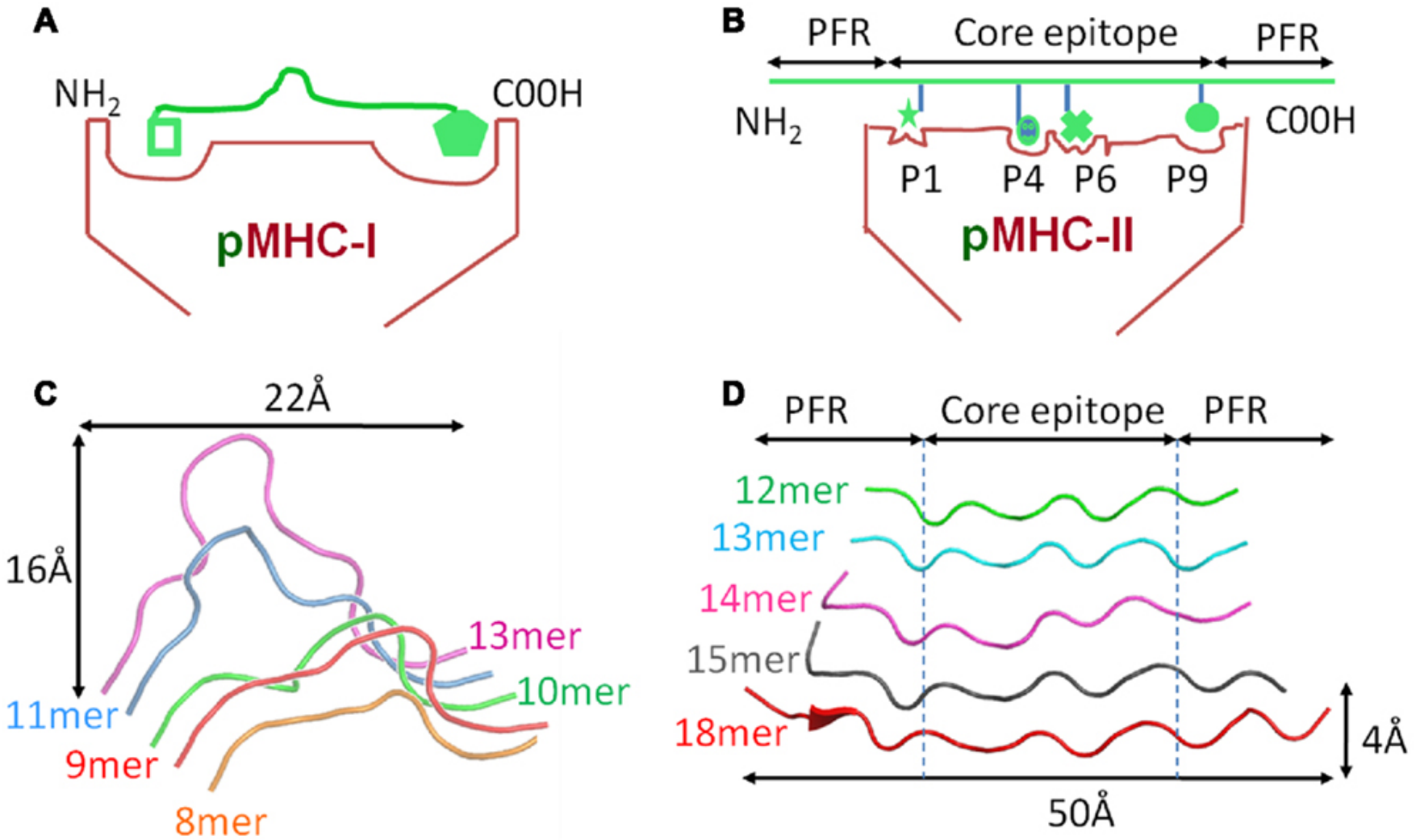
Peptide-bound complex



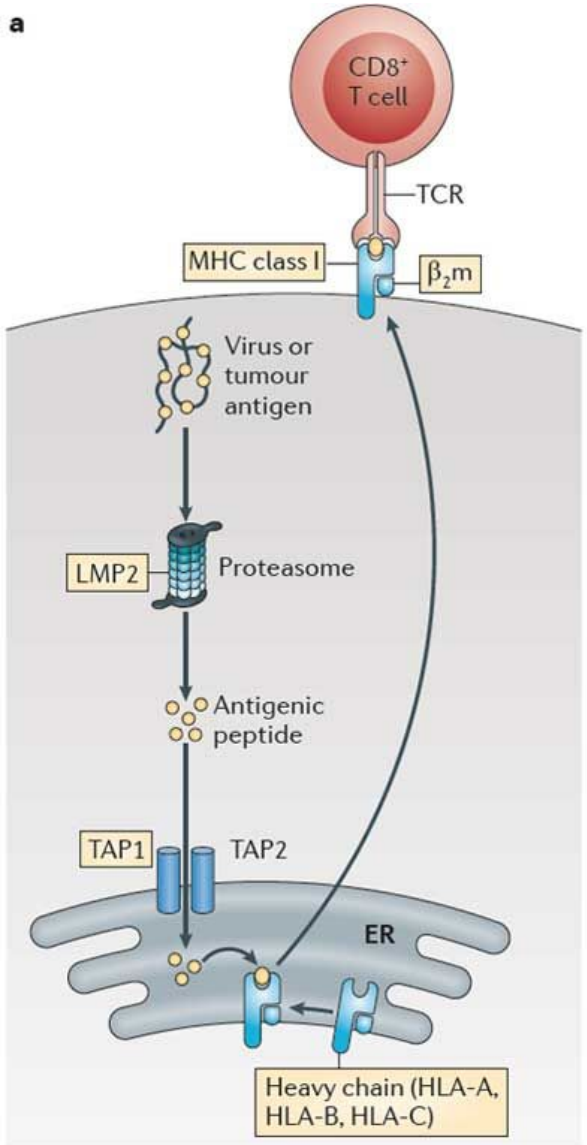


Peptide alone

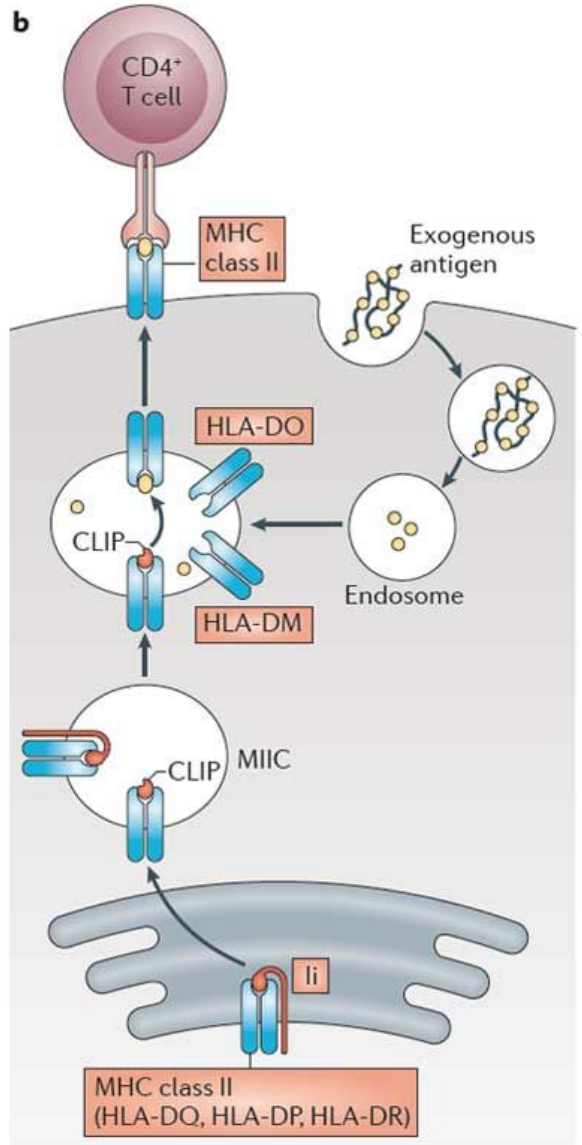
Comparing class I and II peptide binding



Endogenous Ag> MHC class I > $CD8^+$ T cells



Exogenous Ag> MHC class II > $CD4^+$ T cells

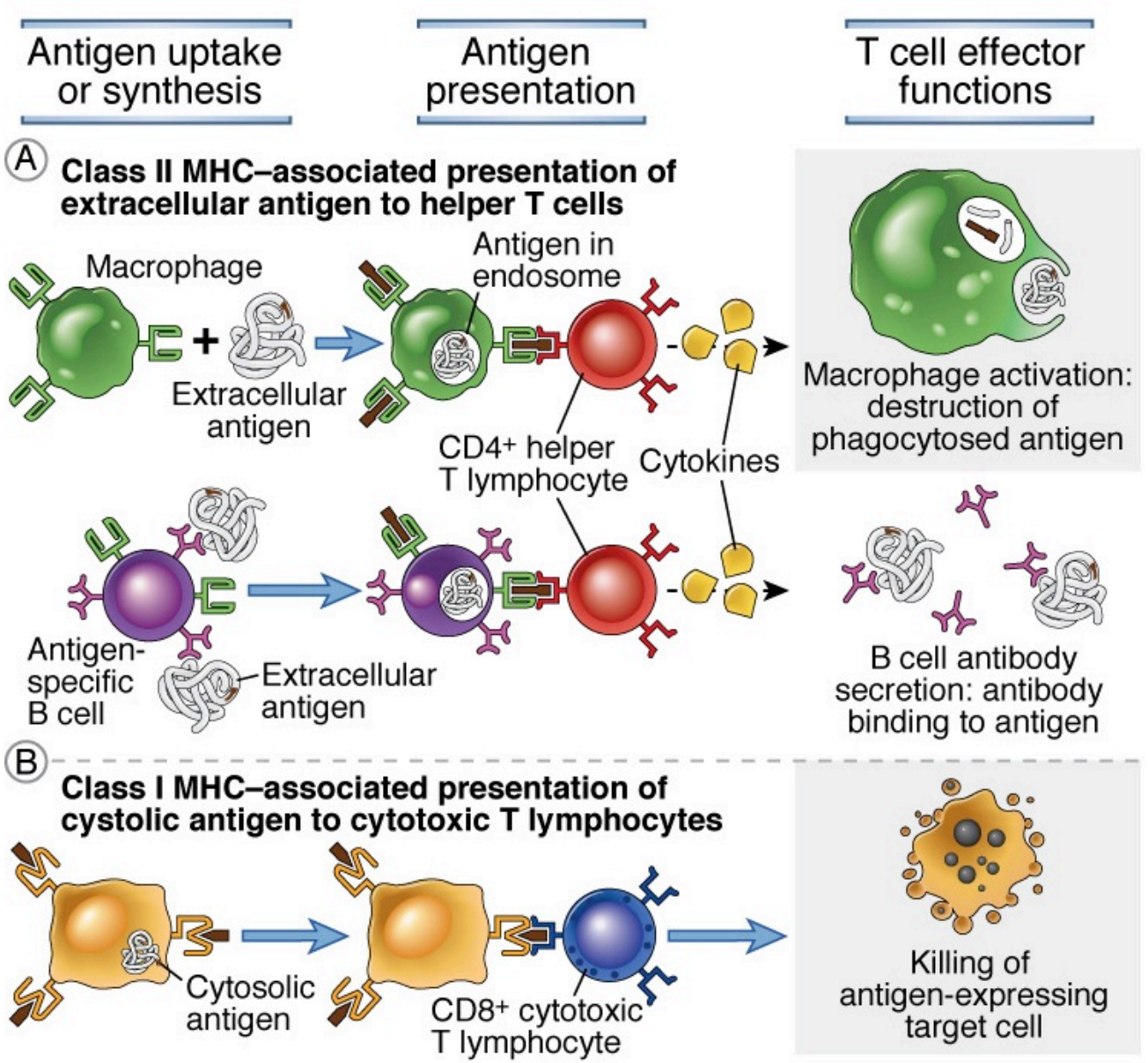


How class I- and class II-associated antigen presentation influences the nature of the T cell response

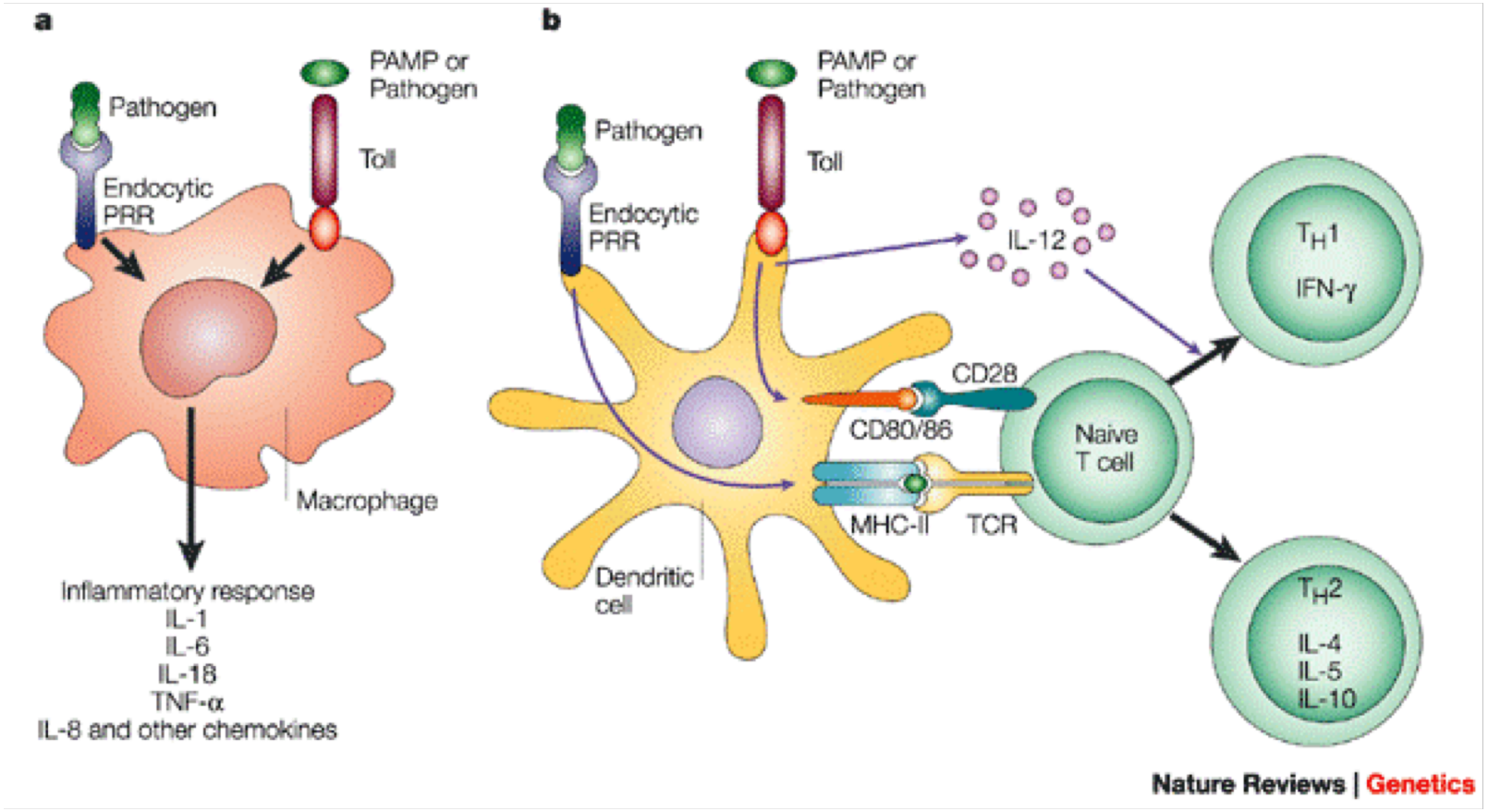
CD4+ T cell

B cell

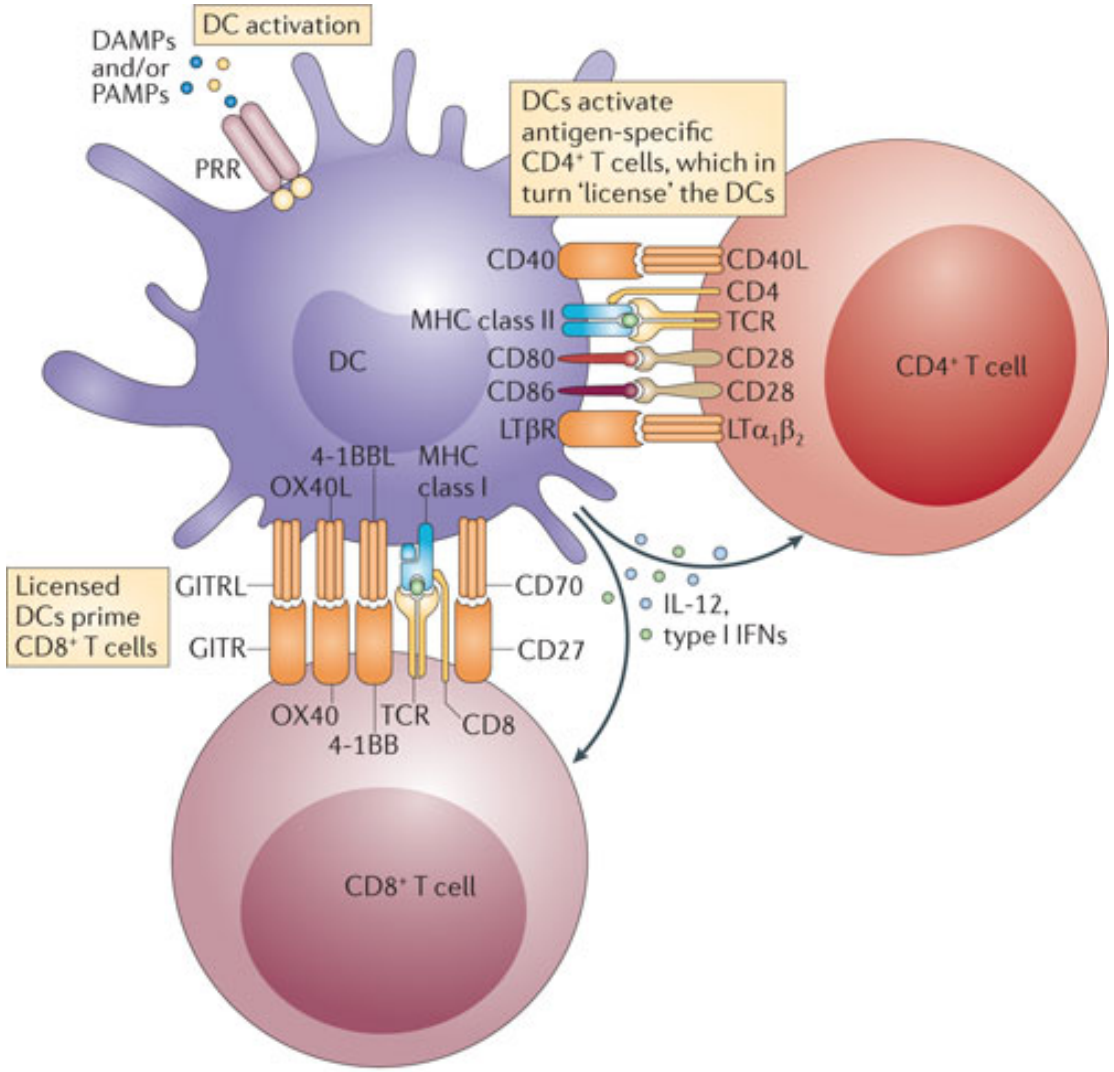
CD8+ T cell



TLR engagement leads to activation of both innate and adaptive immunity



T cell priming involves antigen presentation, costimulatory & cytokine signals



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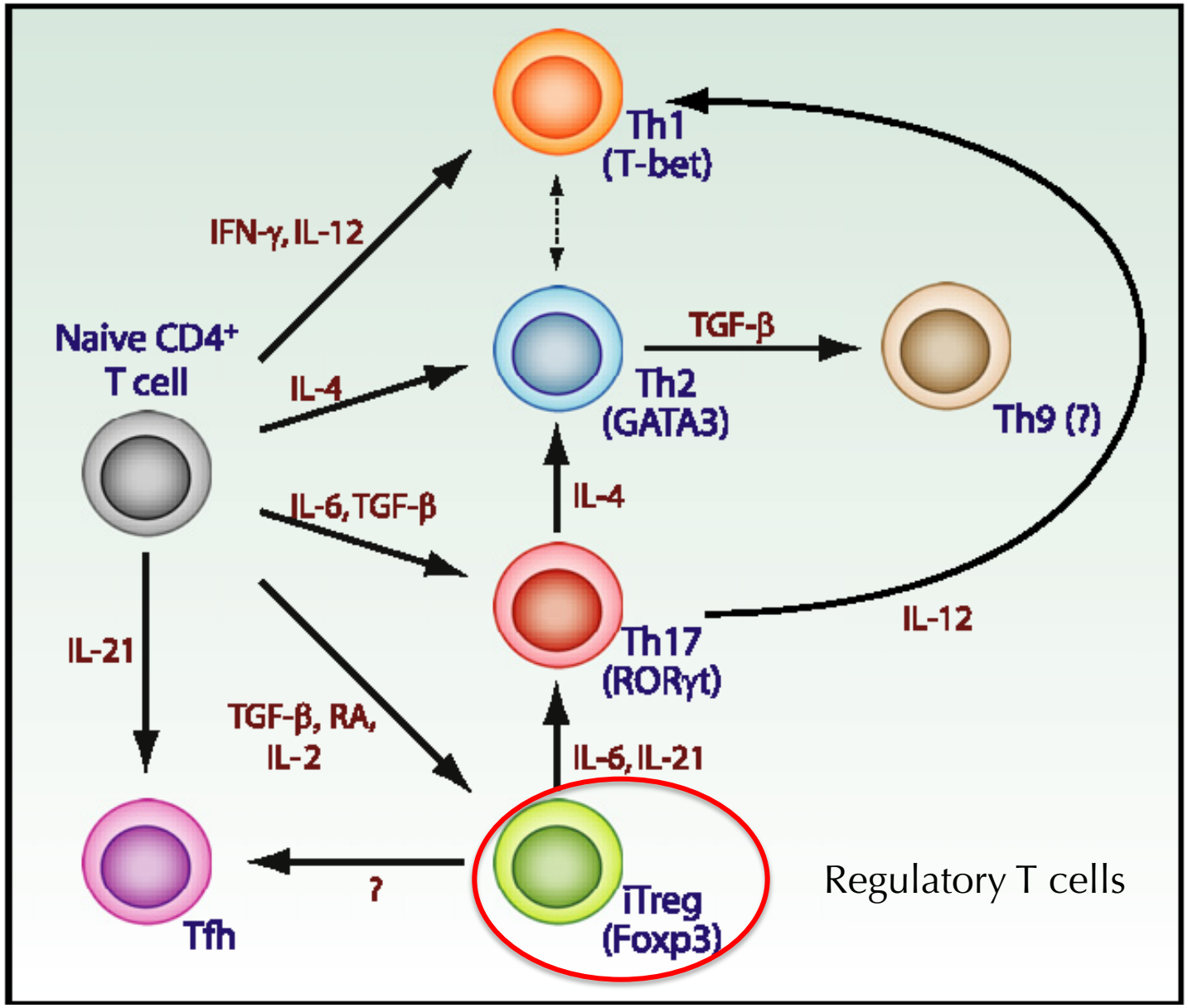
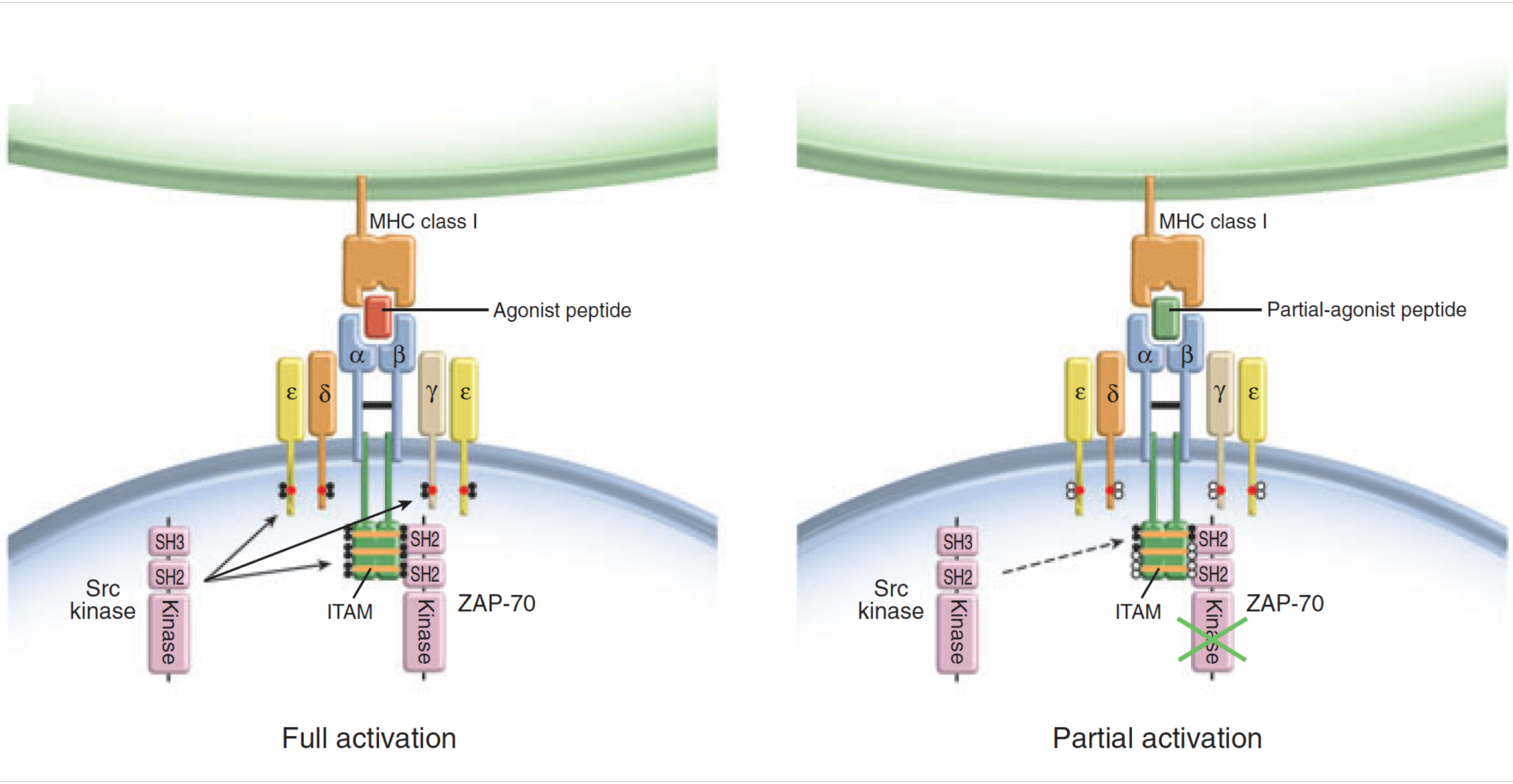
$CD4^+$ T cell plasticity one cell > many possible functional fates

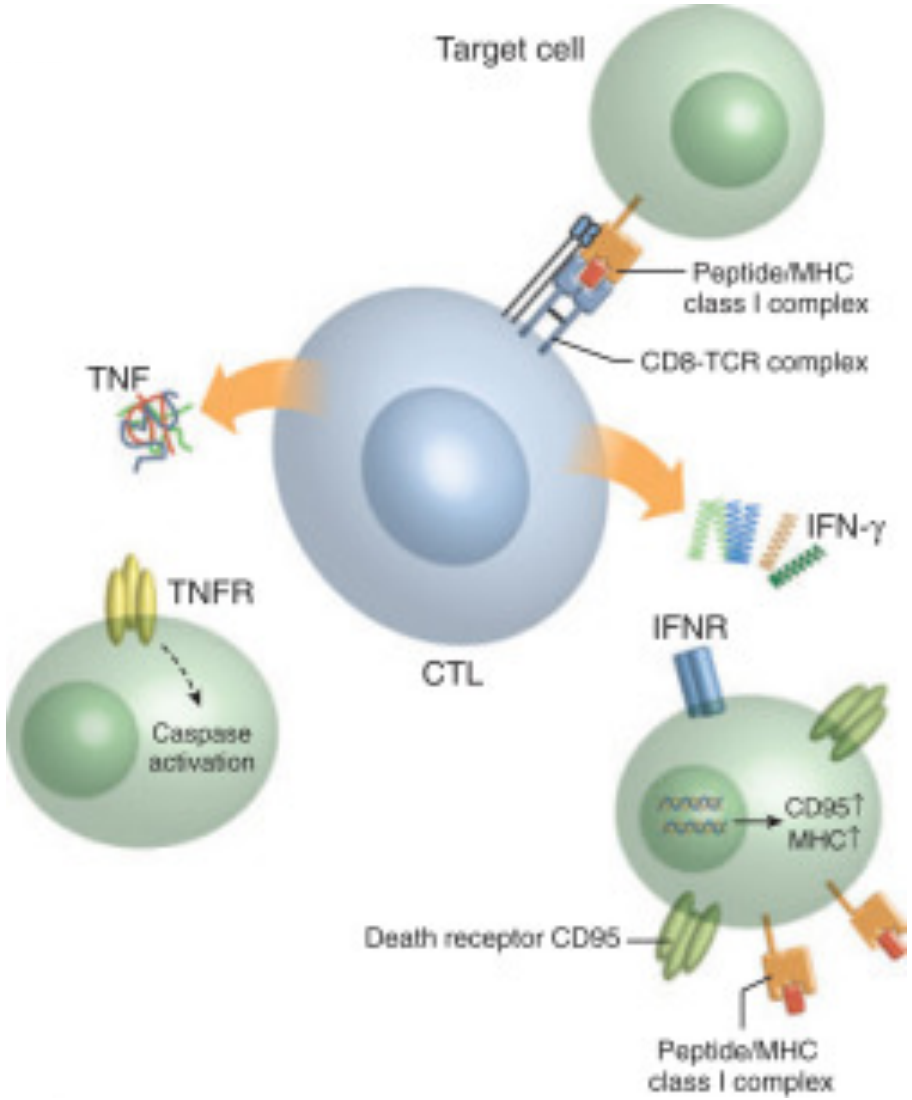


The TCR is a ‘rheostat’, rather than a binary sensor

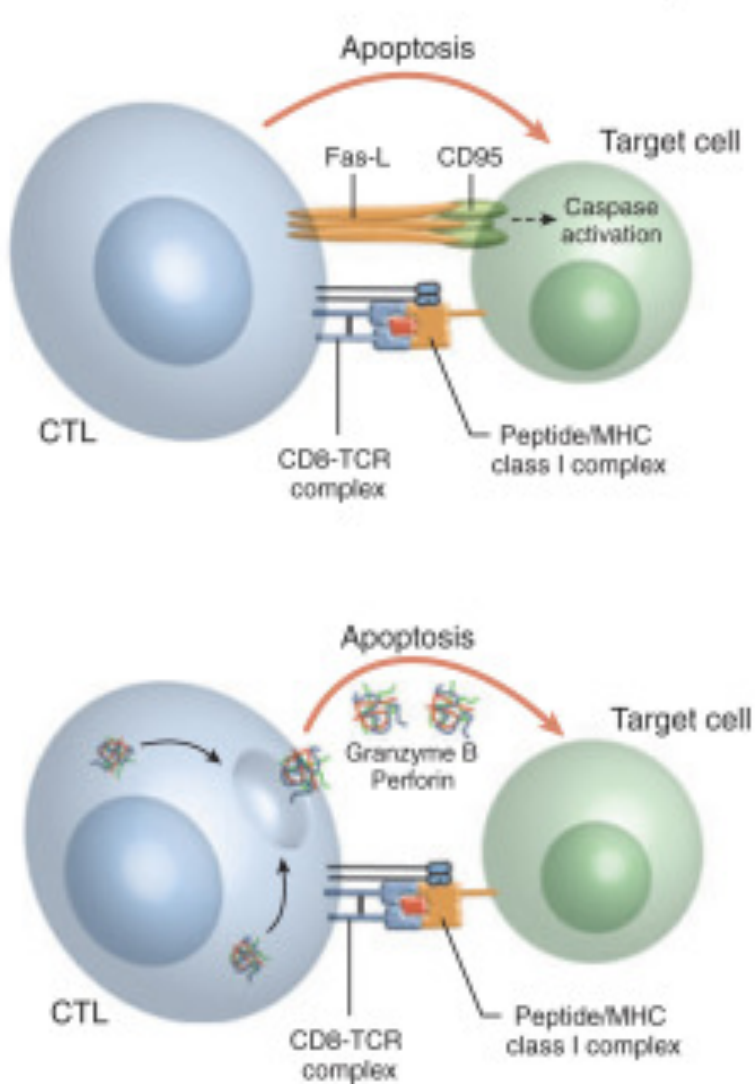


Effector Mechanisms

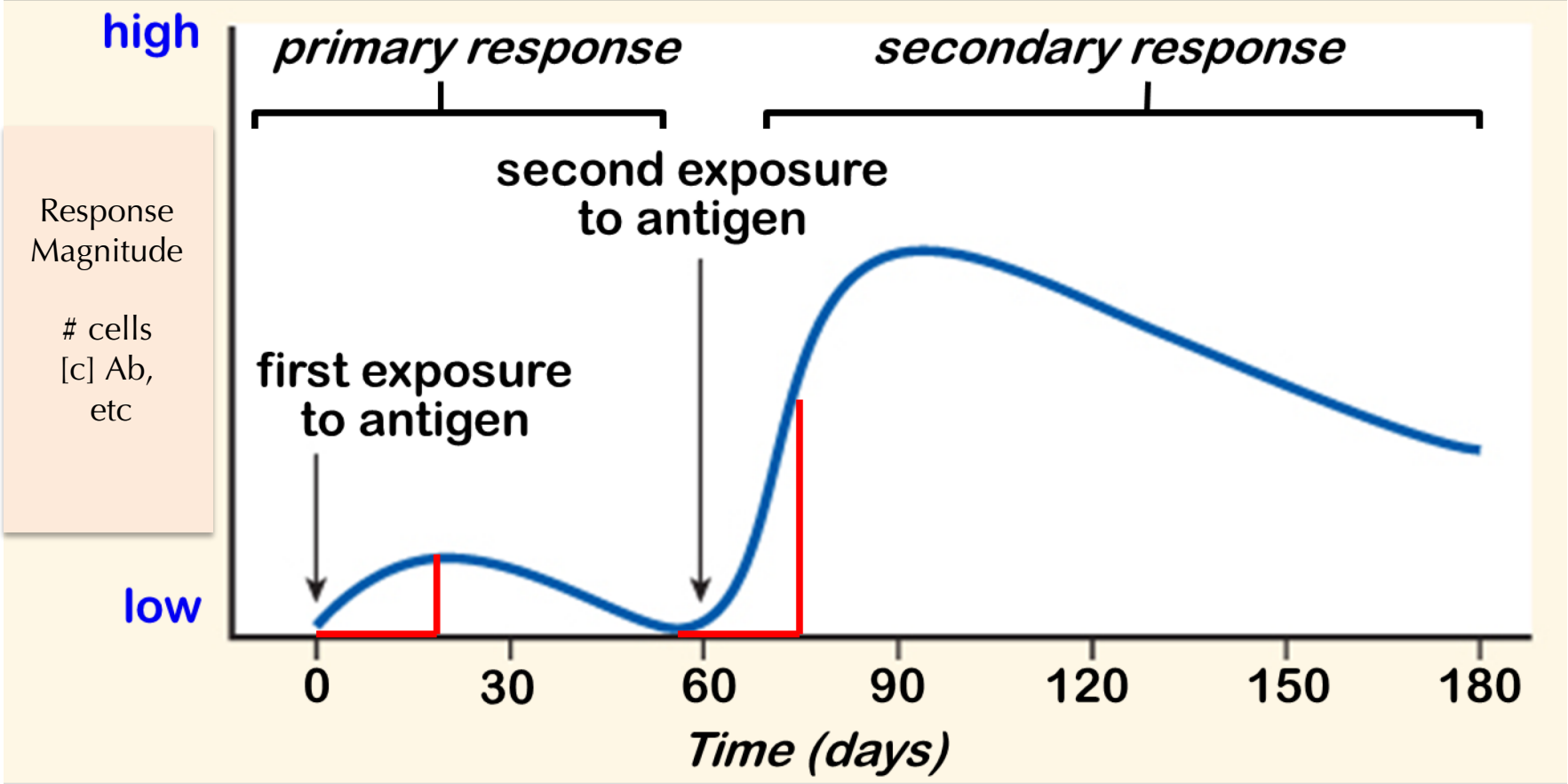
Cytokine mediated



Contact-dependent



Immune memory: a faster and stronger secondary response



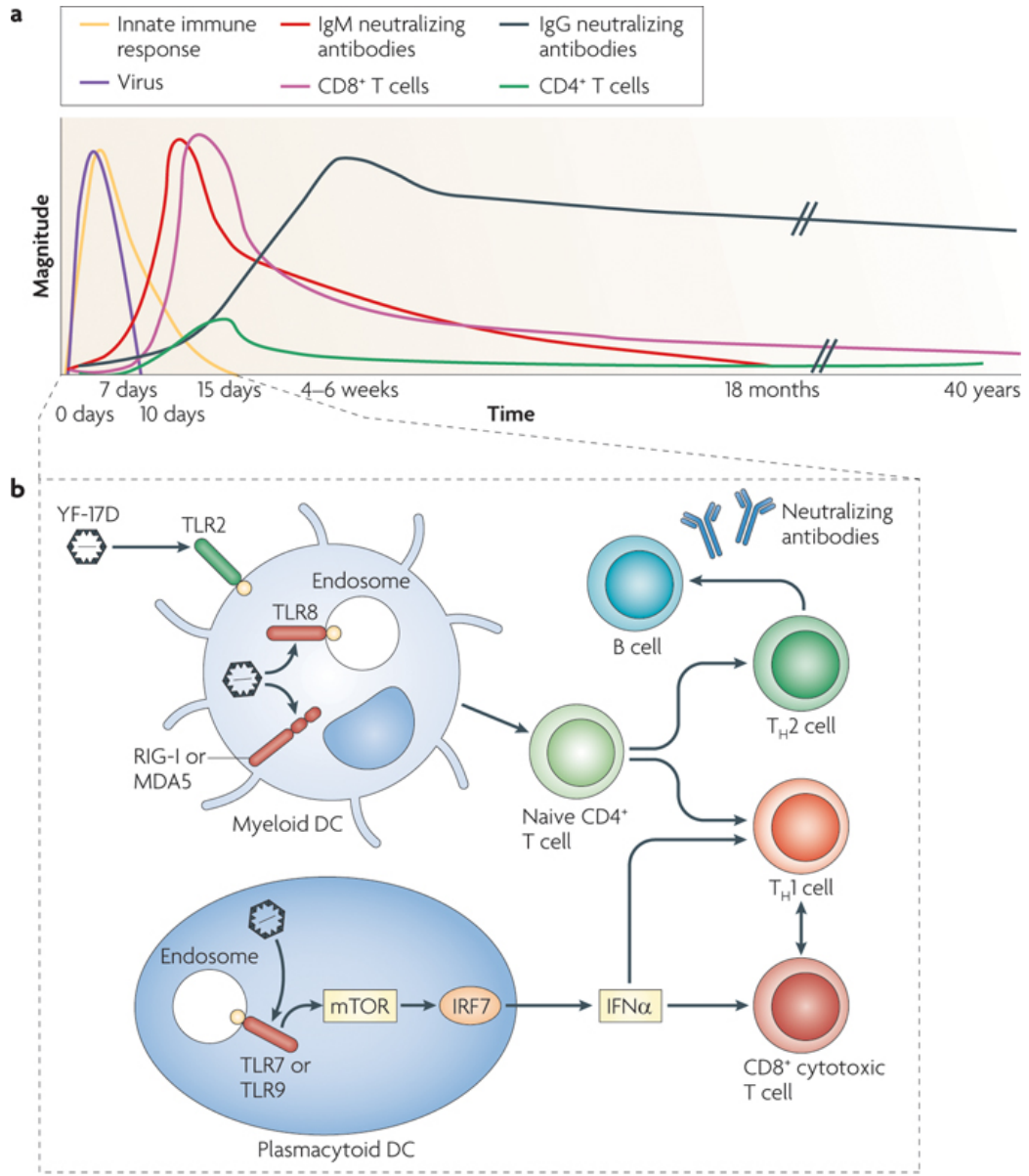
The concept of immune memory

The Plague of Athens, 2nd Peloponnesian War

"The sick and the dying were tended by the pitying care of those who had recovered, because they knew the course of the disease and were themselves free from apprehensions.
For no one was ever attacked a second time, or not with a fatal result"

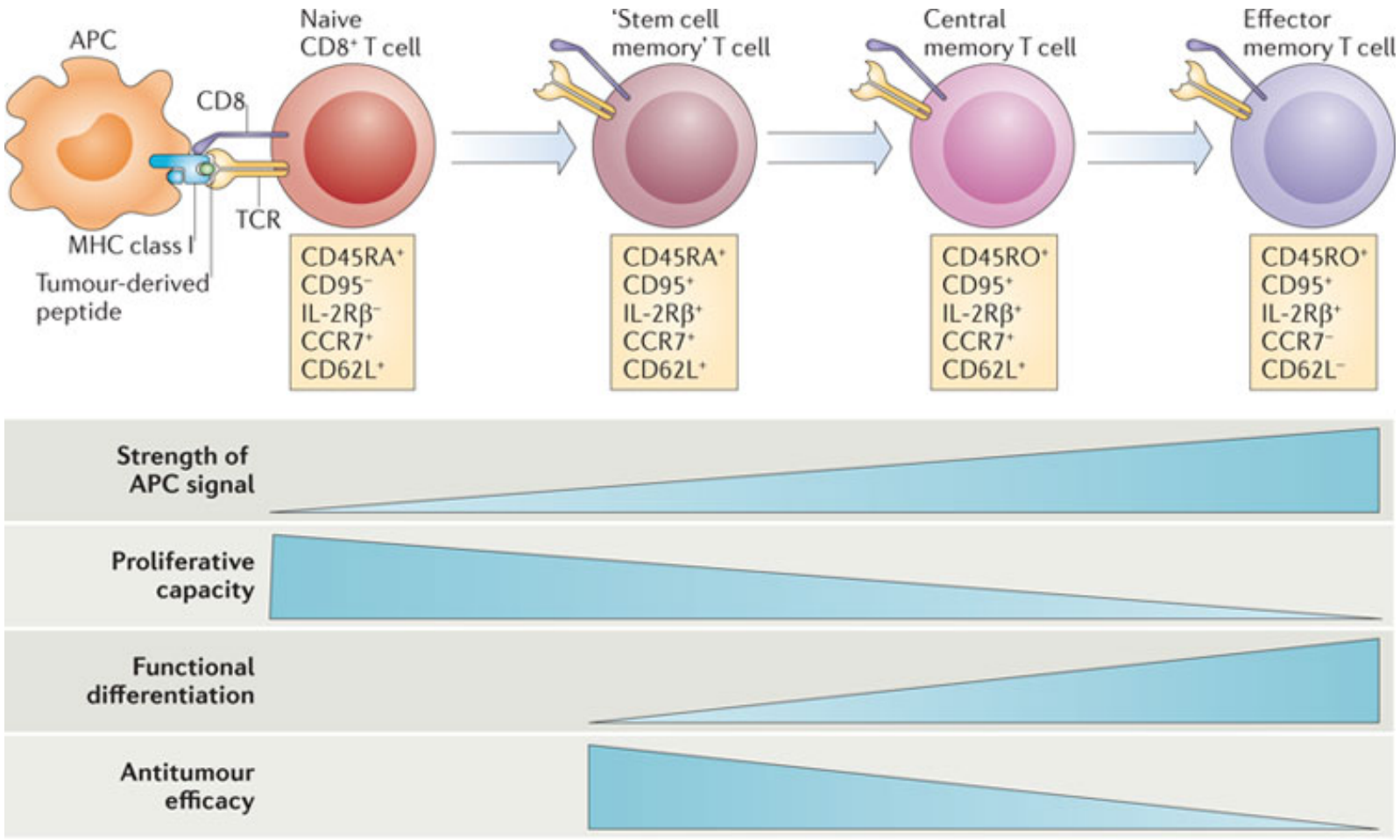
Thuycides, 400 B.C.

Vaccination with Yellow Fever YF-17D induces long-lived immunity in humans



Adapted from B. Pulendran,
Nature Reviews Immunology 2009

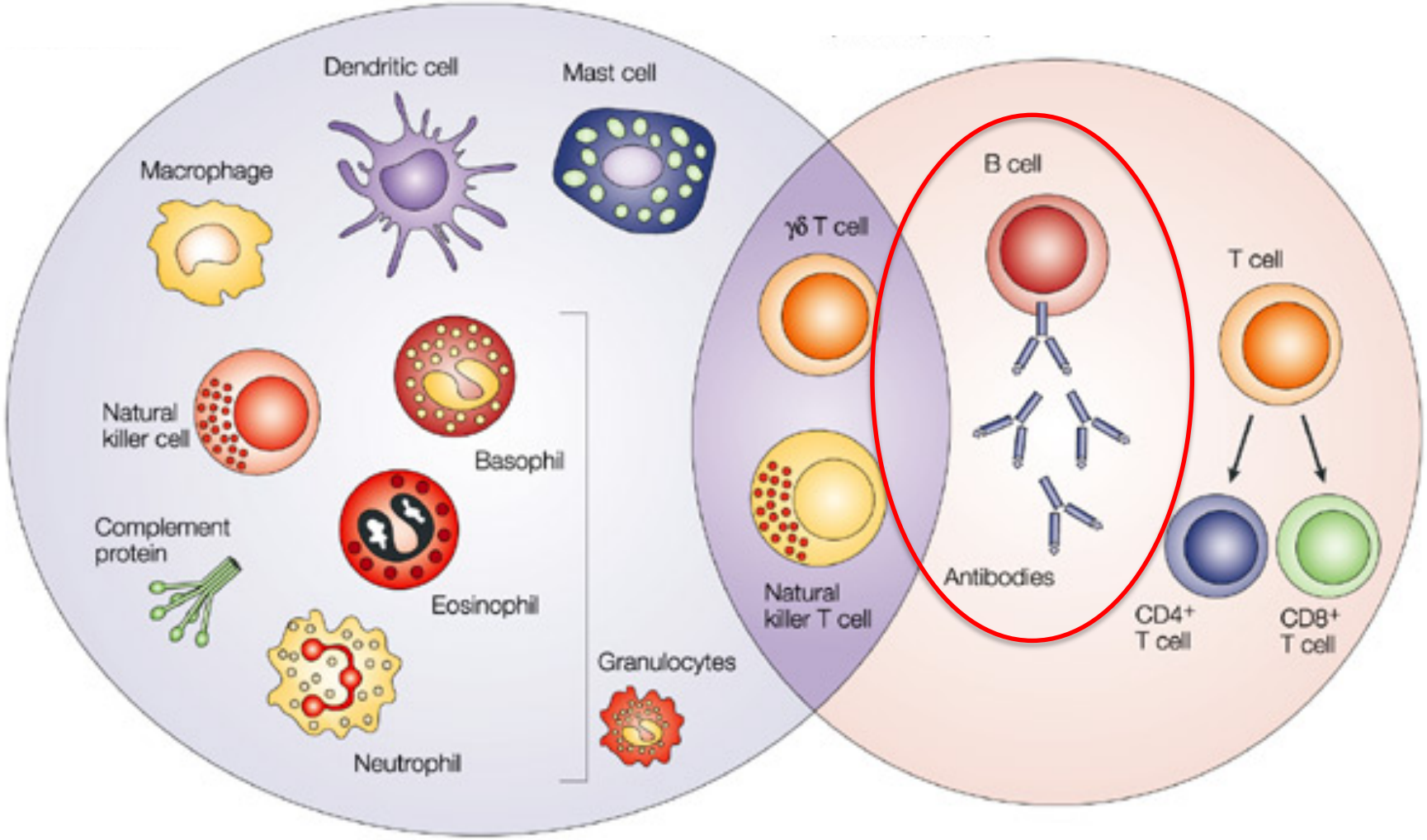
Memory T cell subsets: T_{CM} T_{EM} T_{RM} & T_{SCM}



Innate & Adaptive Immunity

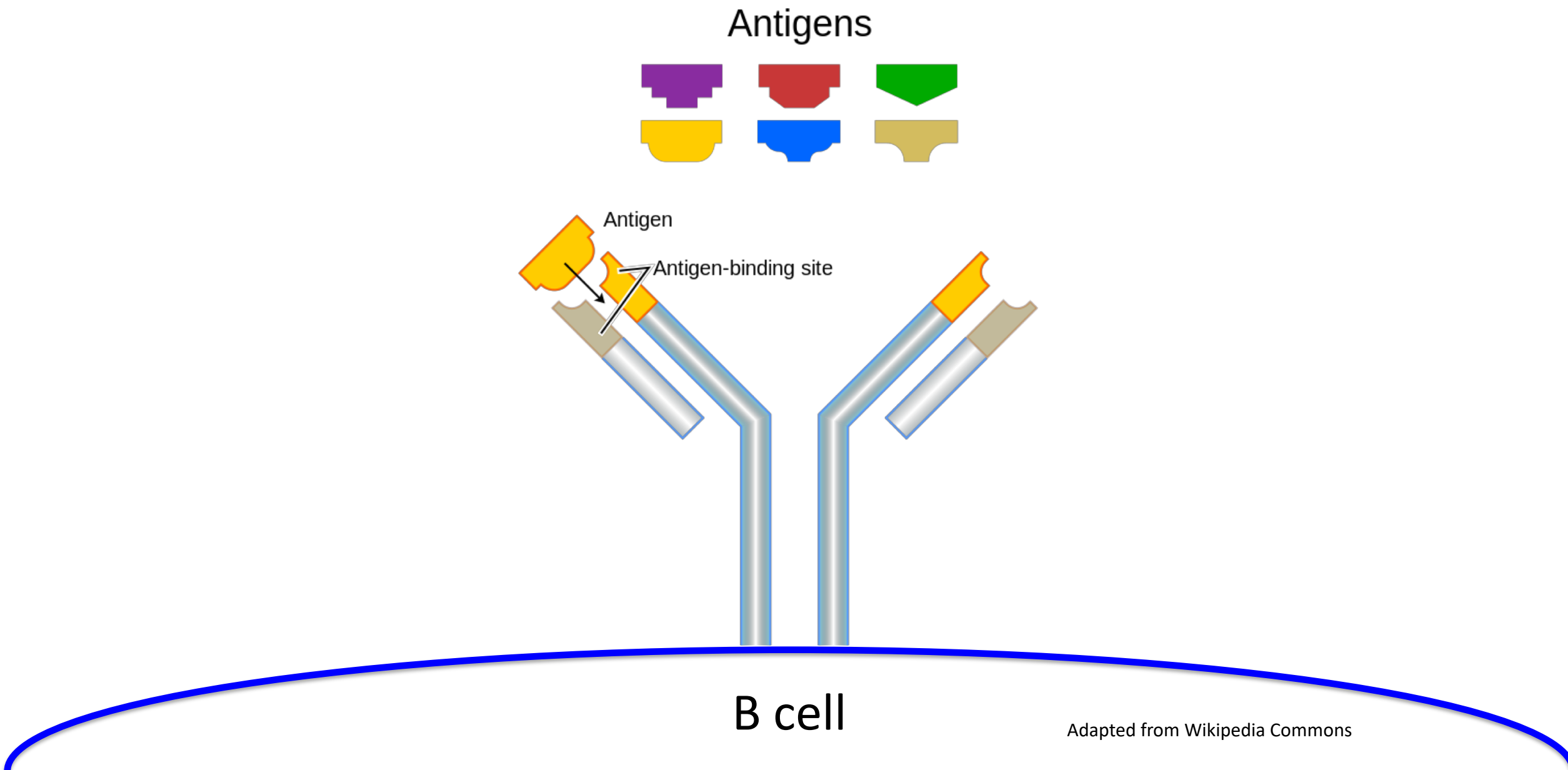
Innate arm
(fast and non-specific)

Adaptive arm
(slower but Ag-specific)



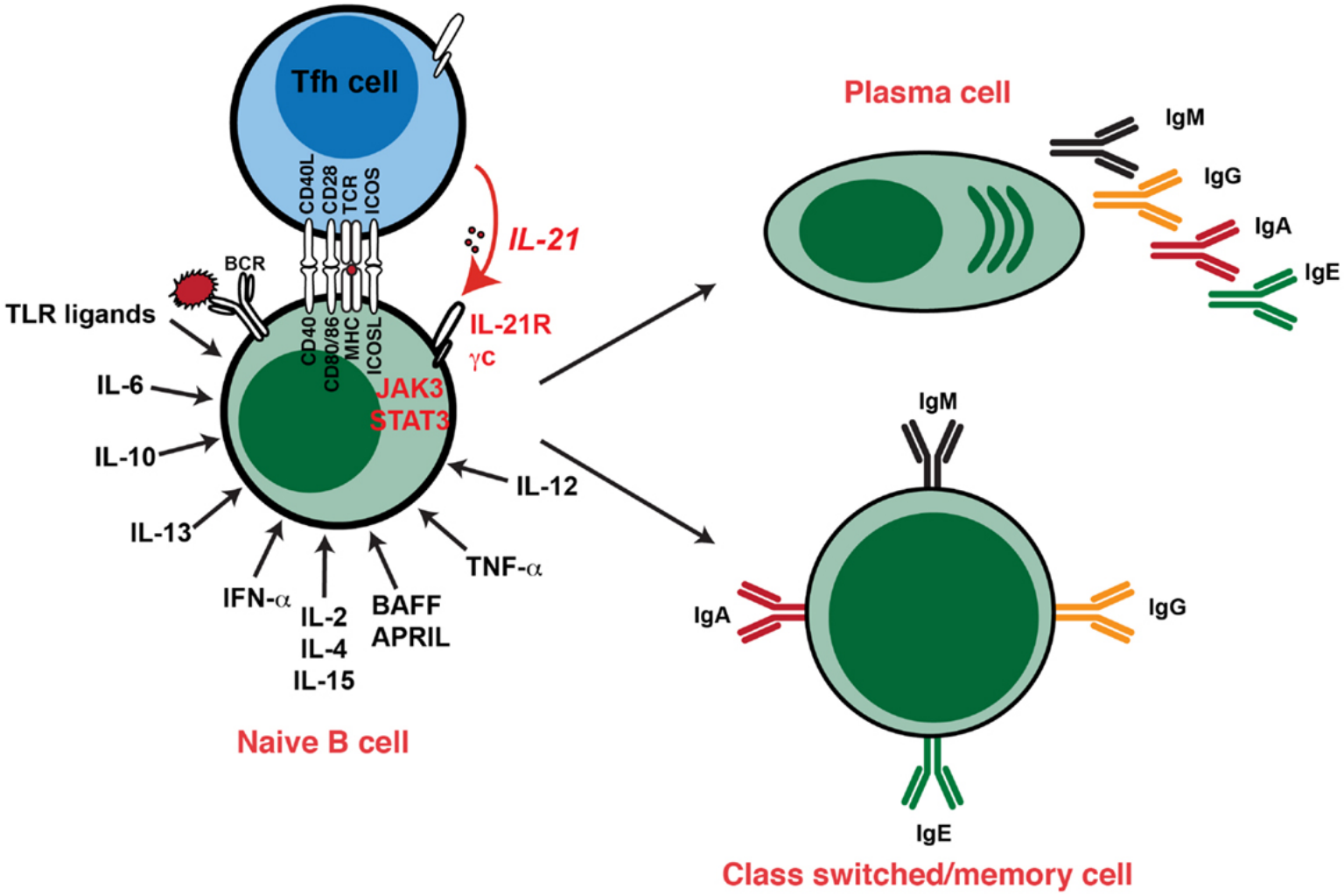
G. Dranoff, Cytokines in cancer pathogenesis and cancer therapy , Nature Reviews Cancer 4:11-122

B cells recognize specific fragments of intact proteins via antibody receptors



Adapted from Wikipedia Commons

Antibodies can be membrane-bound or secreted

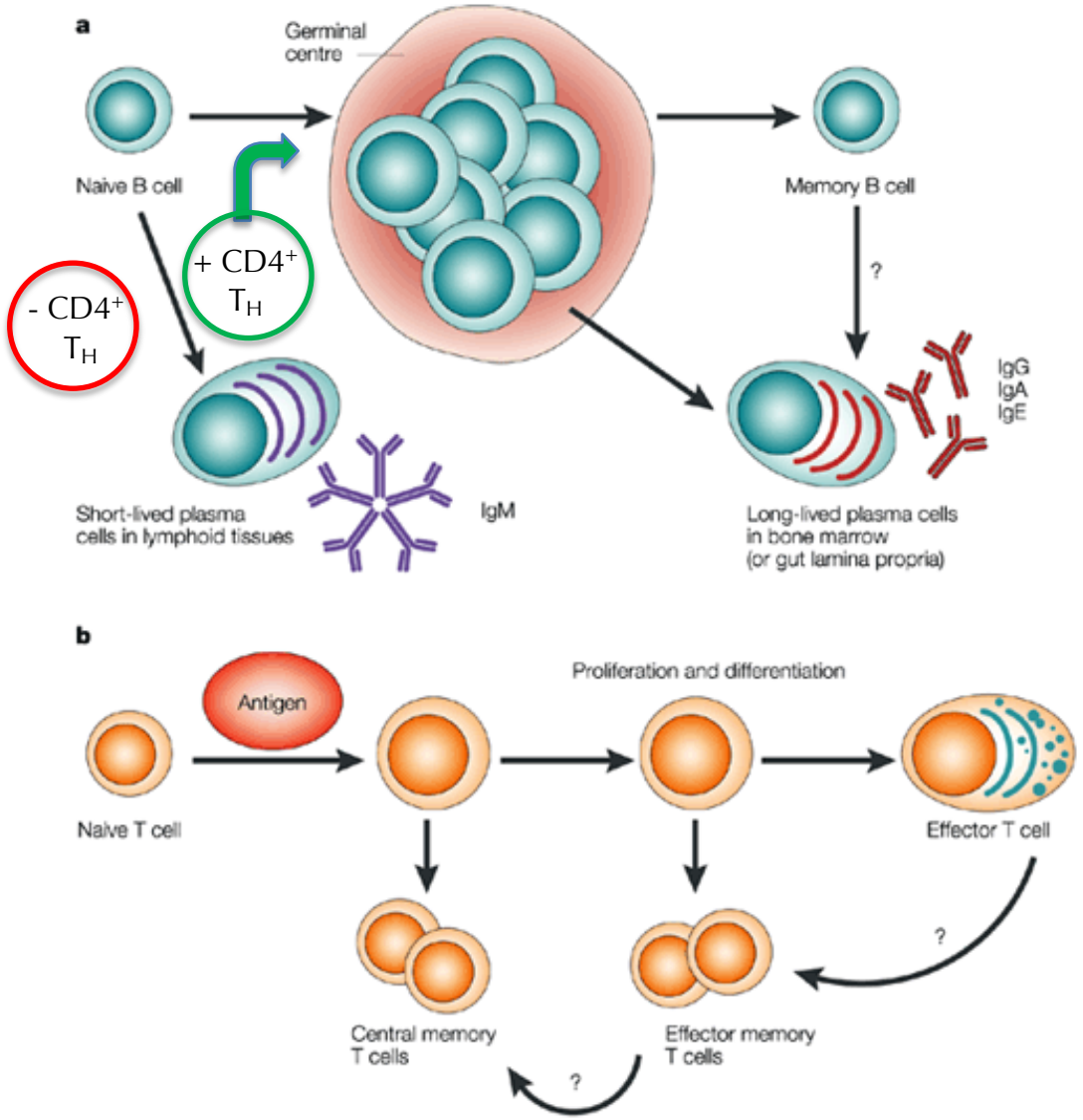


Moens & Tangye, Cytokine-mediated regulation of plasma cell generation: IL-21 takes center stage. Frontiers in Immunology vol. 5 2014

B and T cell responses proceed via distinct pathways

B cells

T cells



Nature Reviews | Immunology David Gray, NRI **2,** 60-65 2002

B cell development

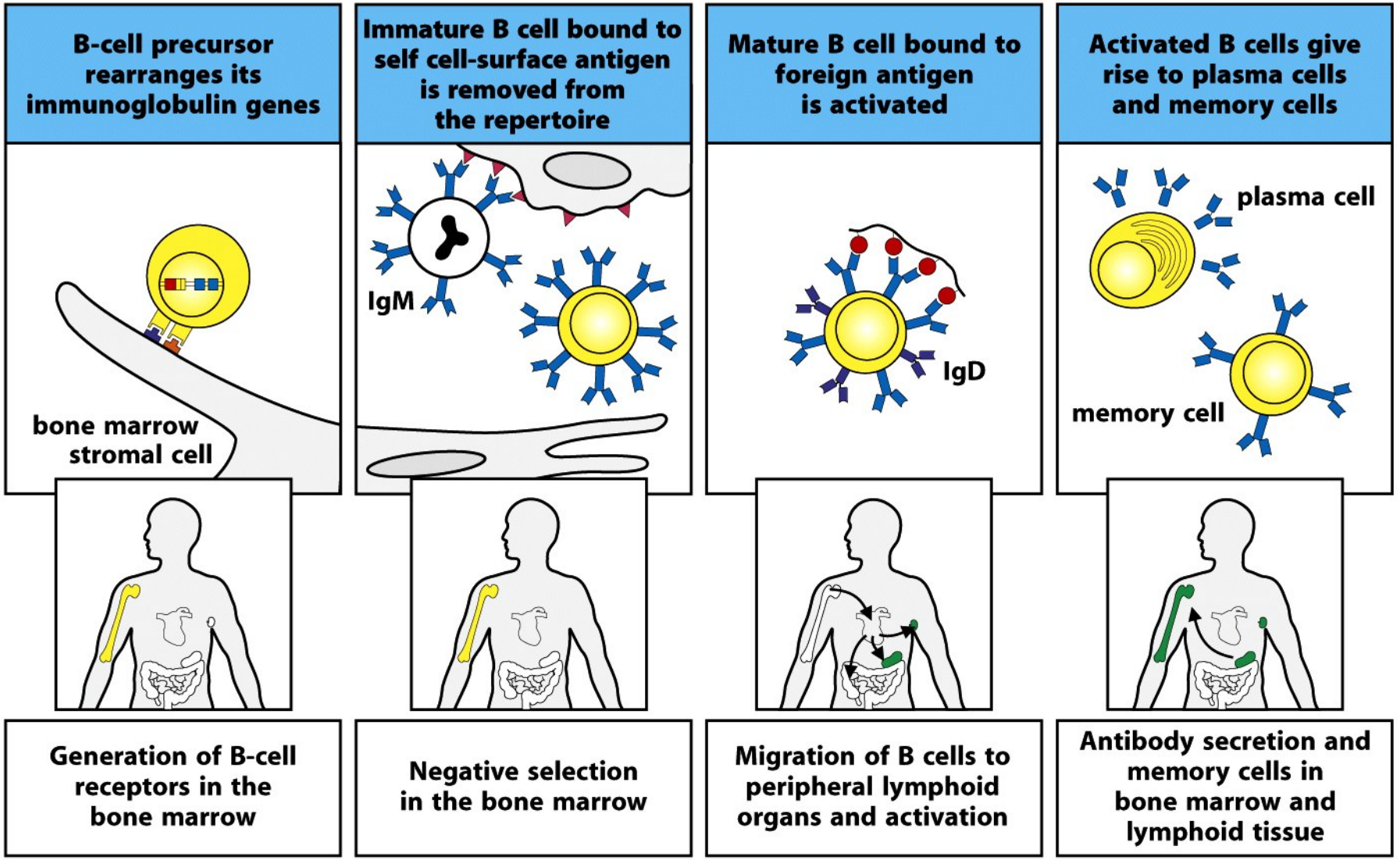
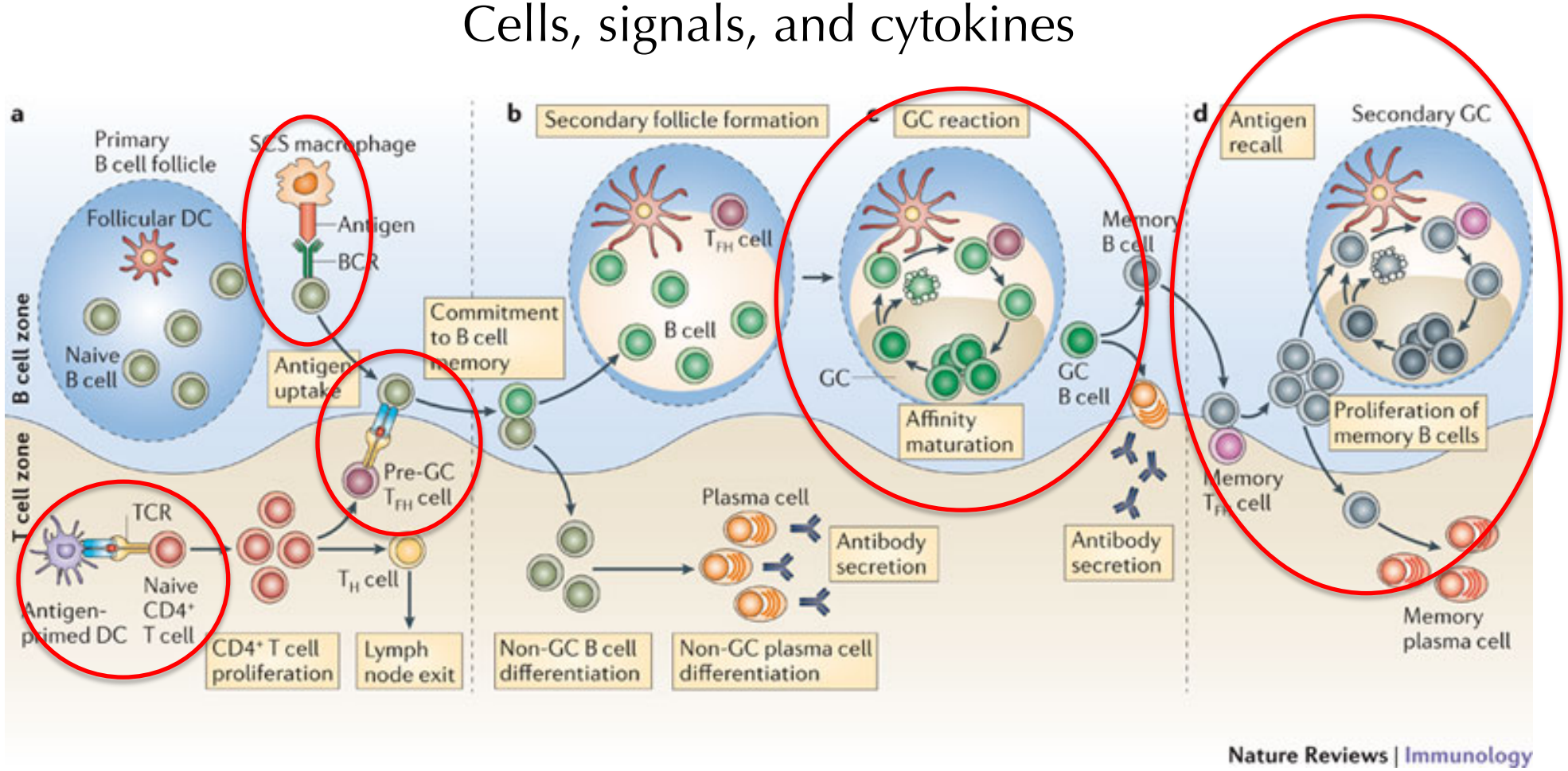


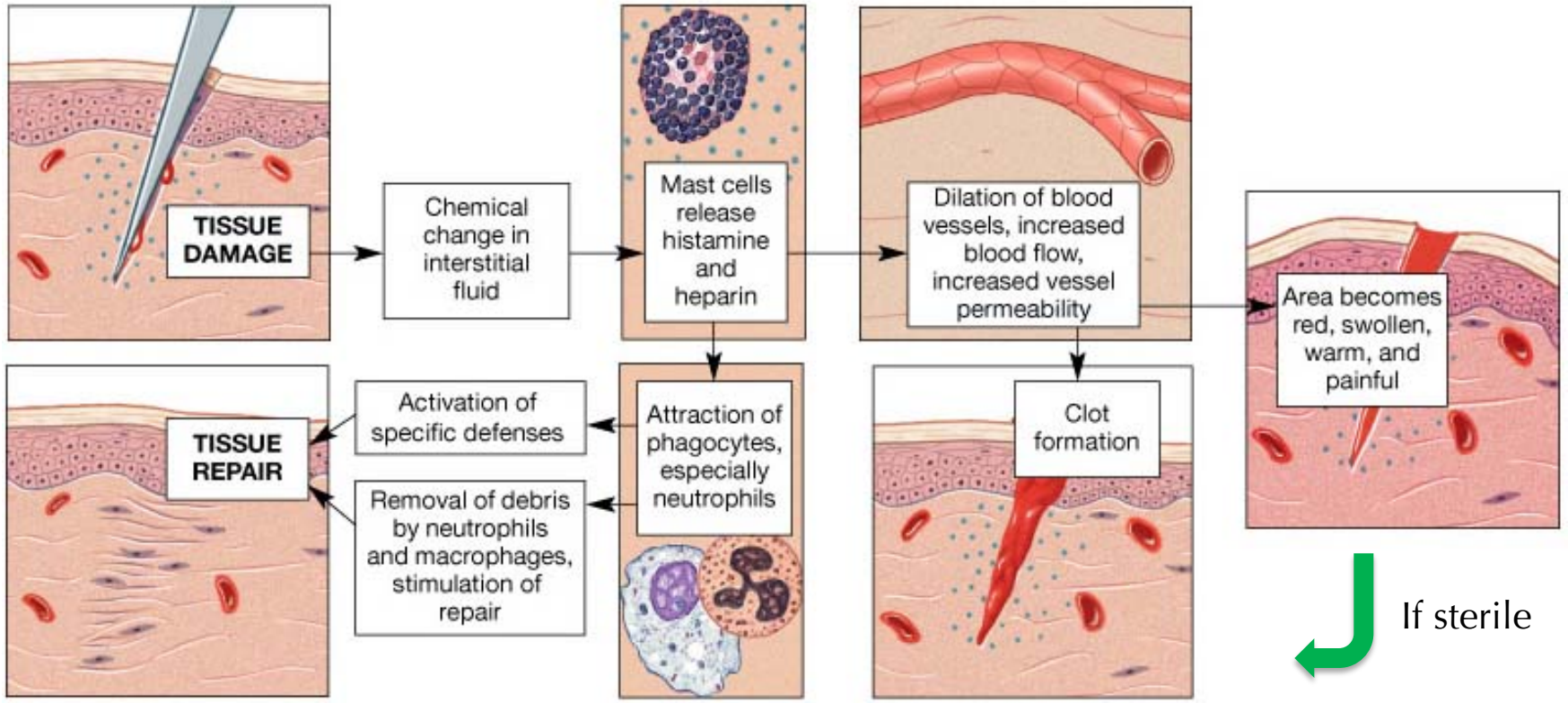
Figure 7-1 Immunobiology, 7ed. (© Garland Science 2008)

Multistep progression to B cell memory:
Cells, signals, and cytokines



McHeyzer-Williams et al, NRI **12** 24-34, 2012

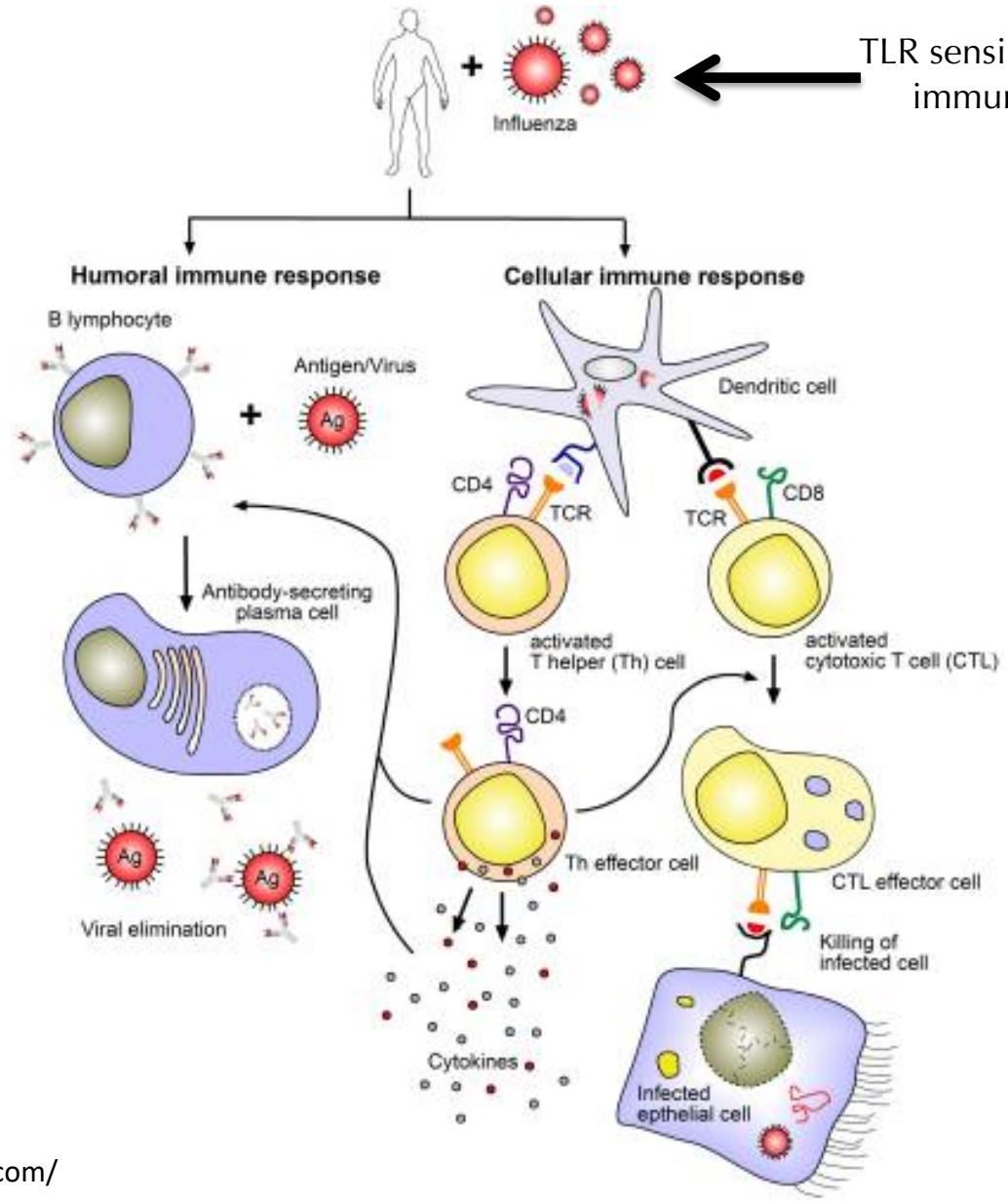
How does an immune response against an infectious pathogen begin?



TLR + damage

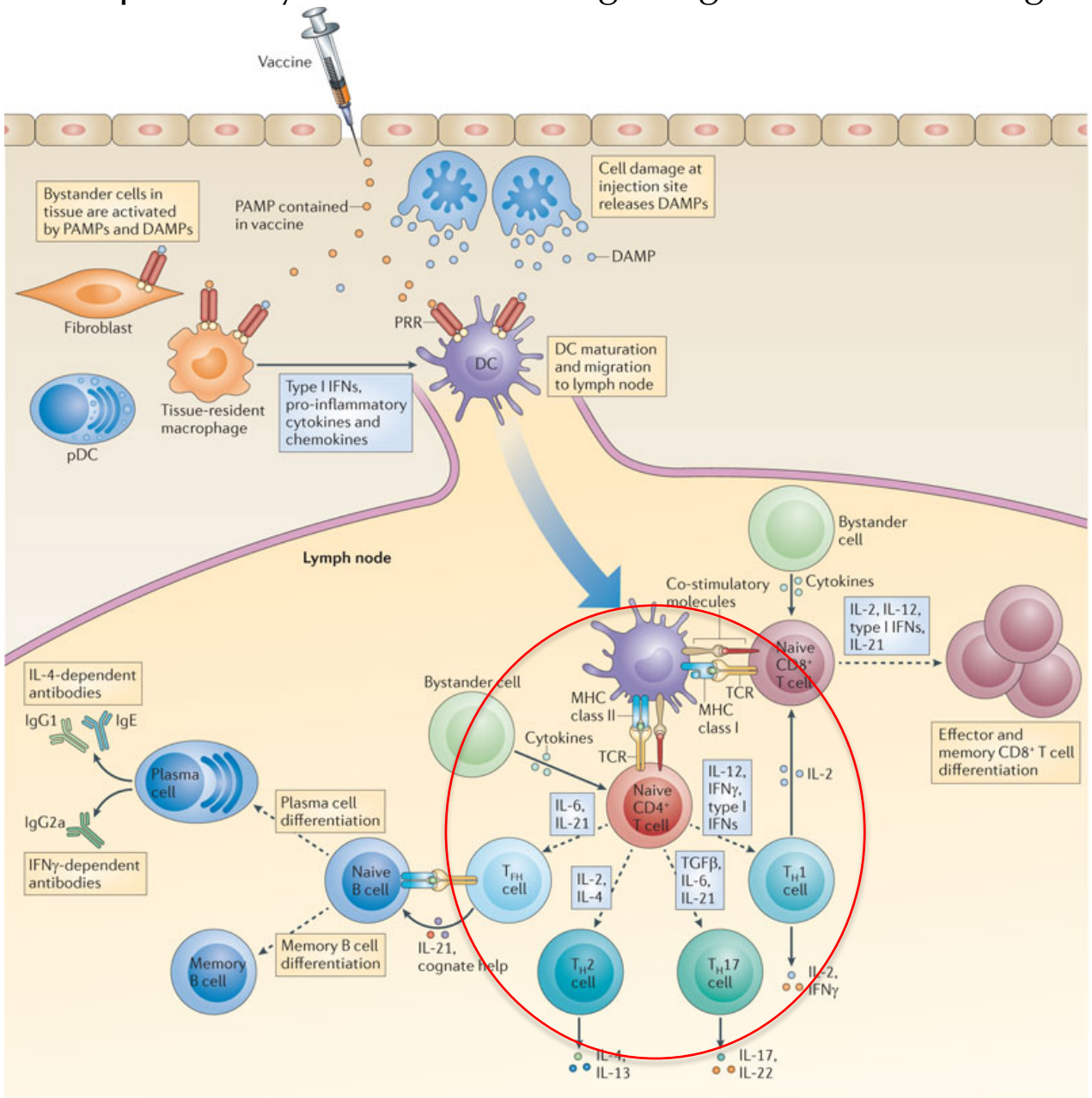
If sterile

The infection-immunity cycle



http://betournay.wikispaces.com/

B and T cells are primed by activated DC migrating from sites of antigen uptake



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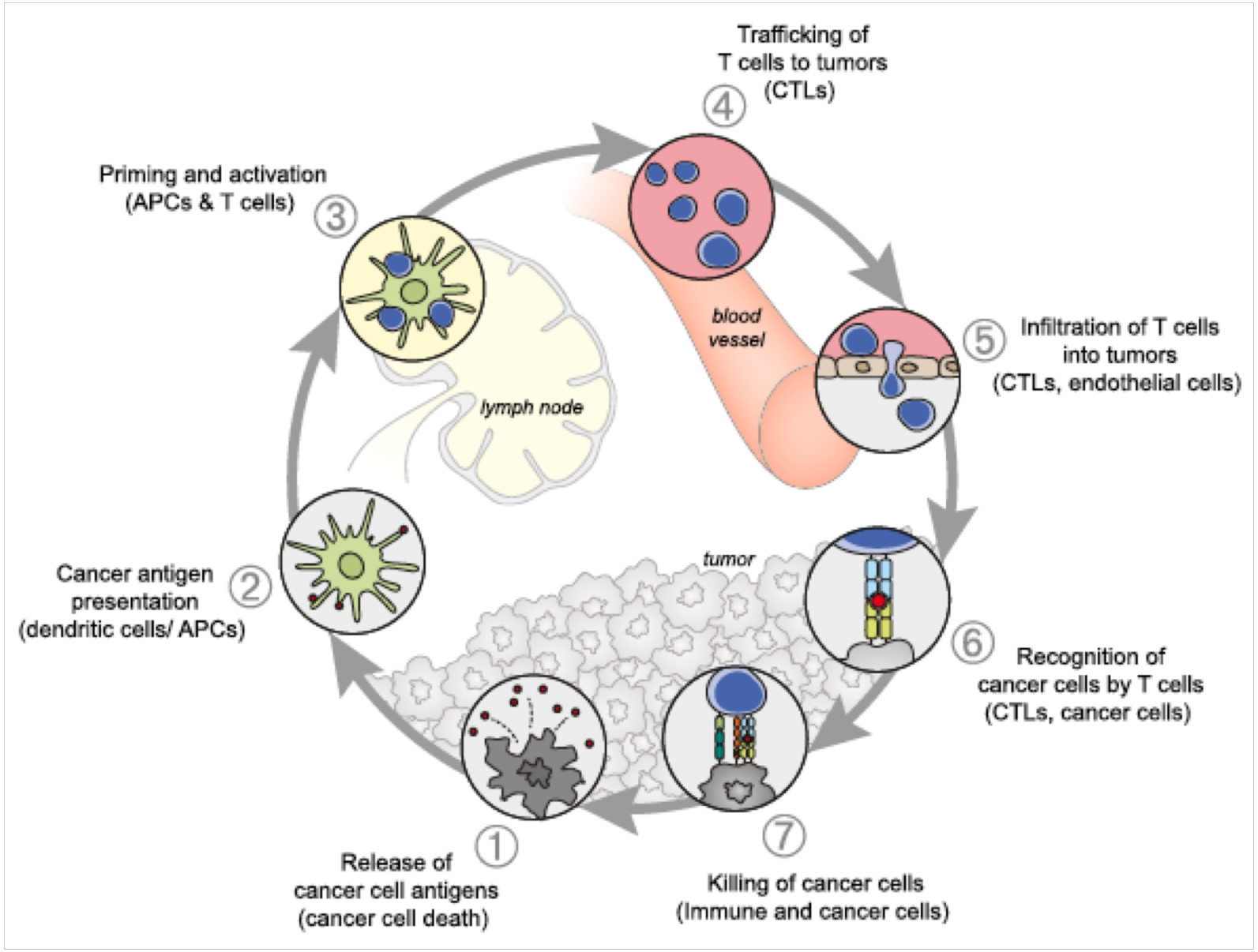
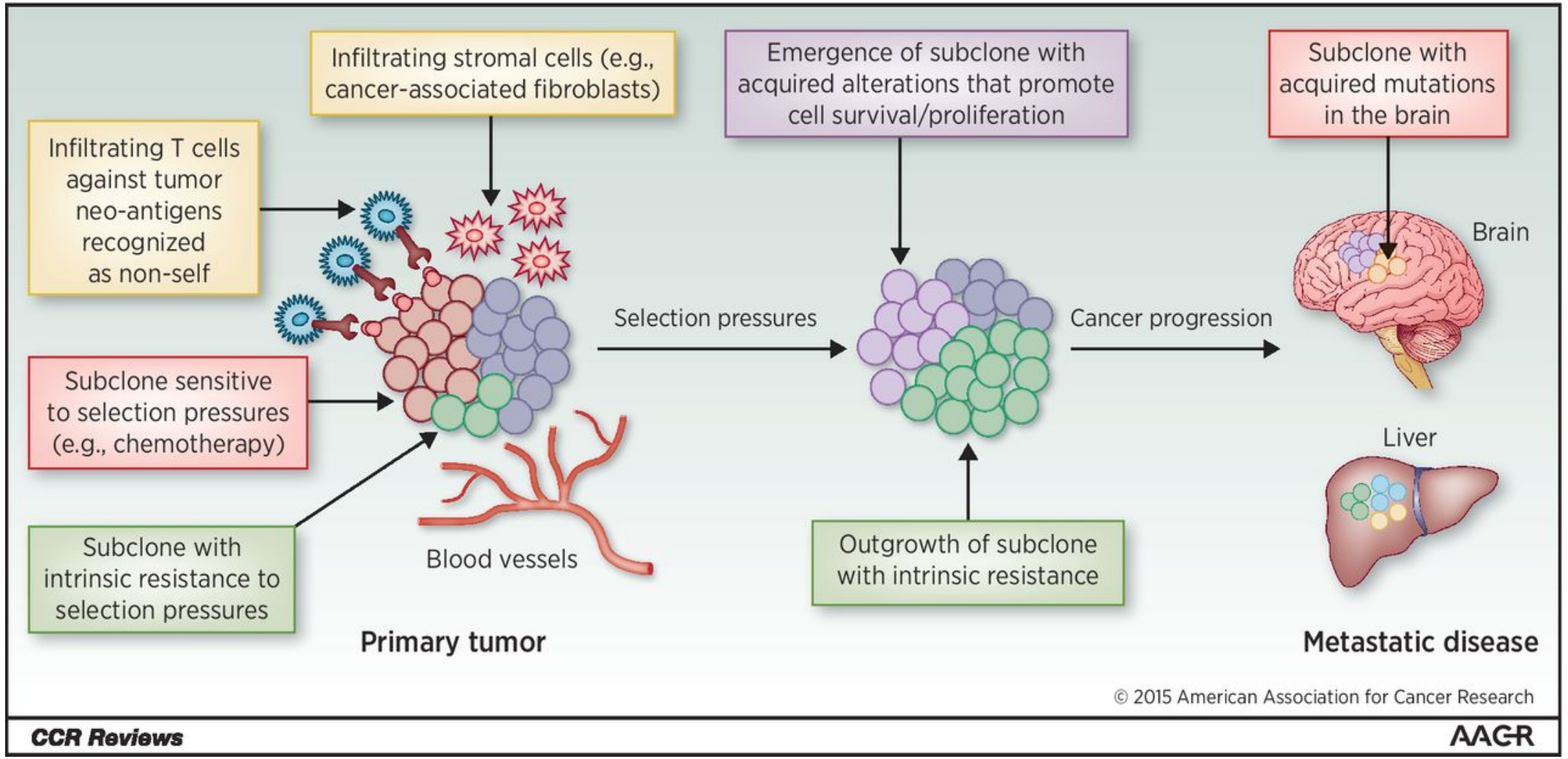
The Cancer-Immunity cycle



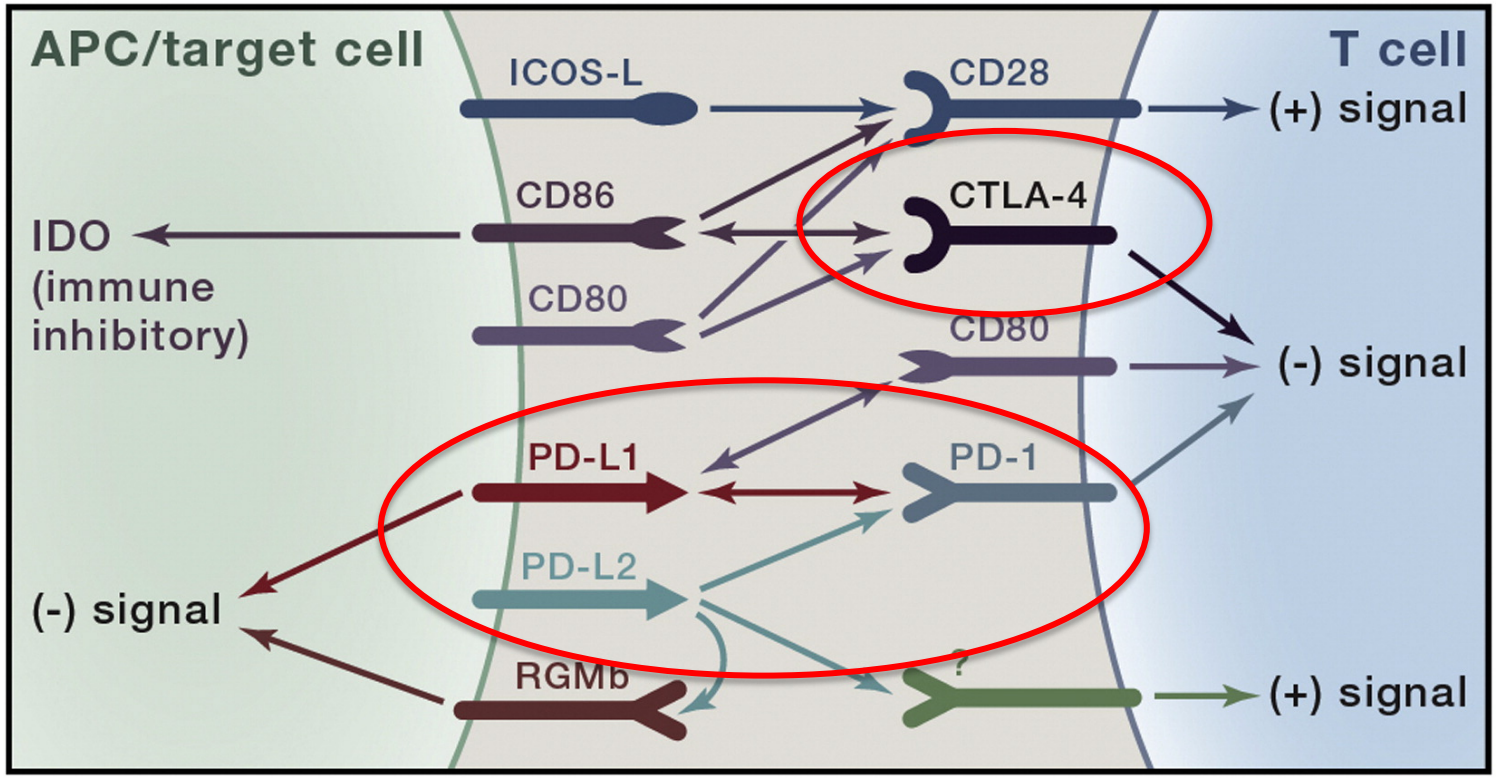
Adapted from "Oncology meets immunology: the cancer-immunity cycle" (2013) DS Chen & I Mellman. Immunity. **39:**1-10

Tumors evolve, adapt, progress, and escape

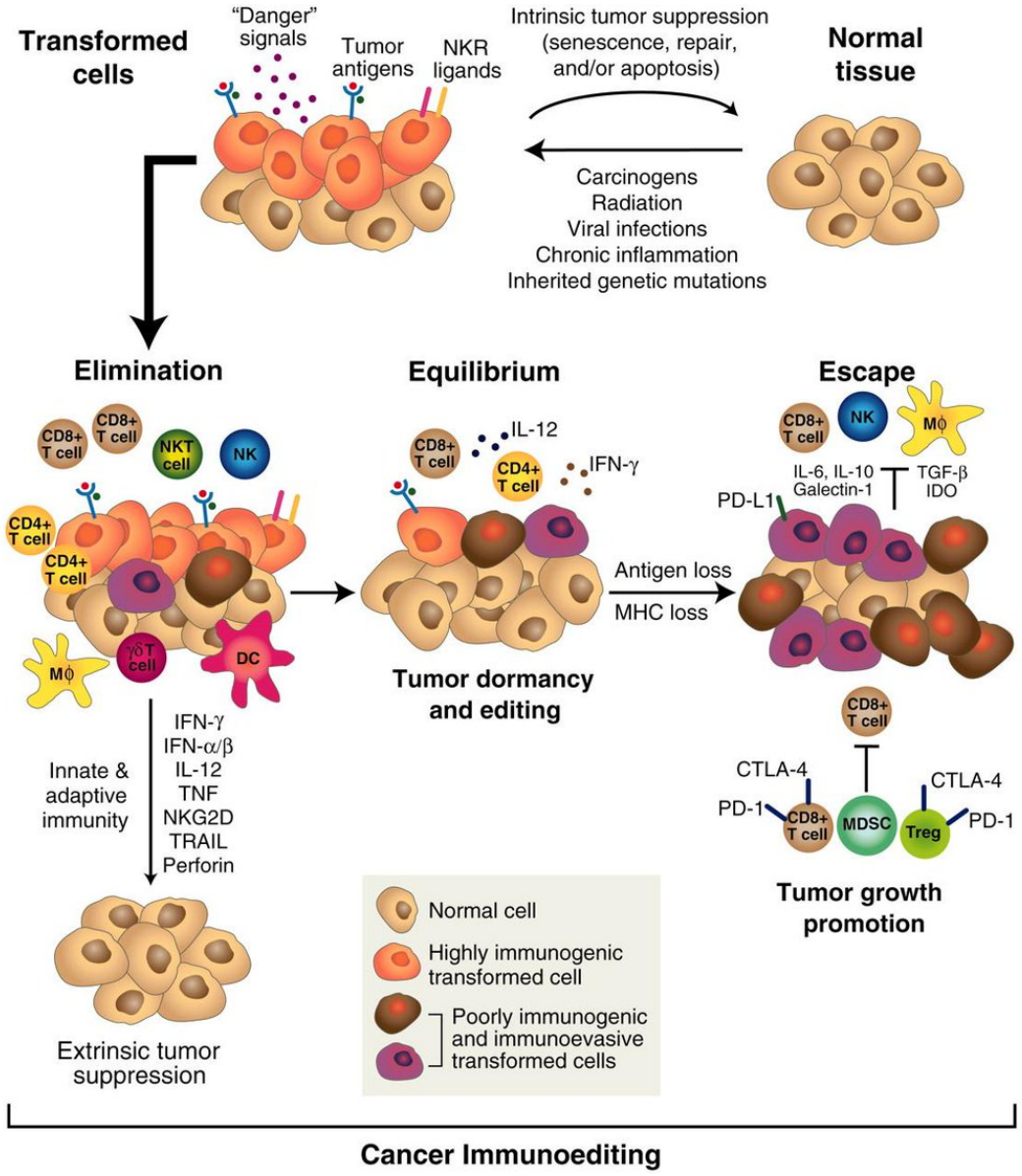


Translational implications of tumor heterogeneity, Jamal-Hanjani et al, Clinical Cancer Research 2015

T cells responses are regulated by both positive and negative signals

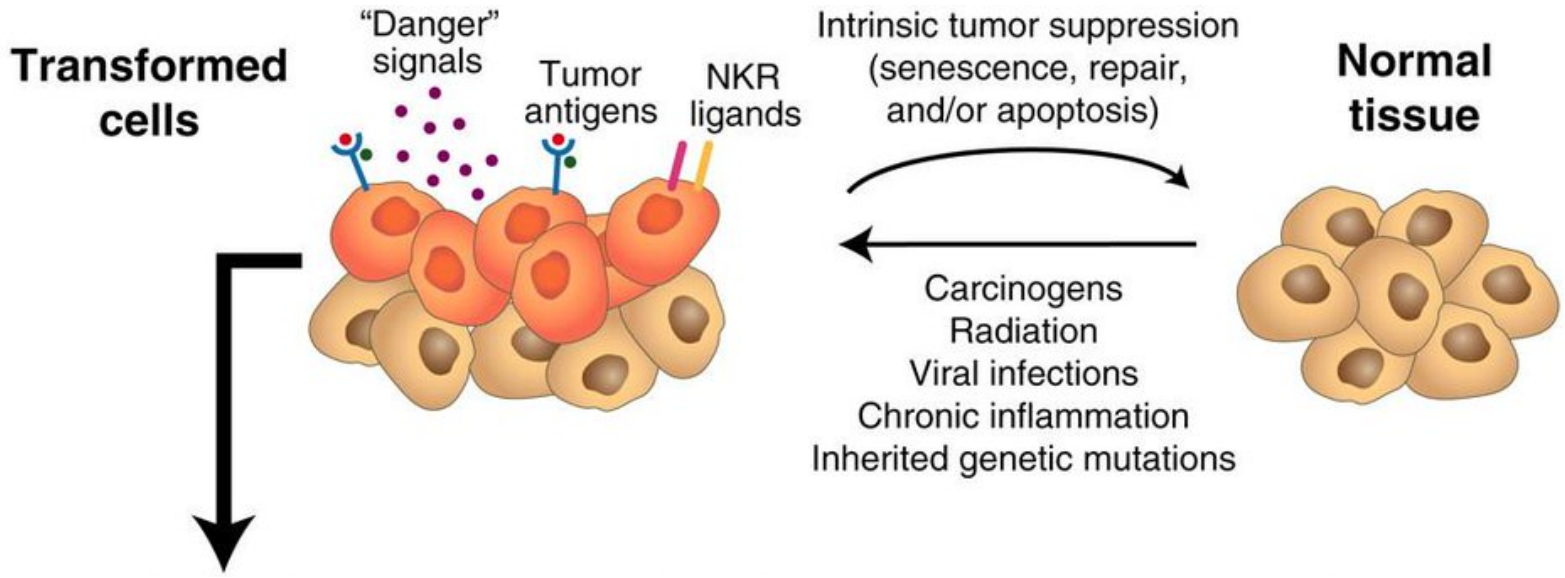


Tumors ‘edit’ the immune response to avoid destruction



Robert D. Schreiber et al. Science 2011;331:1565-1570

Tumors ‘edit’ the immune response to avoid destruction



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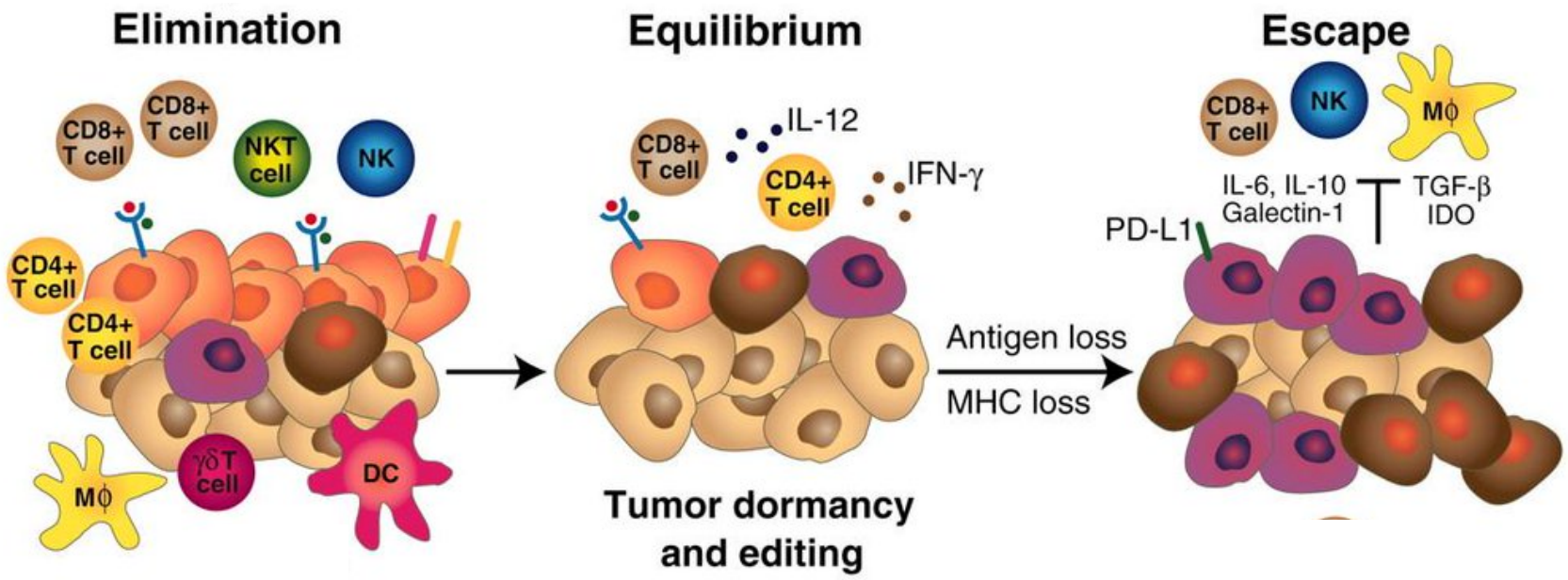
Tumors ‘edit’ the immune response to avoid destruction



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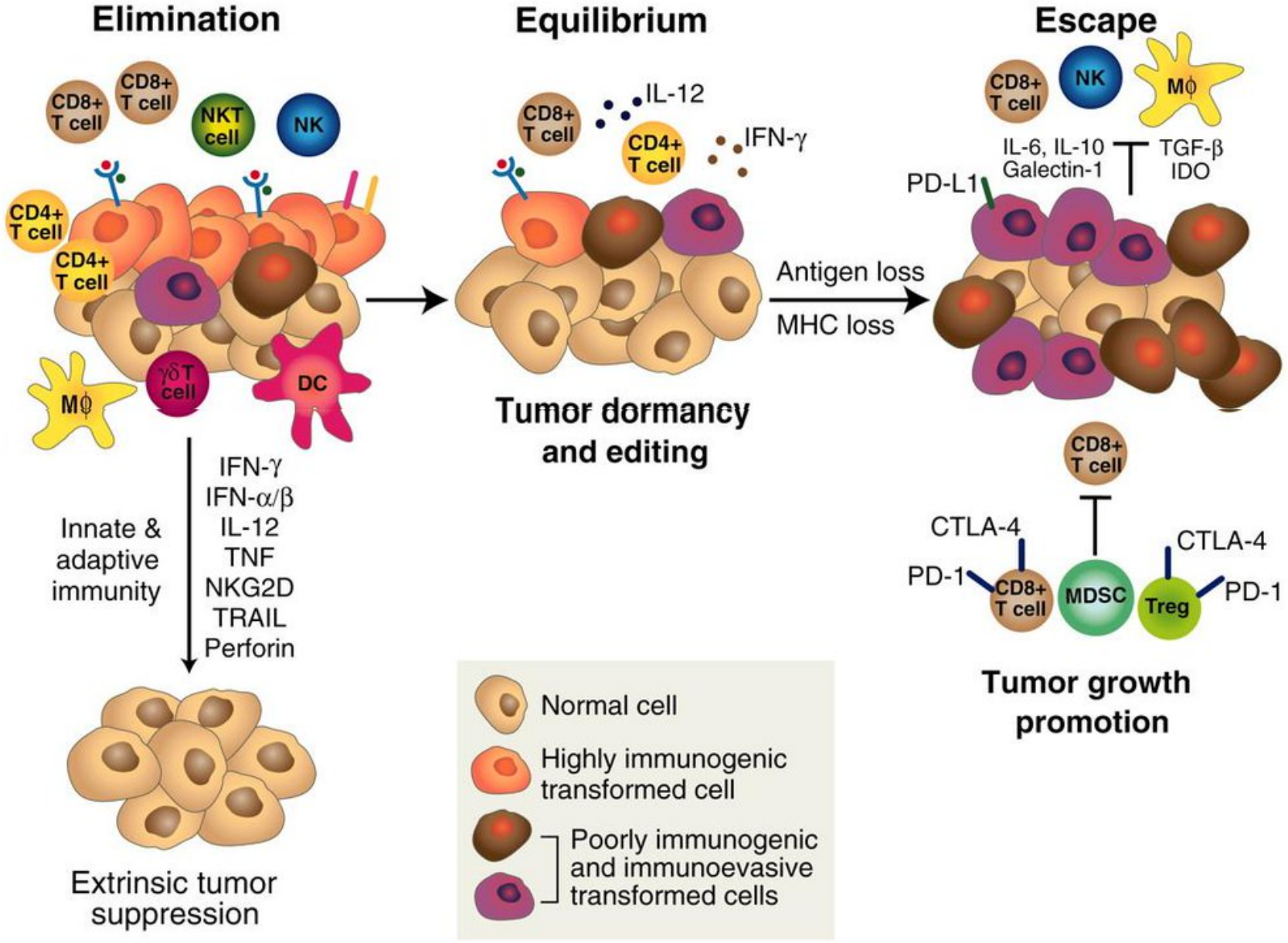

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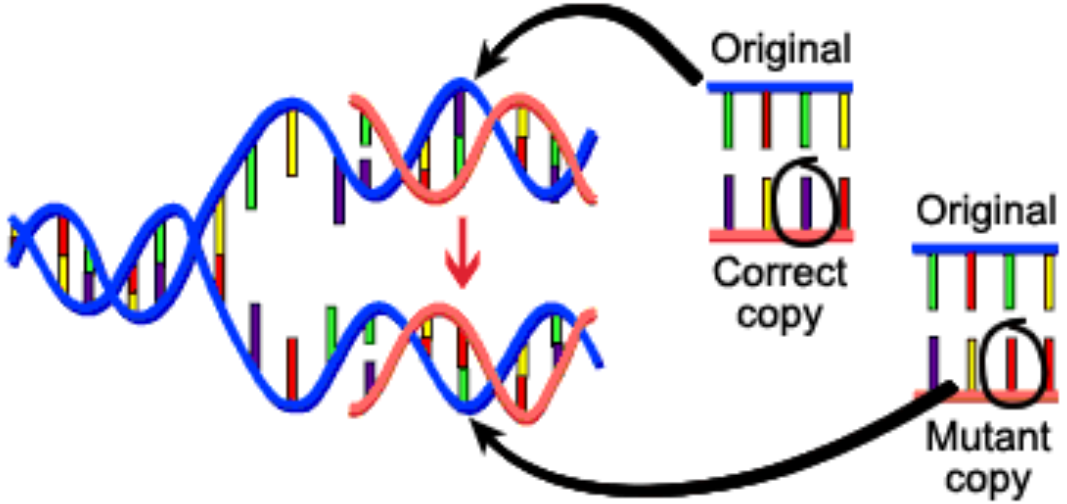
What are the target antigens for anti-tumor immune responses?

- For NK cells: missing MHC and/or stress ligands
- For mAB and CAR-T cells: appropriately-targeted surface proteins (usually non-mutated)
- For $CD4^+$ and $CD8^+$ T cells: tumor-specific mutations *(neoantigens)*

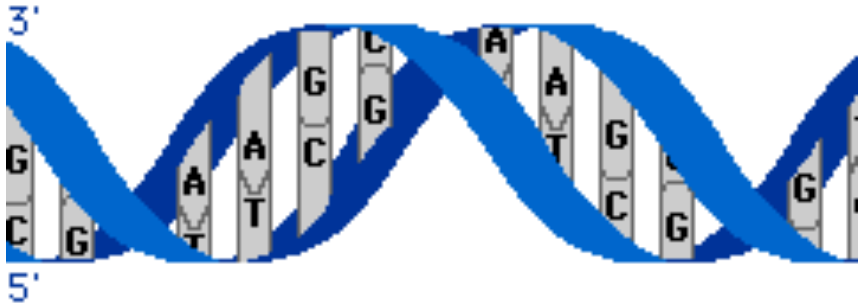
The accuracy of DNA replication is crucial,

but mistakes, called mutations, do happen**



* 1 in 10^{-8} per site/generation * 50-70 billion cells divide per day

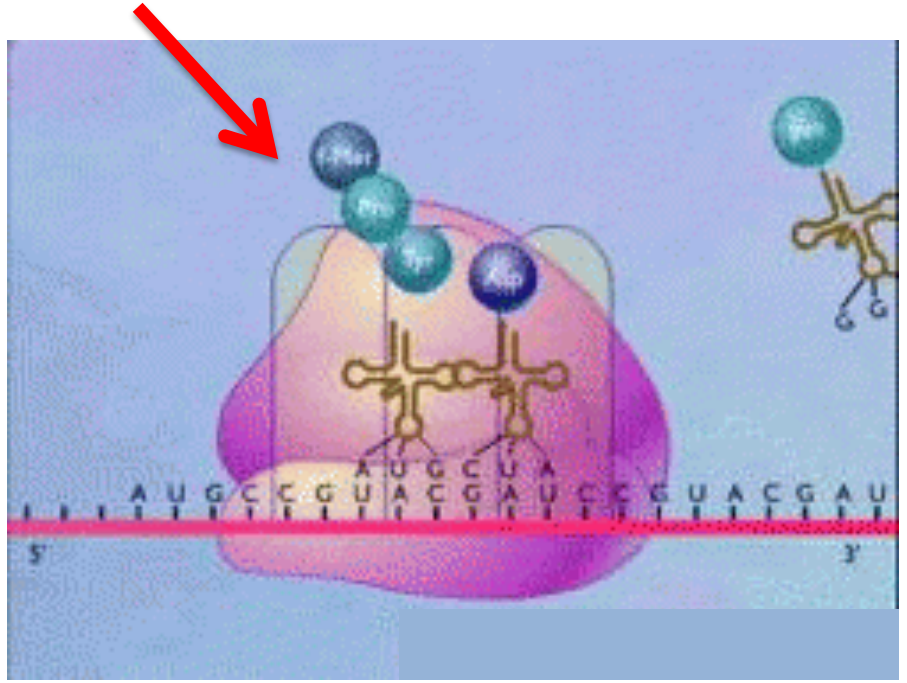
DNA copied to RNA



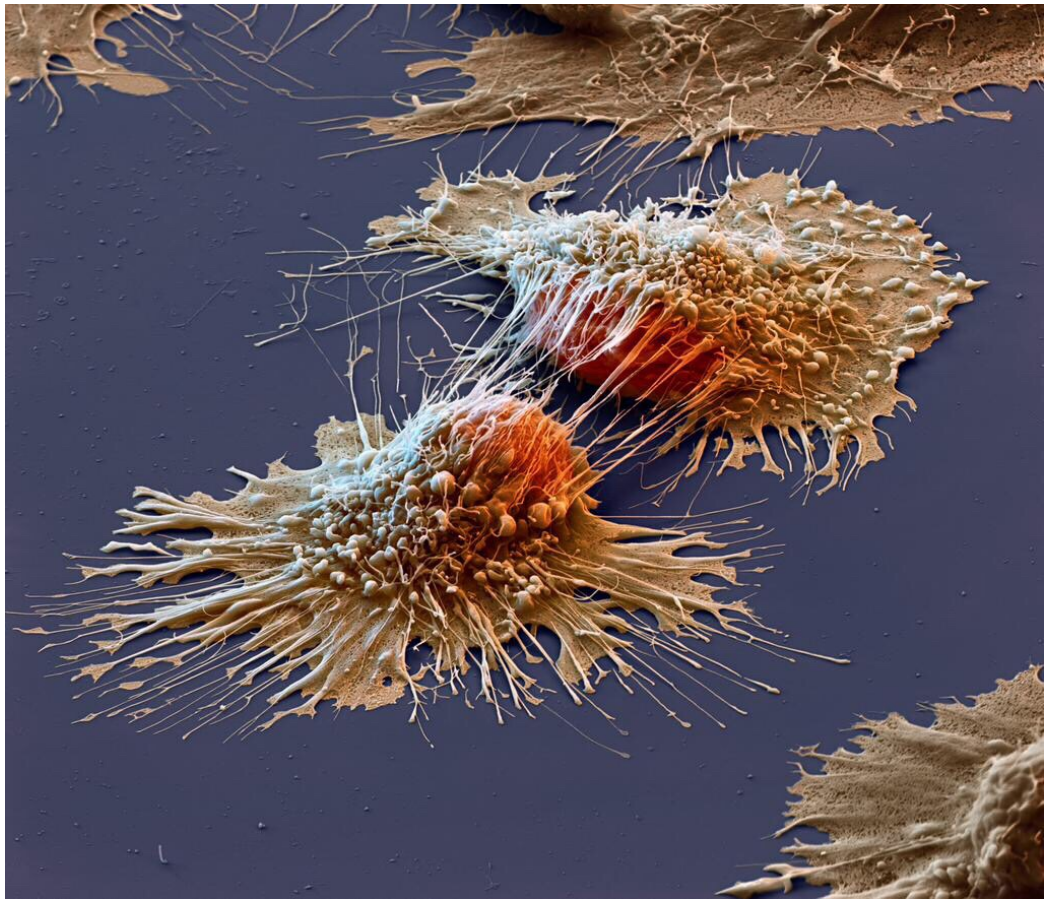
Mutations in protein

RNA instructs protein synthesis

Mutations in DNA/RNA



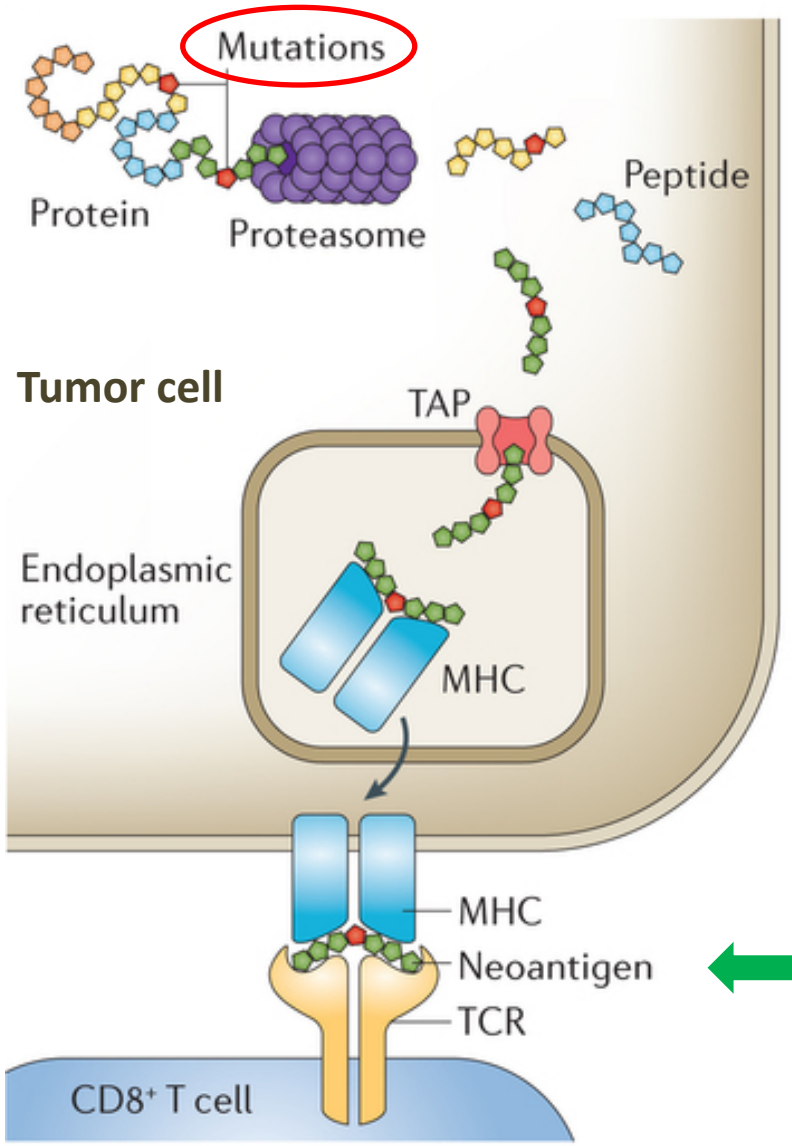
Cancer results from *function-altering mutations*
in the genes controlling cellular life & death



Tumor mutations create targets for T cell recognition = Neoantigens (NeoAg)

NeoAg ID useful for:

1) Vaccines
2) Cellular Therapy
3) Immune monitoring
4) Patient selection for ICB



2 classes of mutations:

"Driver" mutations:

Enable cancer
(Eg: Ras, P53, PIK3CA, etc)

"Passenger" mutations:

Along for the ride?

T cells can 'see' both types

Adapted from Hackl et al "Computational genomics tools for dissecting tumour–immune cell interactions" Nature Reviews Genetics **17**:441-458 (2016)

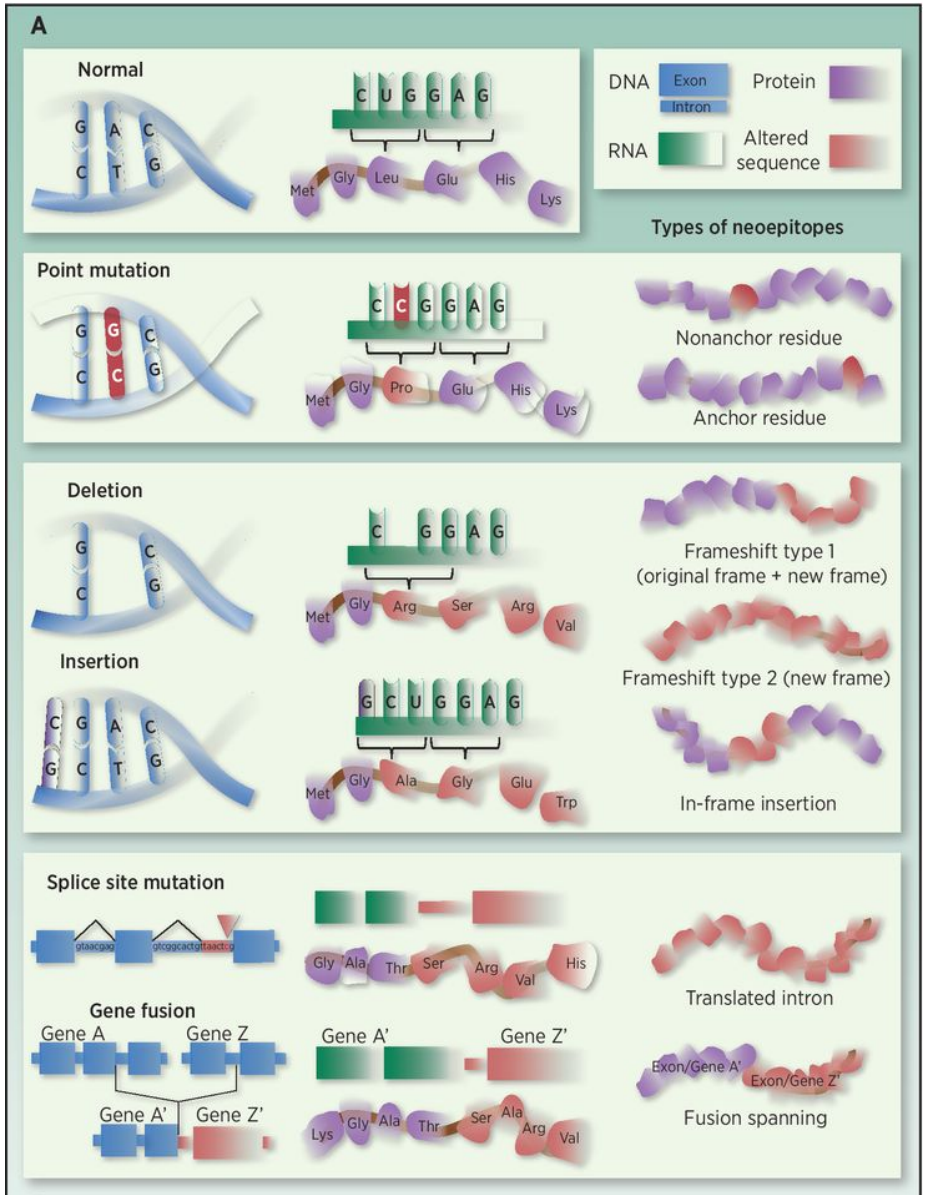
Sources of NeoAgs

Somatic mutations

Missense

Frameshift

Splice variants



nature biotechnology

EDITORIAL

VOLUME 35 NUMBER 2 FEBRUARY 2017

The problem with neoantigen prediction

Personalized immunotherapy is all the rage, but neoantigen discovery and validation remains a daunting problem.

Last December, the newly minted Parker Institute for Cancer Immunotherapy and its venerable East Coast counterpart, the Cancer Research Institute, announced the formation of the Tumor Neoantigen Selection Alliance. This initiative, involving researchers from 30 universities, non-profit institutions and companies, aims to identify software that can best predict mutation-associated cancer antigens, also known as neoantigens, from patient tumor DNA. The hope is that solving the shortcomings of current *in silico* methods for identifying neoantigens will galvanize a new wave of personalized cancer immunotherapies. But,

for a particular allele to build a model with sufficient accuracy. But as many MHC alleles lack such data, 'pan-specific' methods—capable of predicting binders based on whether MHC alleles with similar contact environments have similar binding specificities—have increasingly come to the fore.

Today, a raft of software tools for predicting MHC binders are now available (http://cancerimmunity.org/resources/webtools/). But each of these packages has its own idiosyncrasies, strengths and weaknesses. What's more, it has proven difficult to benchmark which tools and com-

Can immune responses to pathogens be instructive for anti-tumor immunity?

- Tumors are not infections, generally do not express TLR
- NeoAg are altered self, rather than non-self
- T_H requirement: neoantigens for both subset?
- Tumors utilize many physiological mechanism to suppress immune response