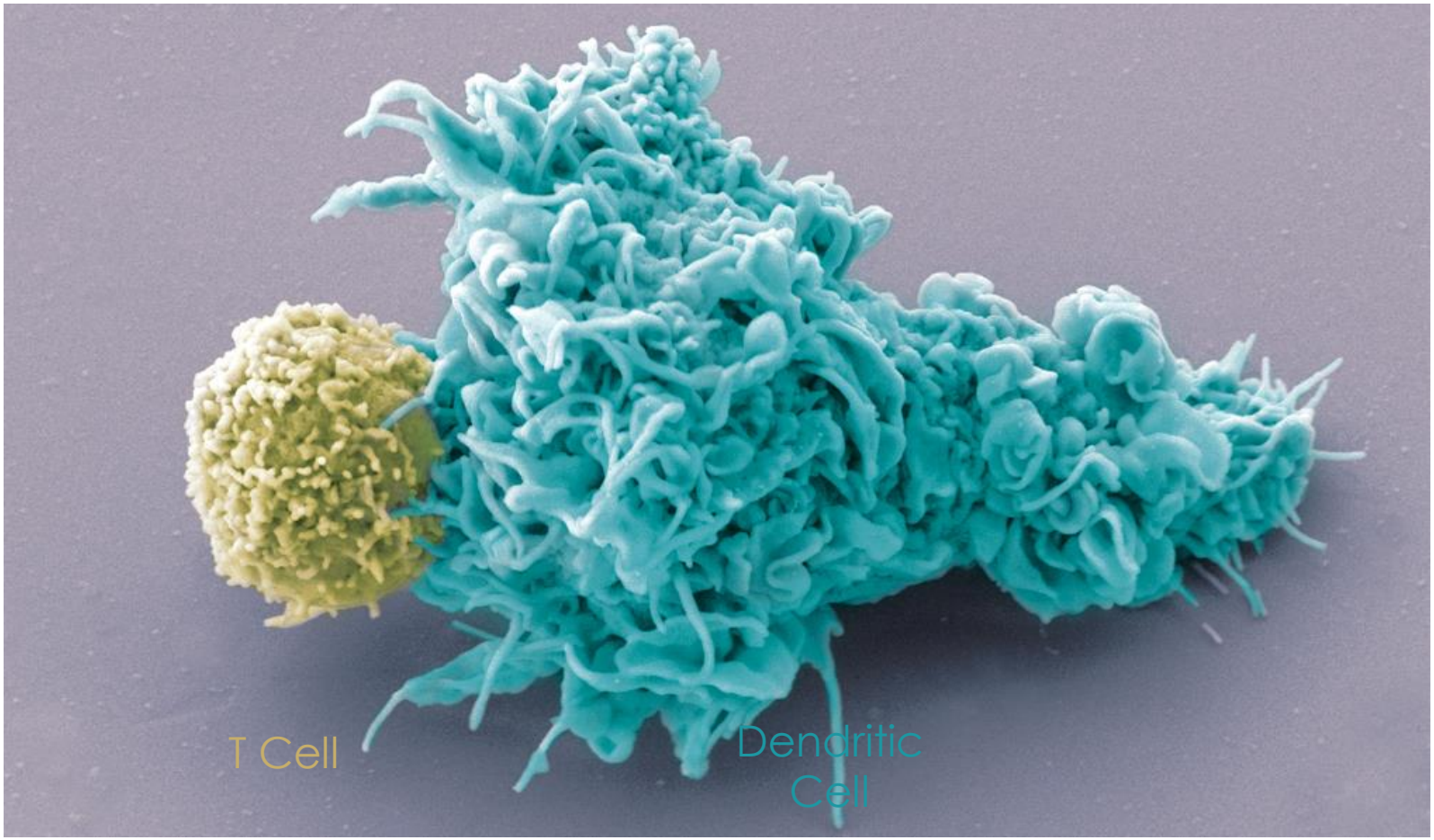
# Introduction to the Immune System

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# Lecture Outline: The Immune System



1. Introduction: what is the immune system?
2. innate immune system
3. adaptive immune system
4. antigen presentation
5. putting it together (very briefly)
6. immunologists tool box
7. questions to think about
8. quiz questions

# Great Resources



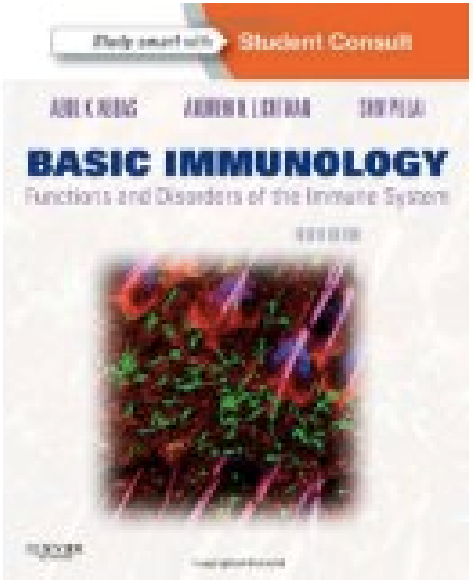
- **Khan academy**
  https://www.khanacademy.org/science/biology/immunology

- **BioGene**
  an information tool for biological research. Use to learn about gene function. Enter a gene symbol or gene name, for example "CDK4" or "cyclin dependent kinase 4" and BioGene will retrieve its gene function and references into its function.
  https://itunes.apple.com/us/app/biogene/id333180084?mt=8

- **CD chart**
  cluster of differentiation or CD nomenclature used for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells. Currently 363 human CD markers.
  http://www.hcdm.org/

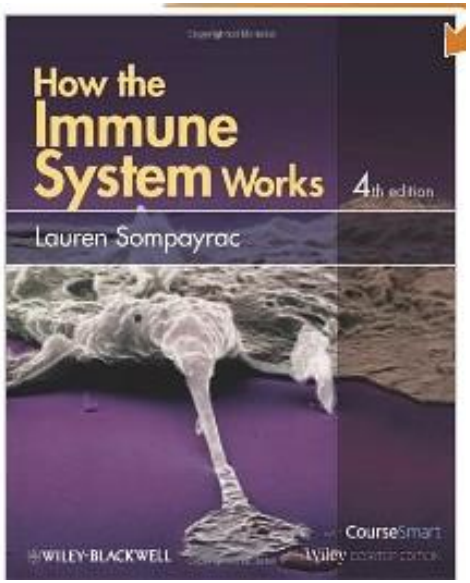
# Other Suggested Resource Material






Basic Immunology – Functions and Disorders of the Immune System
By: Abbas, Lichtman and Pillai

How the Immune System Works
By: Sompayrac



# What is the Immune System?

series of specialized organs, tissues, cells and proteins that work together to:

- keep infectious micro-organisms out of the body
  - pathogenic bacteria
  - viruses,
  - fungi
- destroy any infectious microorganisms that invade the body

# What is Immunity?



The state of having sufficient biological defenses to avoid infection, disease or other unwanted biological invasion – consequence of the immune system

- protection of one organism against other organisms
- elimination or control of the offending organism
- setting up conditions so the offending organism does not cause problems down the road

# Major Roles of the Immune System?



PATHOGENS disease causing organisms: bacteria, viruses, fungus, parasites and prions

problem: the variety of pathogens is endless pathogens are largely made of the same stuff as us

CANCER aberrant cell growth due to mutation

problem: the variety of cancer is endless and cancer cells are made of the same stuff as us

# The Immune System is Broken Down Into Two Parts



| | Innate Immunity | Acquired Immunity |
|---|---|---|
| | Is always working to protect the body and does not require any special preparation to stop infection. | Needs to be 'primed' before it can work to its full effectiveness. Only really effective after it has seen a possible infective agent before. |
| Speed of Response | Immediate | Slow (>week) |
| Response | Non-Specific Antigen Independent | Highly Specific Antigen Dependent |
| Memory | No | Yes |

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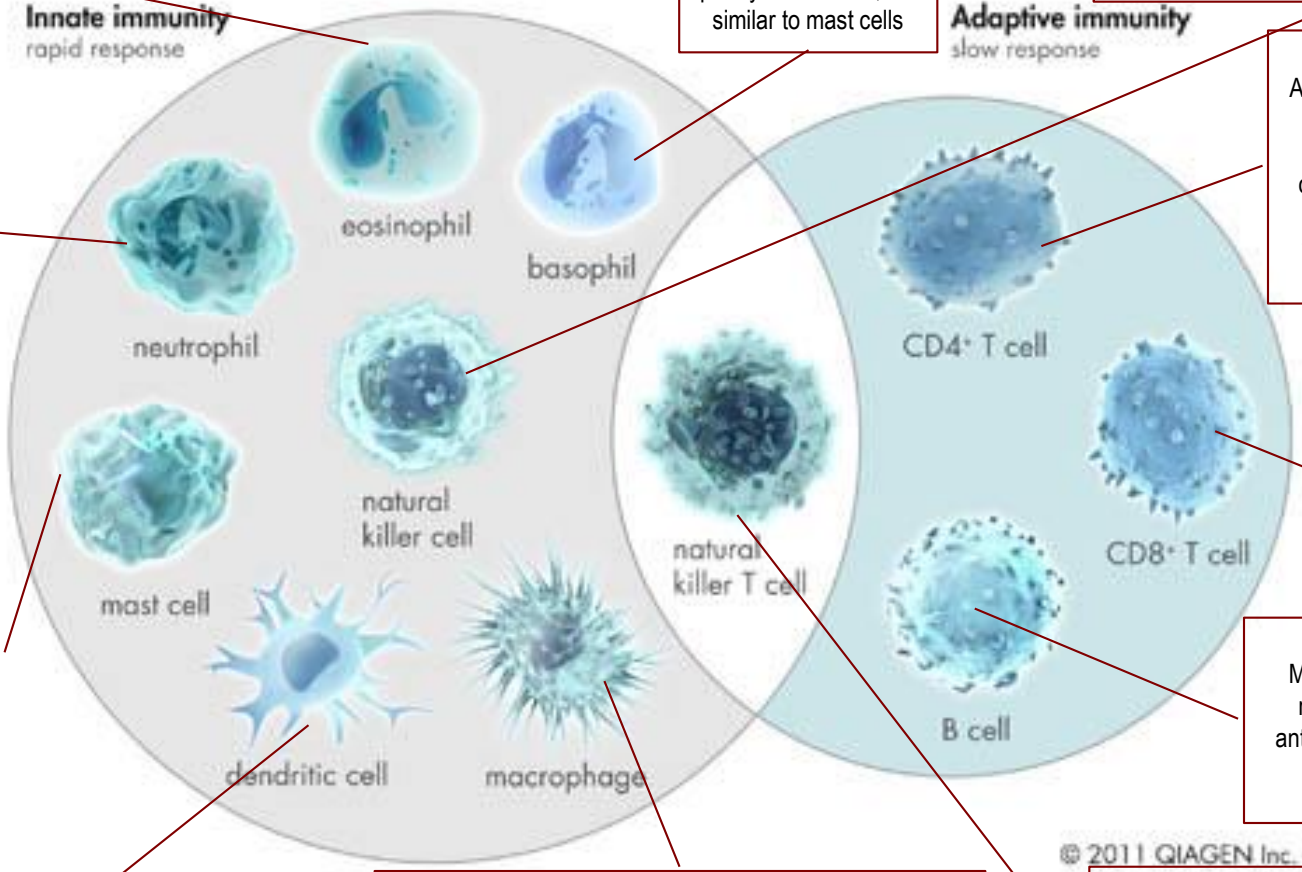
RCSB PDB
PROTEIN DATA BANK

# Innate and adaptive immunity




Have cytoplasmic granules containing enzymes that are harmful to the cell walls of parasites but can also damage host tissues.

Also called polymorphonuclear leukocytes. Mediate the earliest phases of inflammatory reactions. Also highly phagocytic and can eat bacteria

Found in the skin and in the gut. Contain abundant cytoplasmic granules filled with cytokines, histamine and other mediators. When IgE bound to antigen binds to mast cells, they degranulate releasing the contents of the granules, including histamine which may be responsible for symptoms of allergic diseases

Play major roles in innate responses to infections and also links innate and adaptive immune responses. These are the cells that activate T cells via antigen presentation

Recruited into tissue sites where pathogens are present. Function poorly understood, but similar to mast cells

Tissue based phagocytic cell derived from blood monocytes that plays important roles in innate and adaptive immune responses. Activated by microbial products and when activated, phagocytose and kill microorganisms, secrete proinflammatory cytokines and present antigens to helper T cells

Innate lymphocytes that are not T cells and respond early in infection. Function by directly killing pathogen infected cells by lysing (poking holes in) them.

Also called T helper cells. Activated by recognition of MHC class II on antigen presenting cells such as DC. Help other cells of the immune system by sending signals to other cells that then destroy the invading pathogen.

Also called cytotoxic or killer T cells. Activated by recognition of MHC class I on antigen presenting cells. Activation leads to direct killing of infected cells

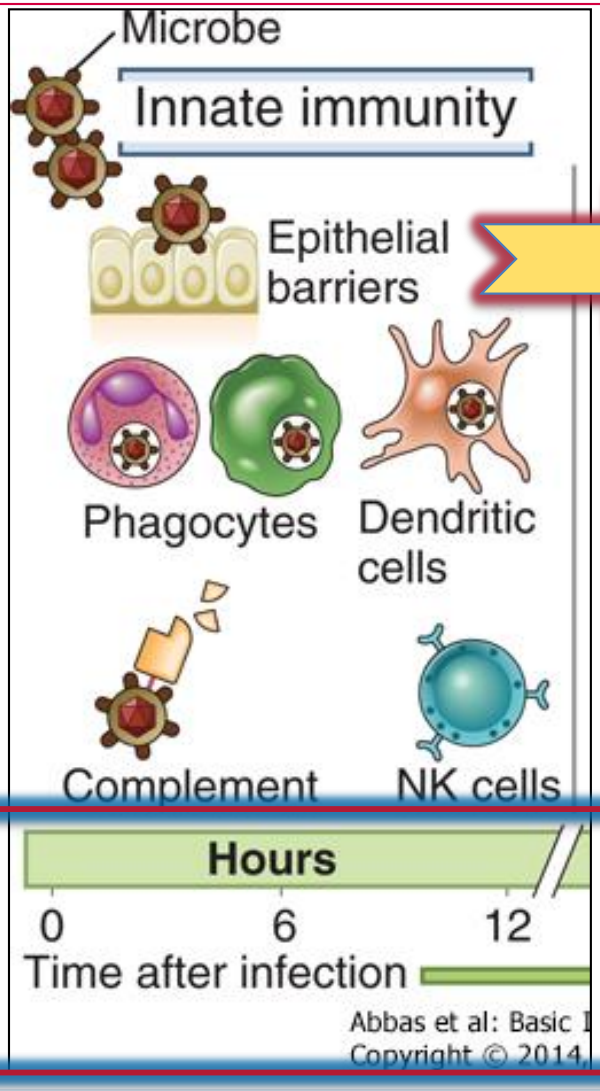
Mediators of humoral immune responses. Cells that make antibody that binds to pathogens and neutralizes them.

Small subset of T cells that are activated very quickly after pathogen infection. Secrete high levels of cytokines that help other cells of the immune system respond to the invading organism.

# innate immunity:
# Provides early line of defense against microbes

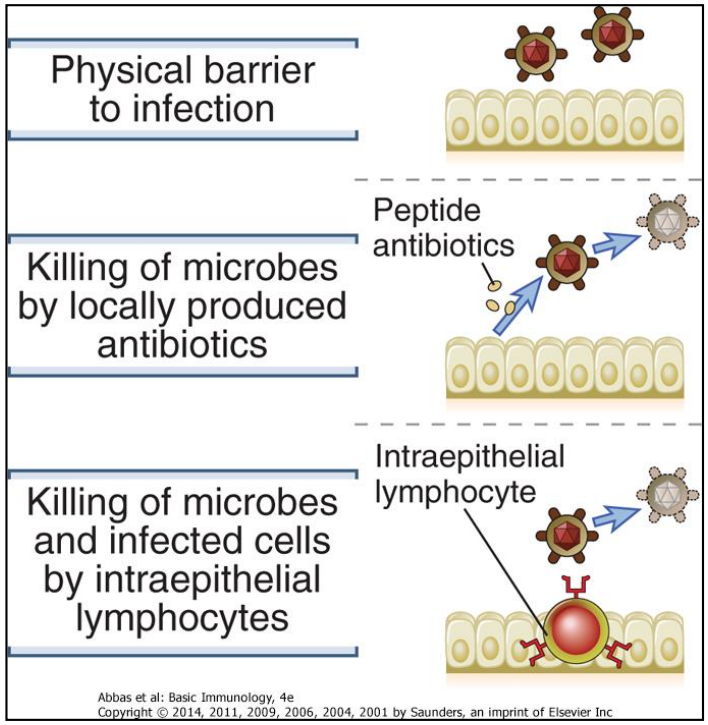
cellular and biochemical defense mechanisms that are always poised and ready to respond

respond to microbes and the products of injured cells. NO MEMORY




**portals of entry for microbes**

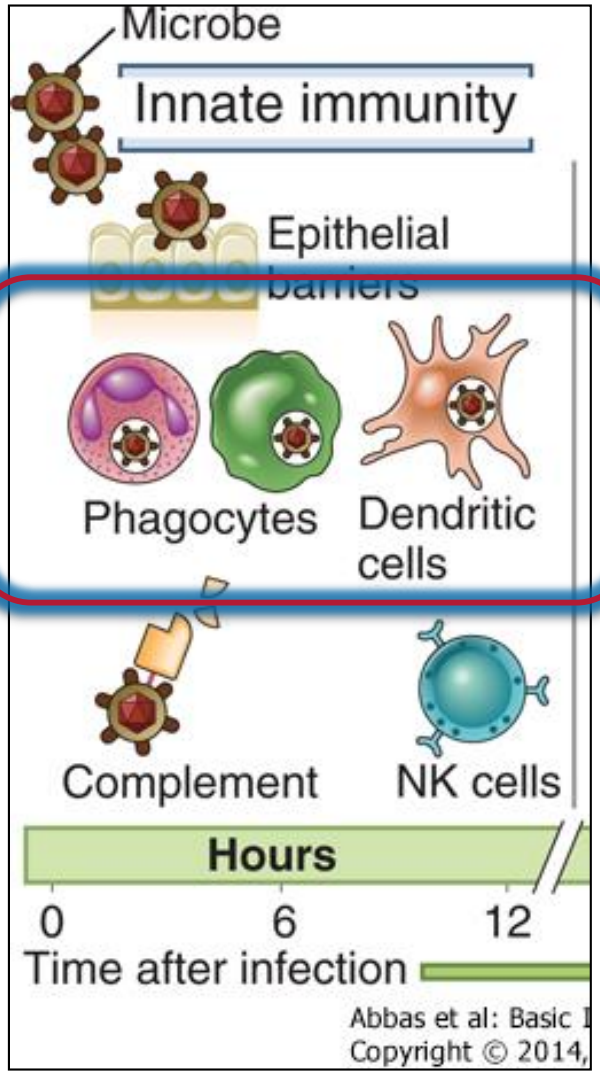
most microbial infections acquired via epithelia of the **skin** & gastrointestinal & respiratory systems.




Barriers such as the eye also contain enzymes such as lysozyme that kills gram positive bacteria by damaging their cell walls

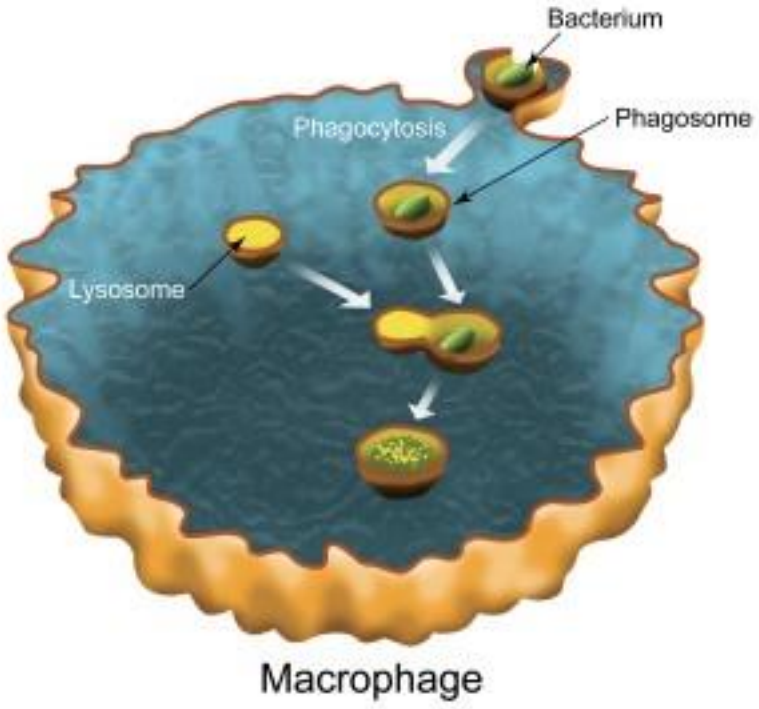
# Innate immunity: Provides early line of defense against microbes






**in tissues:**

microbes that breach epithelia, as well as dead cells in tissues, are detected by resident macrophages, dendritic cells, and other phagocytic cells.



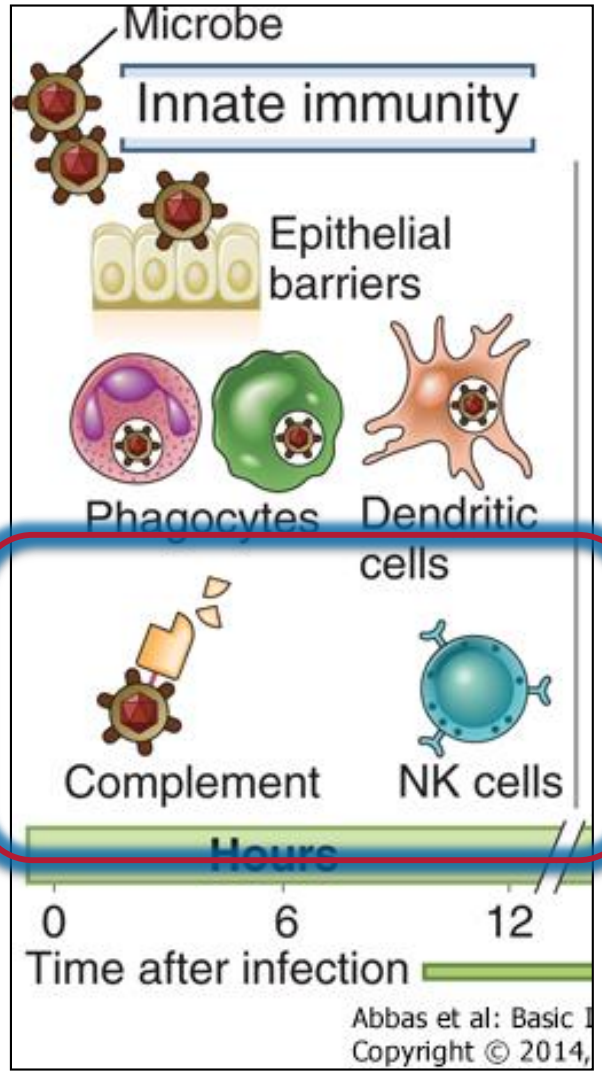

Macrophages are a type of phagocyte.

Phagocytes "eat" the bacteria and destroy them

The precursors of macrophages are monocytes. Monocytes circulate in the blood. Once they move into tissues, they differentiate into macrophages.

# innate immunity: provides early line of defense against microbes






## Complement System:

an enzyme cascade that is a collection of blood and cell surface proteins.

Pathway activation by pathogen triggers three effector systems:

- *Anaphylatoxins* – increase capillary permeability and attracts leukocytes to site of infection
- *the membrane attack complex* – pokes holes in cells or pathgens and damages them.
- *complement receptors on cells* – increases the effectiveness of abilities of antibodies to clear pathogens

## NK or Natural Killer cells:

A type of cytotoxic lymphocyte critical to the innate immune system. The role NK cells play is analogous to that of cytotoxic T cells in the adaptive immune response.

…so they recognize and destroy infected cells (but unlike adaptive response, antigen specificity is lacking).

# Hallmark of Immune System: Self versus Non-Self (pathogen) discrimination

"Easy" to understand how this works for the adaptive (or antigen-specific) immune response, but how does the innate immune response do this?

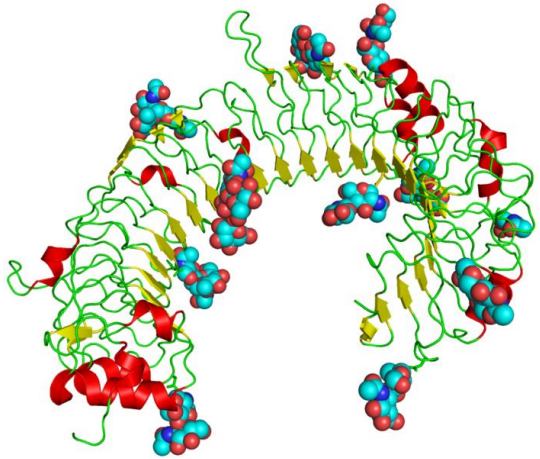
Mediated by the expression of Toll Like Receptors (TLRs) on the surface of innate immune cells such as DCs and Macrophages (+others).

These membrane bound receptors recognize structurally conserved molecules (patterns) derived from pathogens (microbes and viruses) – e.g. parts of flagella, pieces of DNA etc.

TLR ligation results in immune cell activation and initiation of the immune response – this is the basis of innate immune cell self/non-self discrimination.

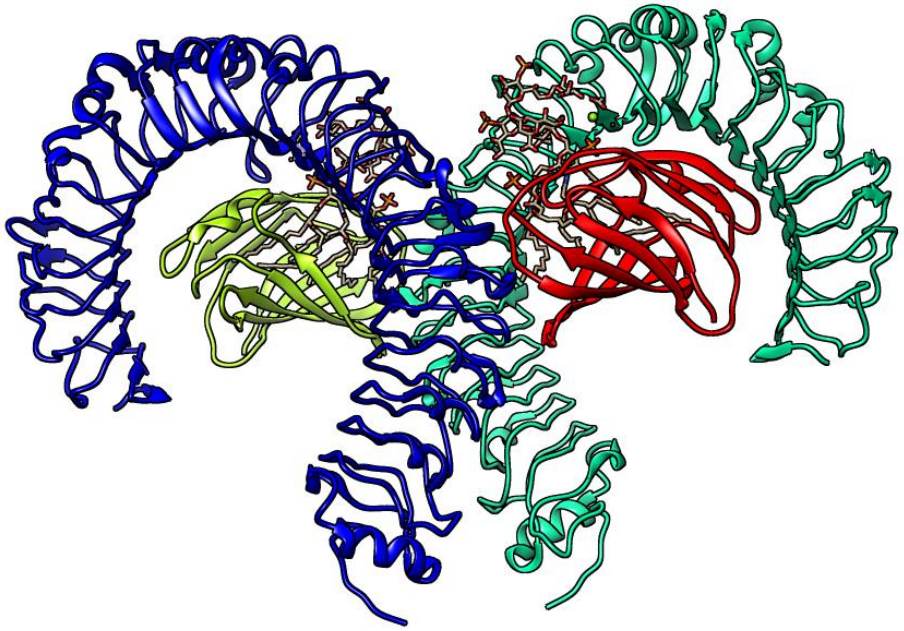
TLRs are a family of structurally related proteins that recognize different pathogen derived factors.

All have curved lysine rich repeats that mediate specific recognition and activation of the pathway.

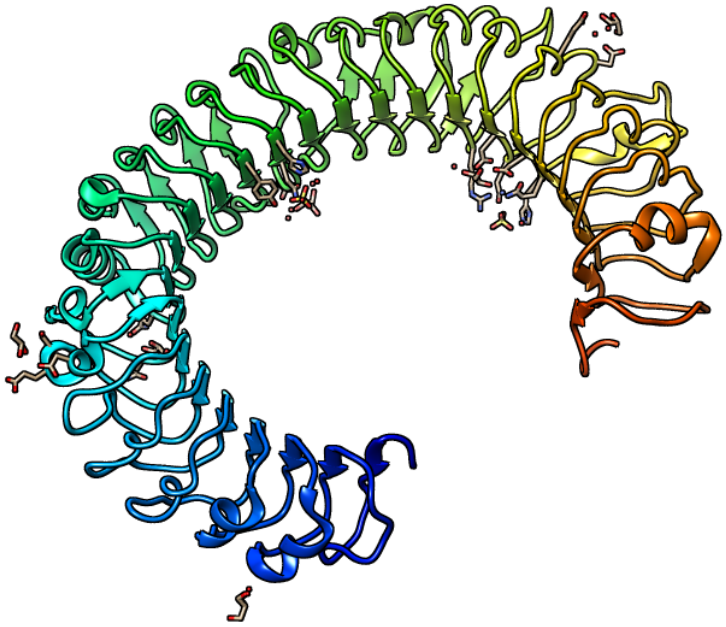


http://en.wikipedia.org/wiki/Image:TLR3_structure.png

# Toll-Like Receptors: Structures



Human TLR-4, PDB ID 3fxi

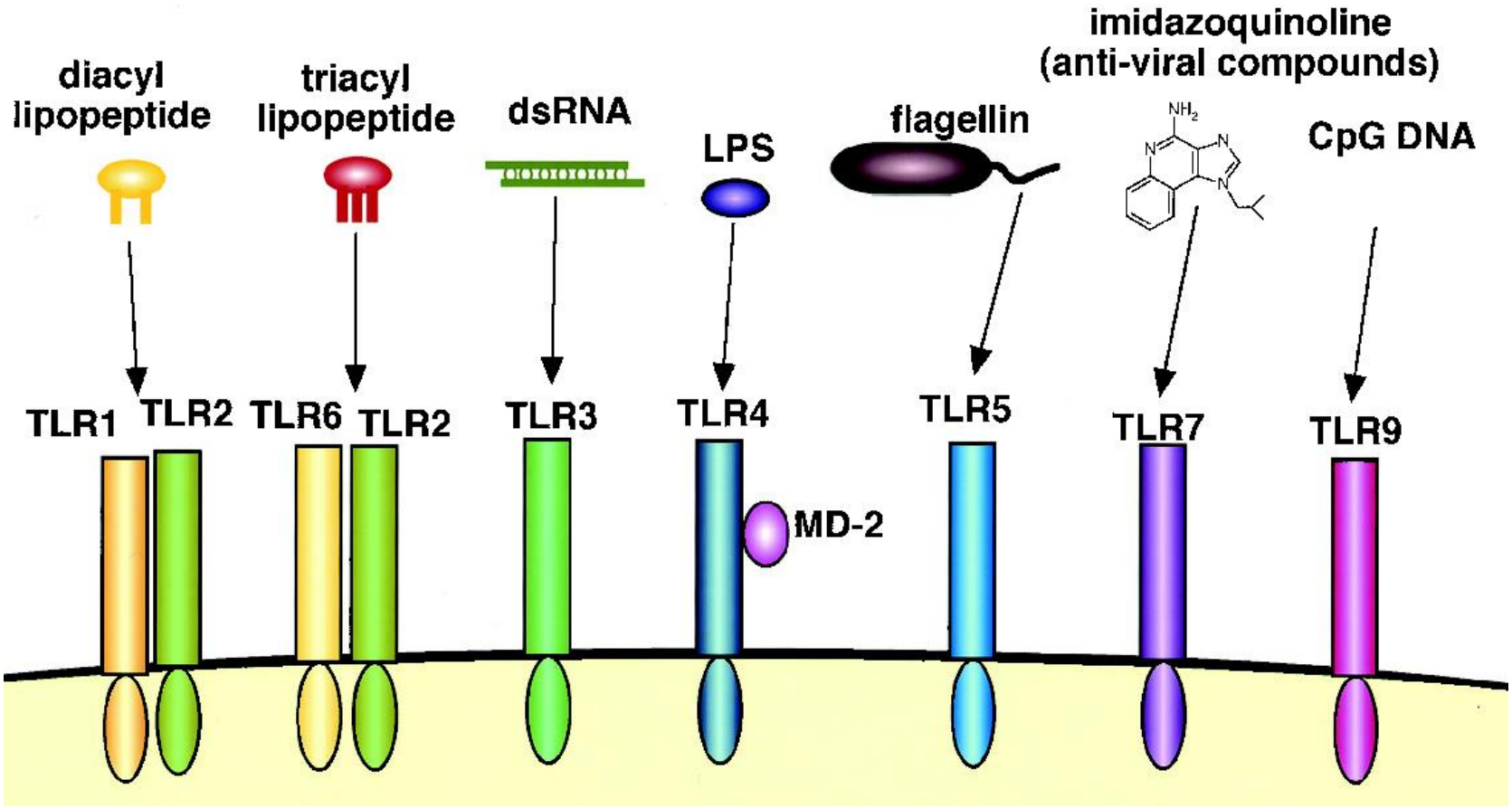


Human TLR-3, PDB ID 1ziw

To examine these structures further go to www.rcsb.org
Type in the PDB ID in the top search box and explore

# Diversity of ligands recognized by TLRs






**Ligands such as LPS also activate the immune system by functioning as pyrogens.**

**Pyrogens are substances that induce fevers. They can be derived from bacteria (like LPS) or from the body itself. Cytokines (see later slide) are examples of small proteins that are secreted by immune cells and induce immune activation and fevers.**

# TLRs

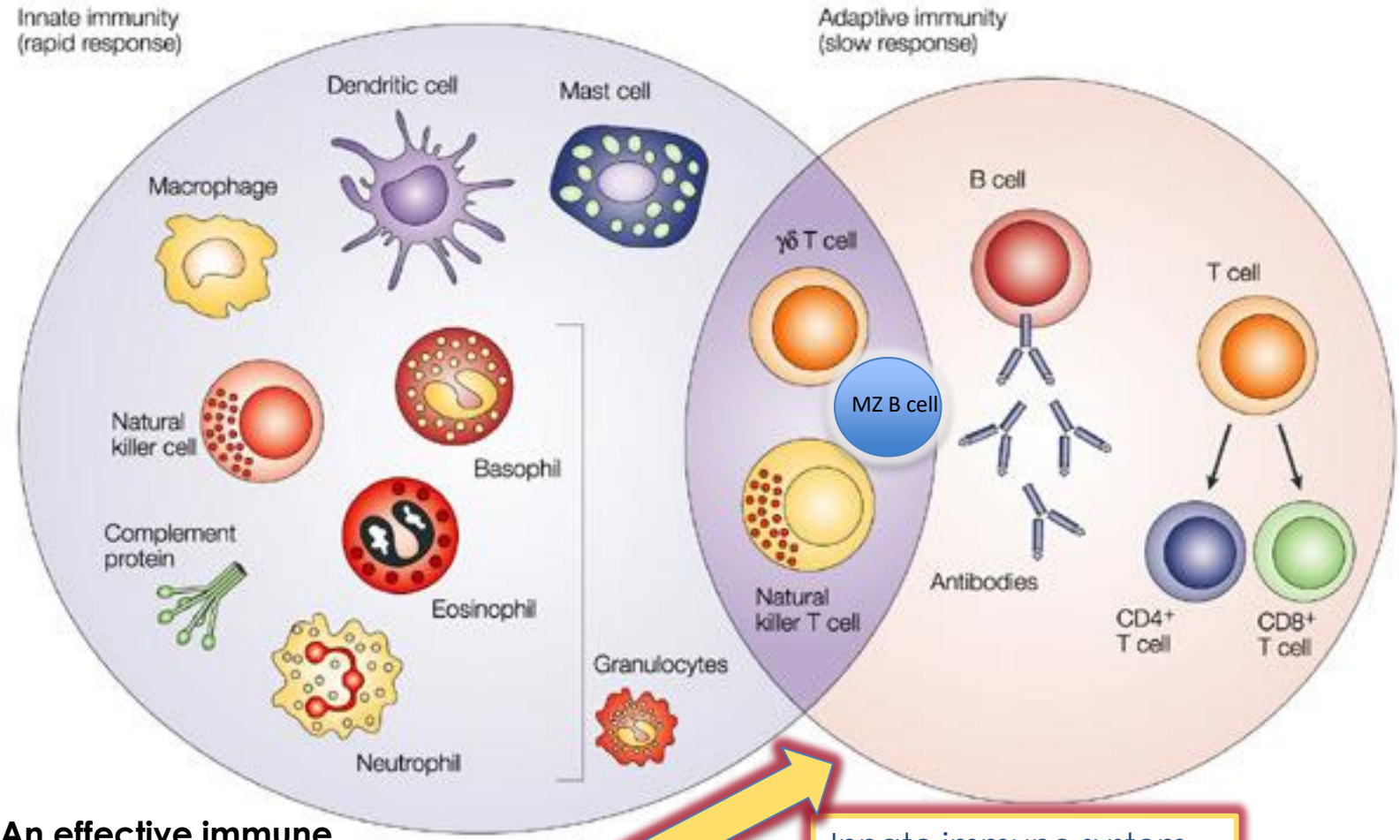


## ALERT the innate immune system that something is wrong


Image from www.invivogen.com

# Innate immune system activates the adaptive immune response






**Note: An effective immune response almost always requires multiple arms of the immune system.**

Innate immune system provides the signals that activate the adaptive immune system

# Cytokines



Small proteins released by cells that has a specific effect on the interactions between cells, on communications between cells or on the behavior of cells and in regulating immune functions.

The cytokines include:

- interleukins,
- lymphokines
- cell signal molecules

Examples:

- tumor necrosis factor (TNF)
- interferons (alpha, beta, gamma)
- Interleukin-2

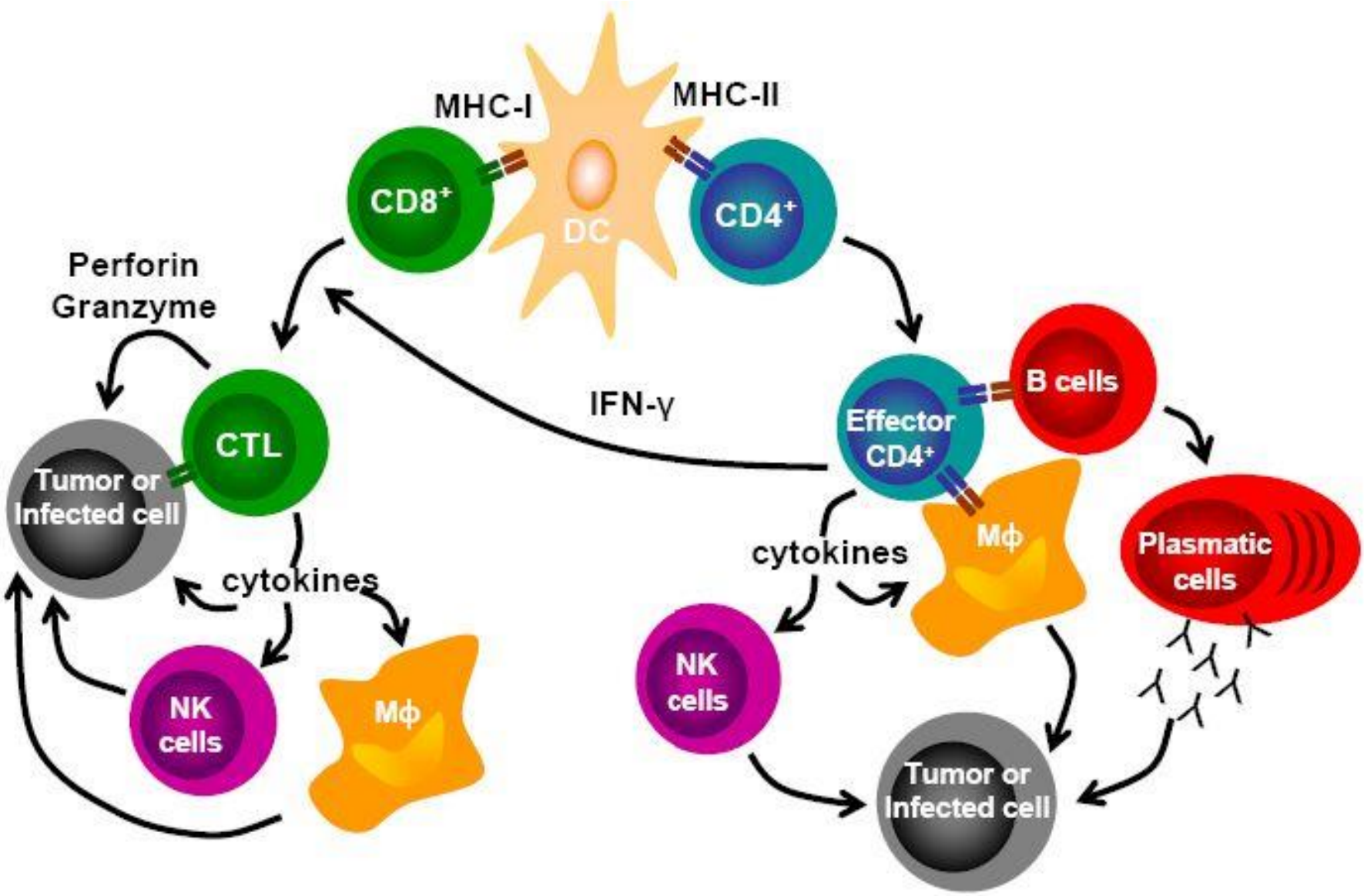
They trigger inflammation and respond to infections.

Cytokines regulate all the immune functions we discussed today.

CD4 T helper cells help mainly by secreting cytokines

# Cytokines orchestrate immune responses





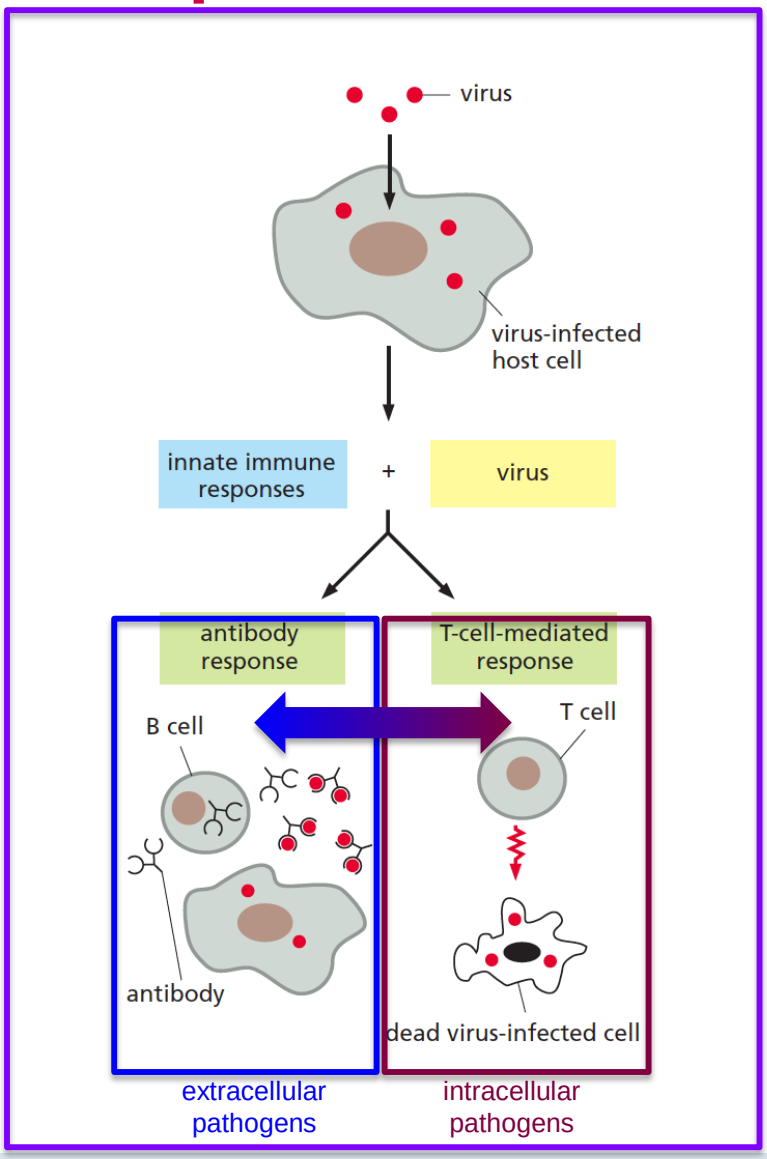
# Two main classes of adaptive immune responses

1. humoral immunity (antibody response)

2. cell mediated immunity (T-cell mediated response)

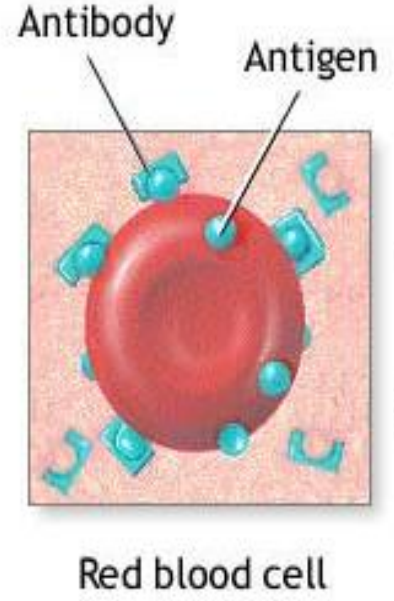
Mediated by two different types of cells call lymphocytes

- B lymphocytes (B cells)
- T lymphocytes (T cells)

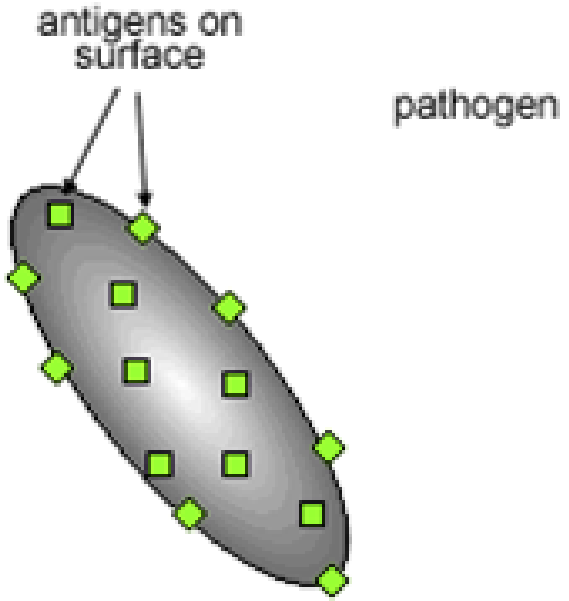


# What is an Antigen?

- The term antigen derives from the two words **anti**body **gene**rator
- Most antigens are proteins or large polysaccharides
- Often a component of invading microbes, such as the capsule, cell wall, flagella, toxin
- THESE ARE THE THINGS THE IMMUNE SYSTEM RECOGNIZES




An antigen is a substance that induces the formation of antibodies because it is recognized by the immune system as a threat

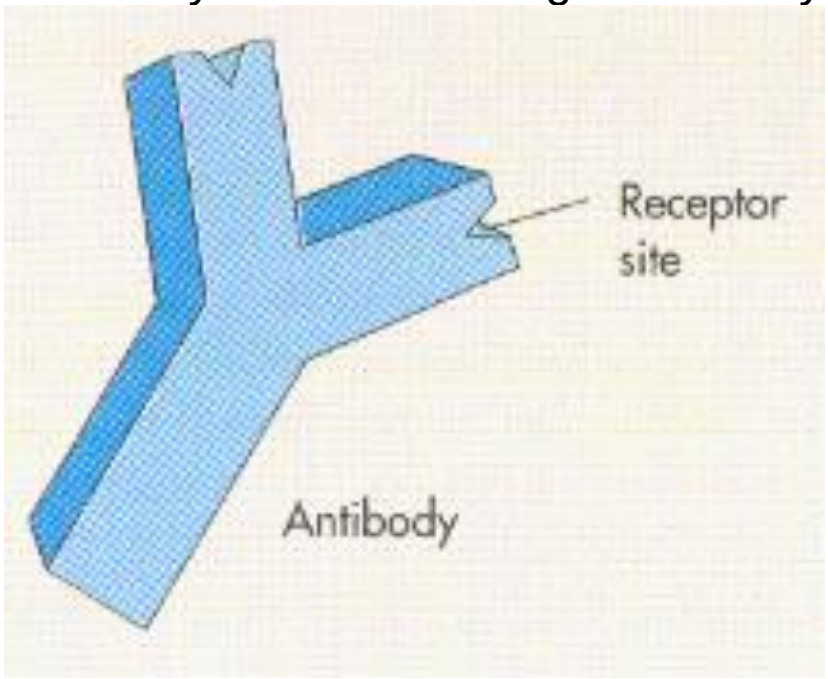


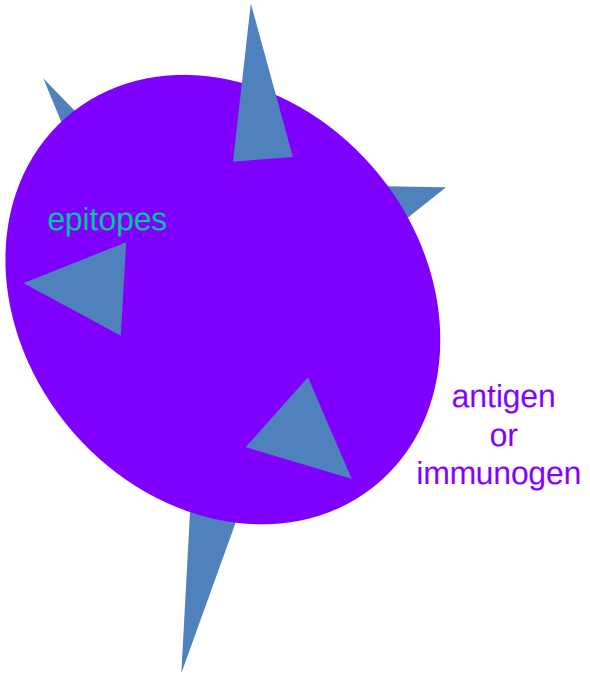
# Antigens – more terminology



- An antigen that elicits an immune response it is often referred to as an immunogen

- Epitope is the reactive portion of the antigen that reacts chemically with an antibody to form the antigen-antibody complex or immune complex



# How can the immune system deal with so many different pathogens?

The immune system has no prior “knowledge” of what all these different pathogens might be

> The immune system of a newborn cannot know that it might someday travel to jungles of Africa and encounter strange new pathogens it has never seen before

The pathogens are always changing (mutating/evolving)

> An “arms race” – immune system changes/evolves, the pathogen mutates. And pathogens can change a lot faster than we can. (e.g. HIV!)

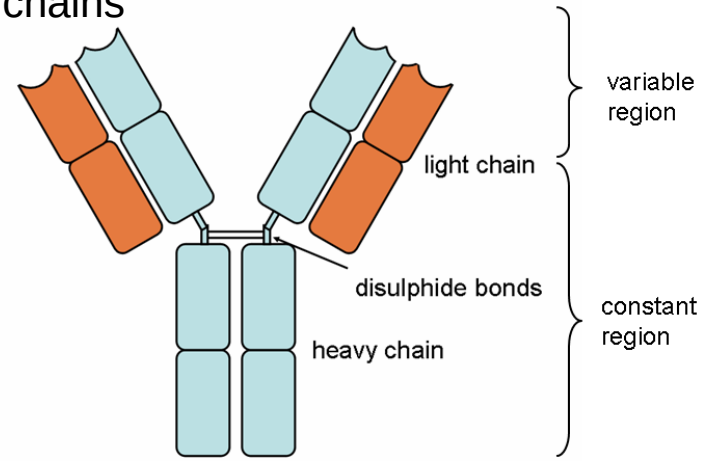
The immune system must anticipate new problems and adapt to an ever changing world of pathogens.

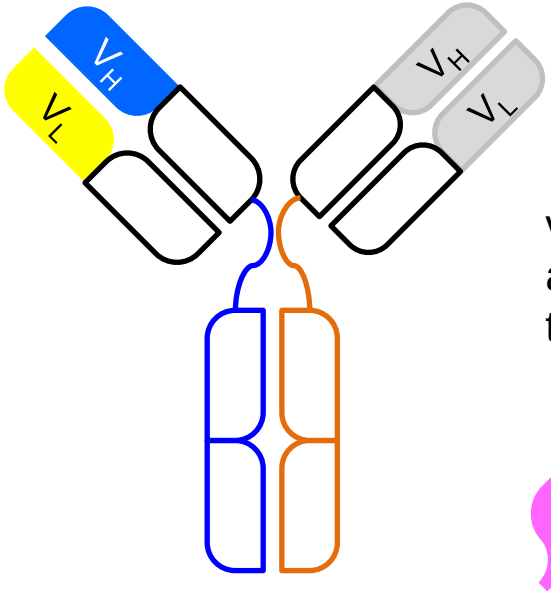
The “anticipatory” and specific immune system is called adaptive immunity or the adaptive immune system

# B cells make and secrete antibodies.

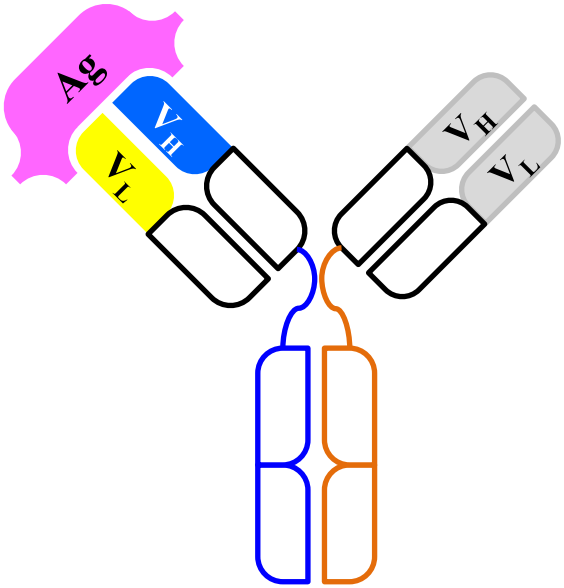
## Antibodies bind to antigens

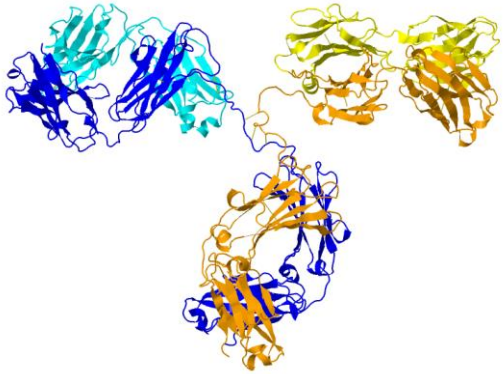
Antibodies are disulfide linked heterodimeric proteins composed of heavy (IgH) and light (IgL) chains



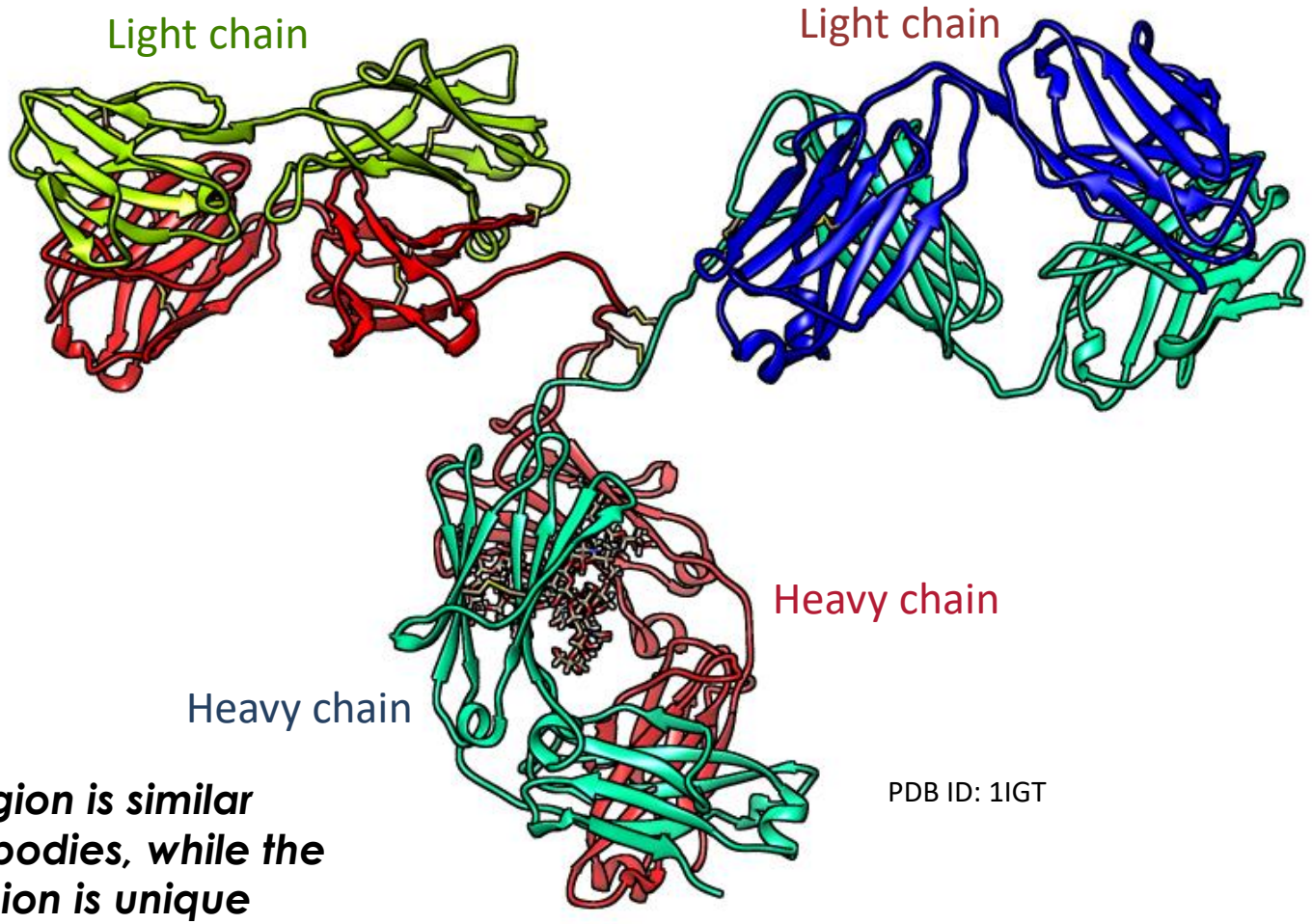




variable regions ($V_L$ and $V_H$) are portion of Ab that bind to antigen



# Antibody Structure: IgG




PDB ID: 1IGT

***Constant region is similar among antibodies, while the variable region is unique***

To examine this structure further go to www.rcsb.org
Type in the PDB ID in the top search box and explore

# Antigens Antibody Binding

- Antigen can bind in pockets, grooves, or on extended surfaces in the binding sites of Antibodies (Abs)
- Abs can bind almost anything… Proteins, DNA, etc.
- Can form binding sites compatible to almost any kind of antigen
- >$10^7$ possible Ab specificities in your body!

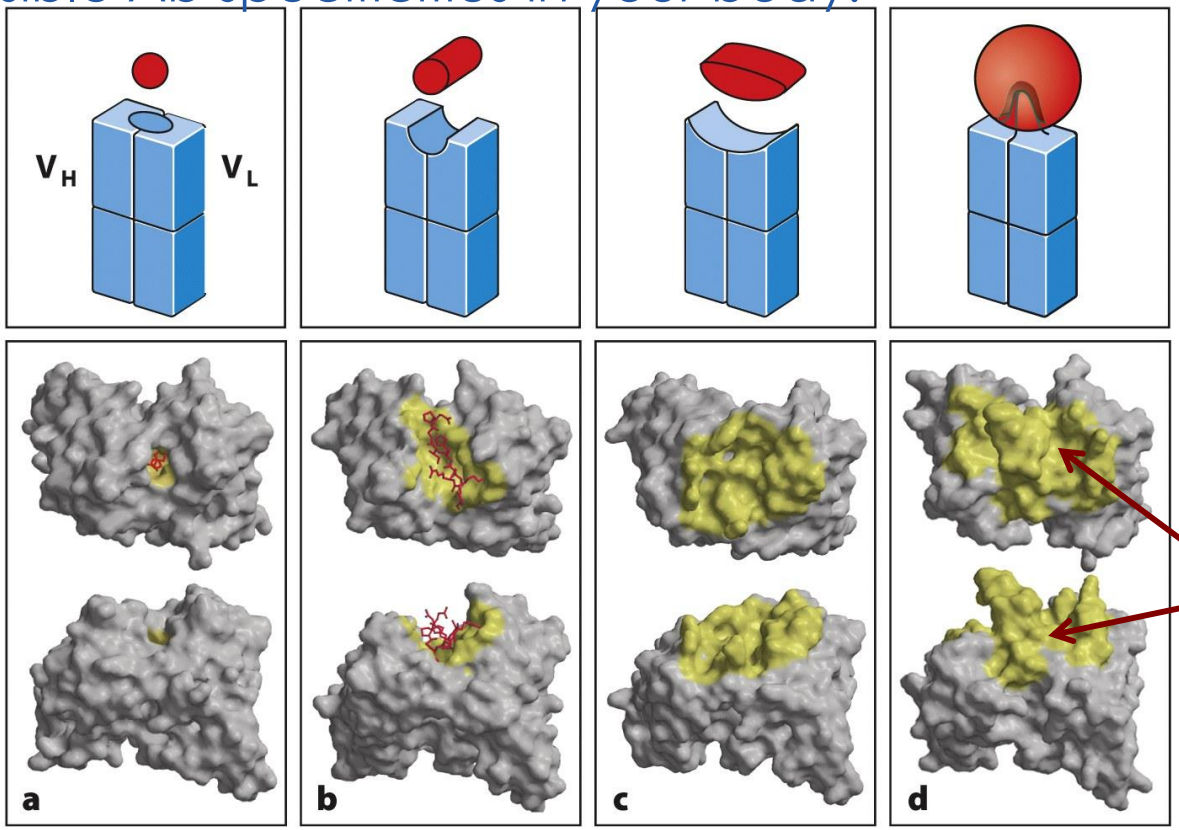
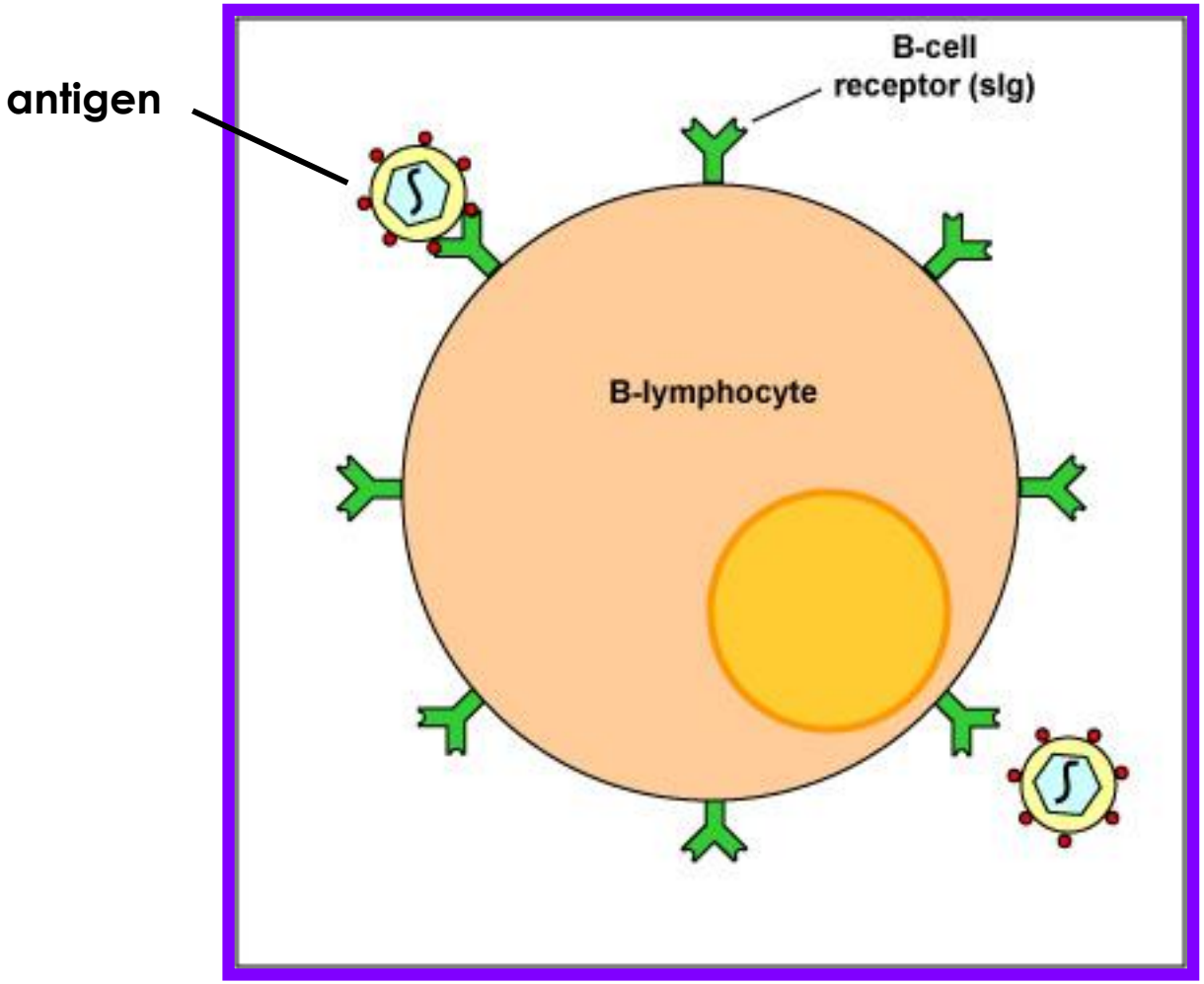
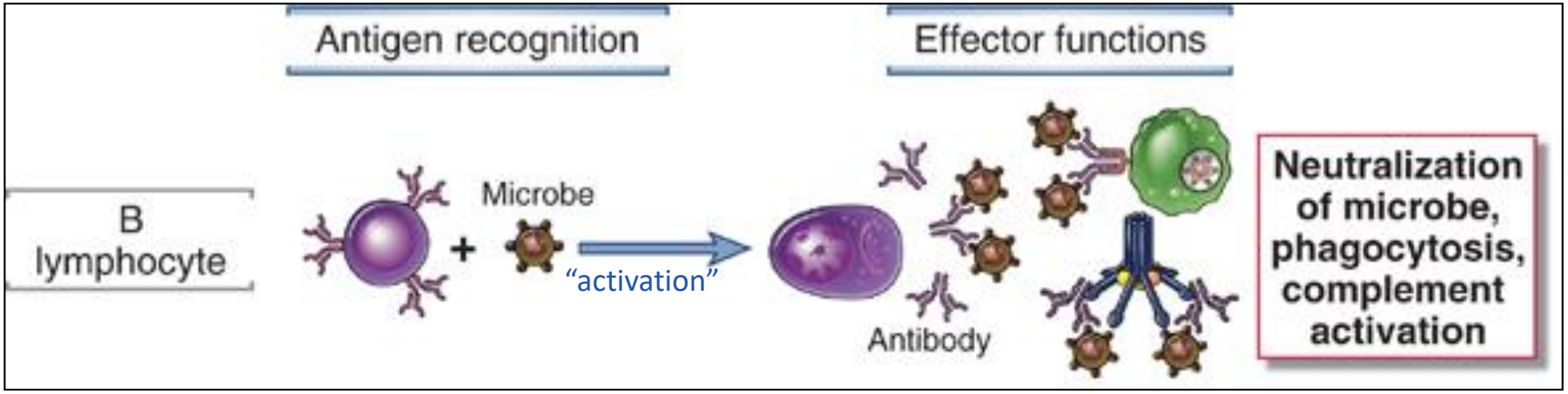



Figure 4.8 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

# Antibodies are the secreted version of the B cell antigen receptor (BCR).



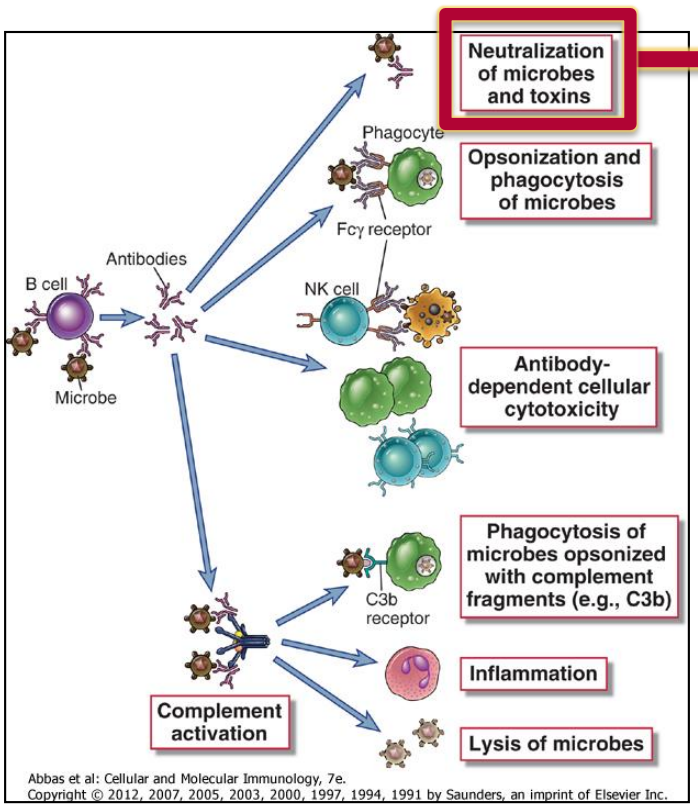
# The Function of Abs in the Immune Response (How do they clear infection?)

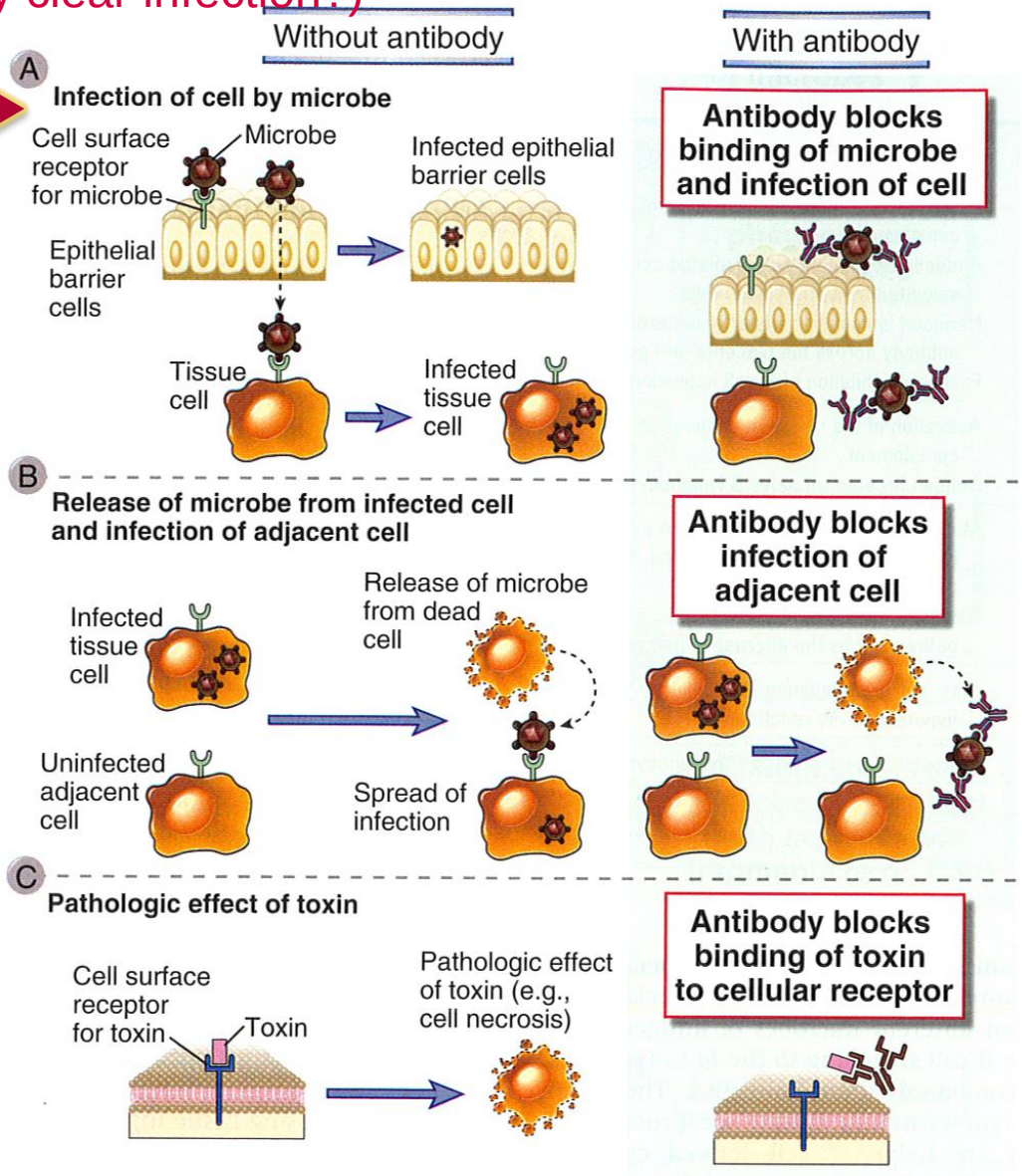



- ***B cells look for their cognitive antigen (the one its surface receptors can bind to)***
- ***Binding stimulates the B cell to enter the cell cycle and develop, by repeated mitosis, into a clone of cells with identical BCRs***
  - ***Switch from synthesizing their BCRs as integral membrane proteins to a soluble version;***
  - ***Differentiate into plasma cells that secrete these soluble BCRs, which we now call antibodies.***
- ***Antibodies bind to antigens and neutralize (prevent it from going from cell to cell)***

# The Function of Abs in the Immune Response
## (How do they clear infection?)





# Vaccination



**TABLE 12–2 Vaccine-Induced Humoral Immunity**

| Infectious Disease | Vaccine | Mechanism of Protective Immunity |
|---|---|---|
| Polio | Oral attenuated poliovirus | Neutralization of virus by mucosal IgA antibody |
| Tetanus, diphtheria | Toxoids | Neutralization of toxin by systemic IgG antibody |
| Hepatitis, A or B | Recombinant viral envelope proteins | Neutralization of virus by systemic IgG antibody |
| Pneumococcal pneumonia, *Haemophilus* | Conjugate vaccines composed of bacterial capsular polysaccharide attached to a carrier protein | Opsonization and phagocytosis mediated by IgM and IgG antibodies, directly or secondary to complement activation |

Selected examples of vaccines that work by stimulating protective humoral immunity are listed.

***Neutralization effect is the goal of vaccination.***
***Antibodies can promote phagocytosis by binding to pathogens – facilitates take up of antigens by macrophages.***

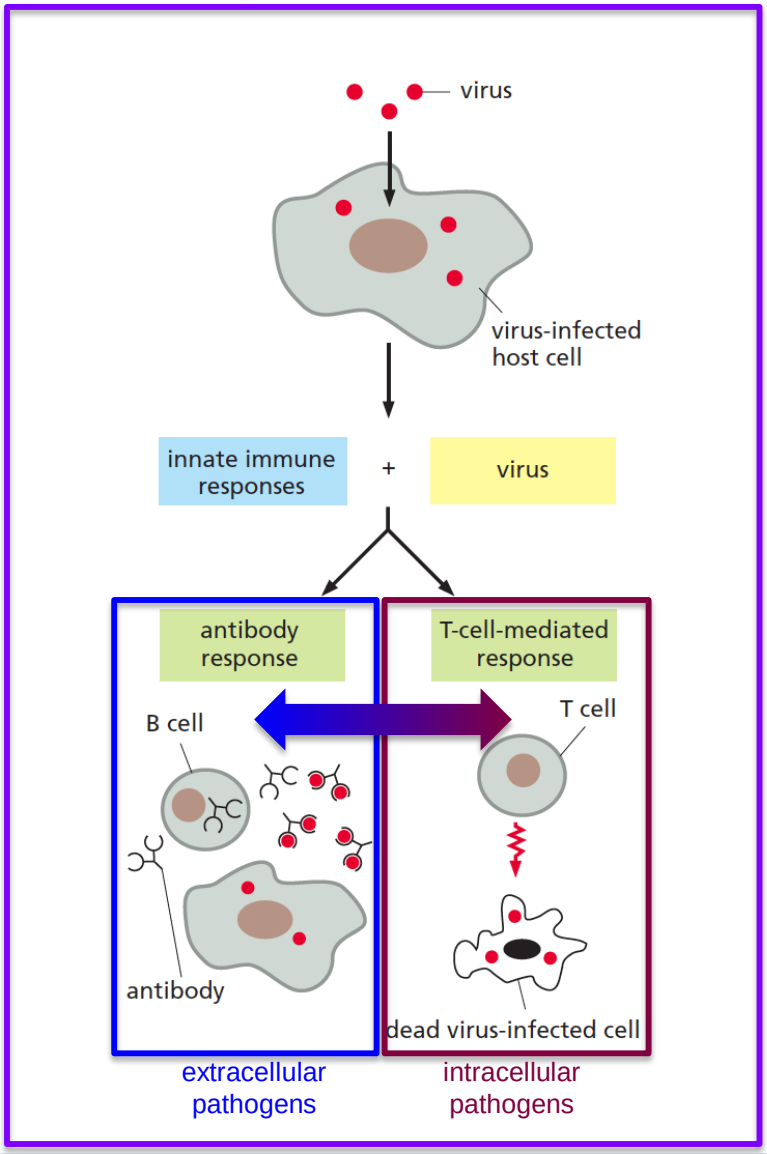
# Two main classes of adaptive immune responses

1. humoral immunity
   (antibody response)

2. cell mediated immunity
   (T-cell mediated response)

Mediated by two different types of cells call lymphocytes

- B lymphocytes (B cells)
- T lymphocytes (T cells)



# The T cell antigen receptor (TCR)

- Overall structure of TCR and BCR (Antibody) are similar
- The TCR is NOT secreted like an antibody
- The antigen binding site is also highly variable
- Like B cells, T cells recombine (scramble) genes to generate much diversity.
- T cells are even MORE diverse than B cells.
- B cell – one receptor for one antigen (high specificity)
- T-cell receptor binding site is similar to B-cell binding site. However, it is never secreted.

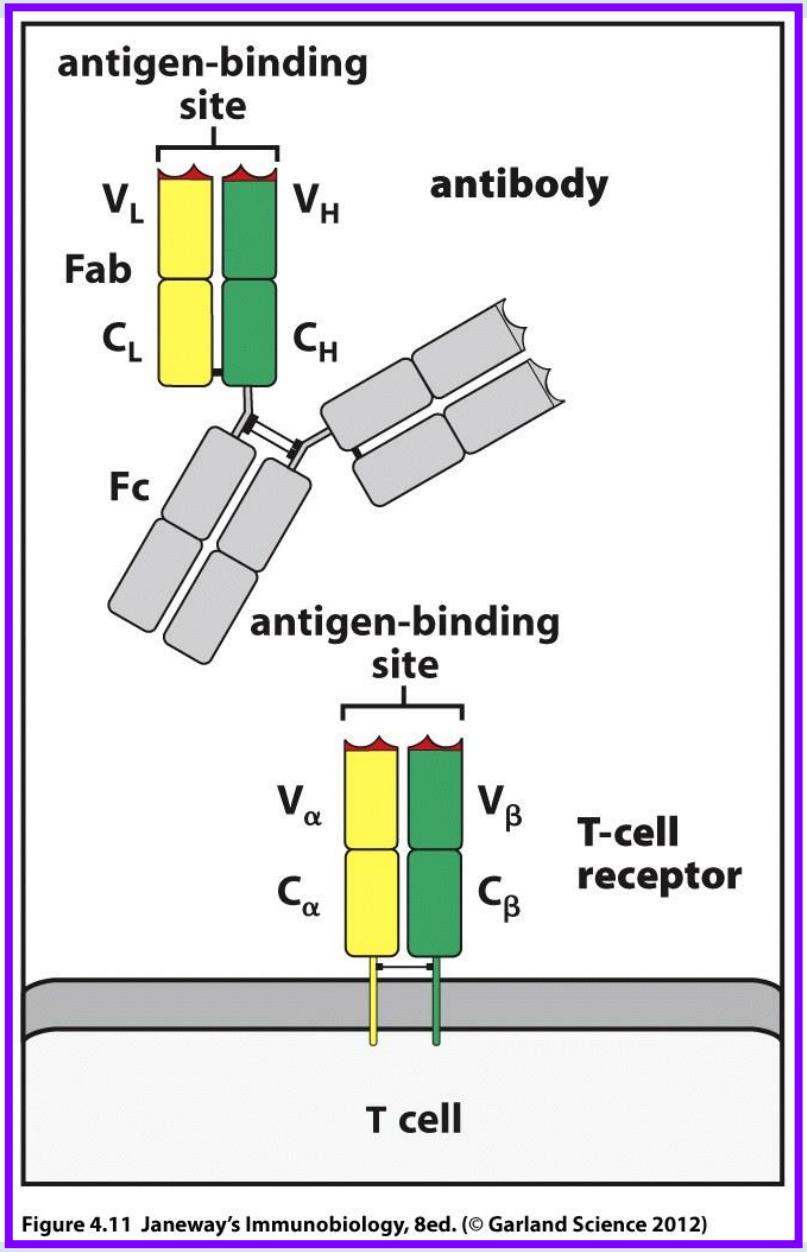
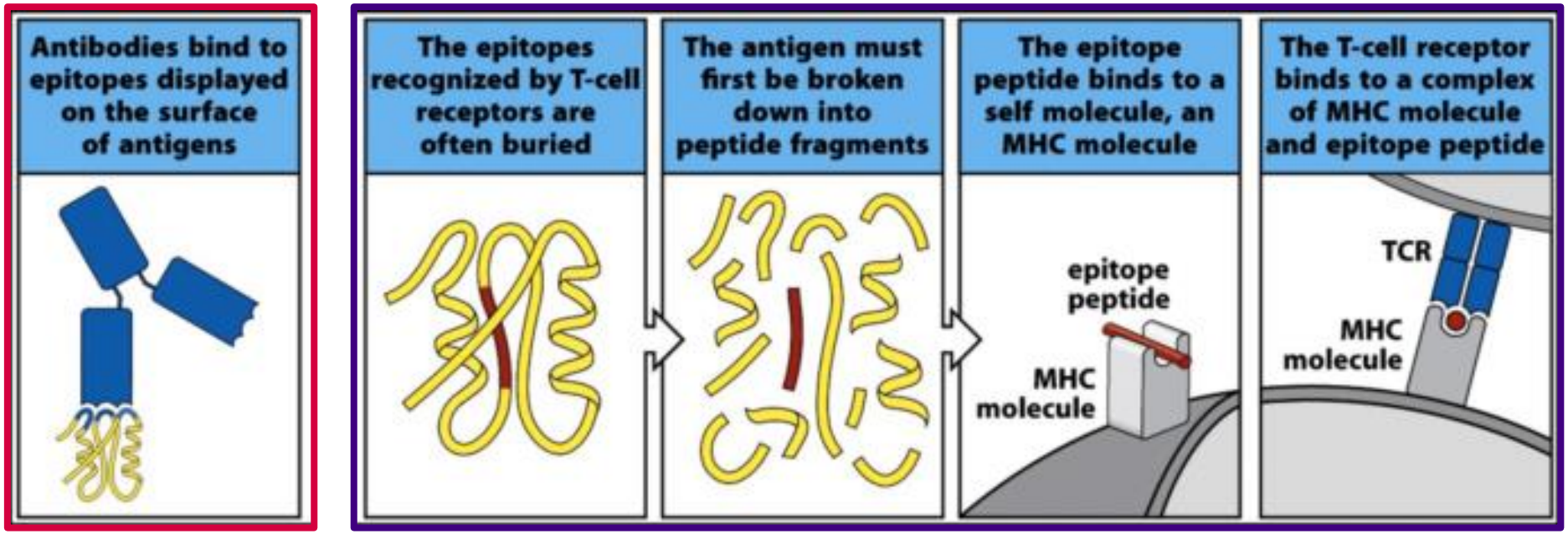


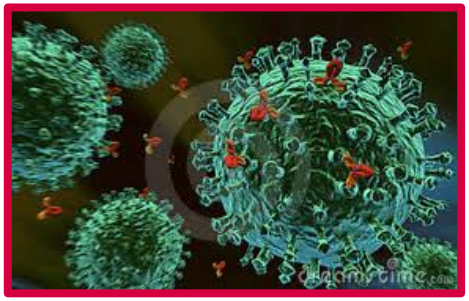

Figure 4.11 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

# Fundamental difference – antigen recognition between TCR and BCR (or Ab)

BCRs (Abs) recognize the (unprocessed) whole antigen

TCRs recognized processed antigen in the context of MHC class I or Class II



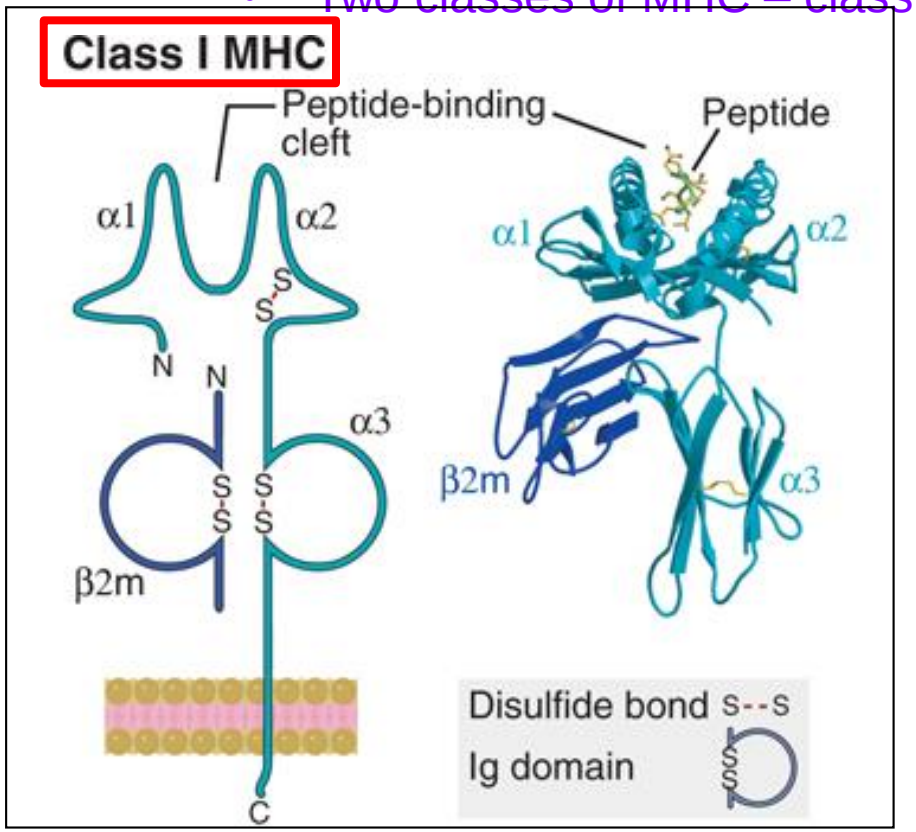



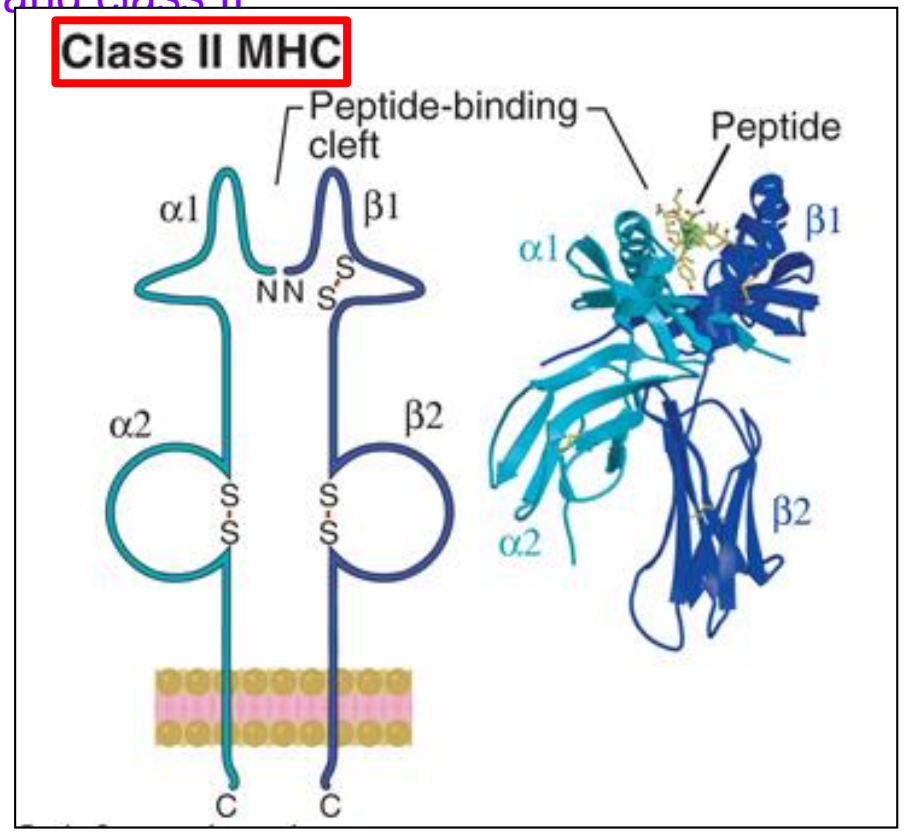
***B cells recognize the whole antigen (native protein)***
***T cells recognize only a piece of the antigen (peptide). Antigen must be broken down (processed) before it can be recognized (peptide is presented to the TCRs)***

# Antigen are presentation



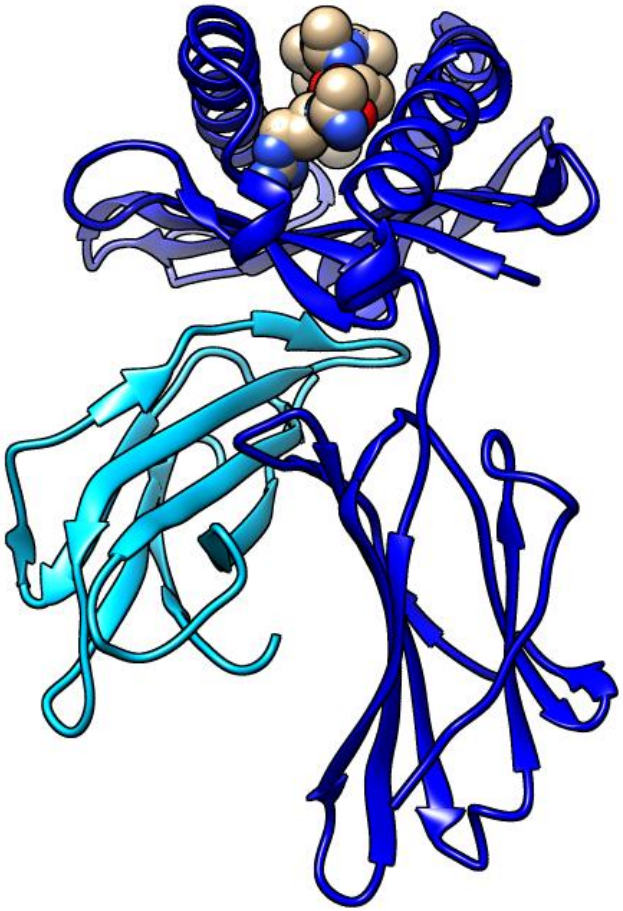
- The peptide fragments of antigen are presented to the TCR bound to proteins called Major Histocompatibility Complex (MHC) molecules
- In human called Human Leukocyte Antigens (HLA)
  - Two classes of MHC – class I and class II



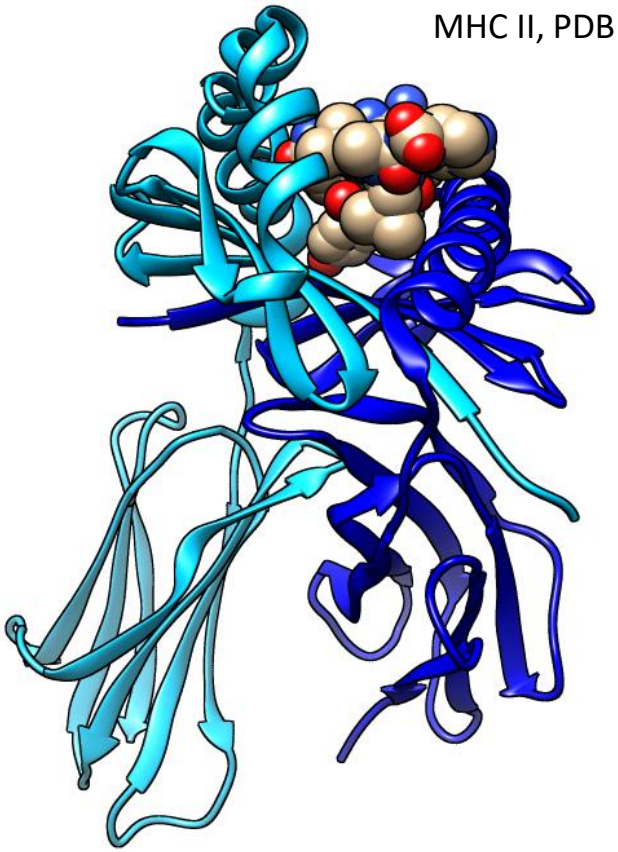



# MHC proteins

MHC I, PDB ID 1HSA



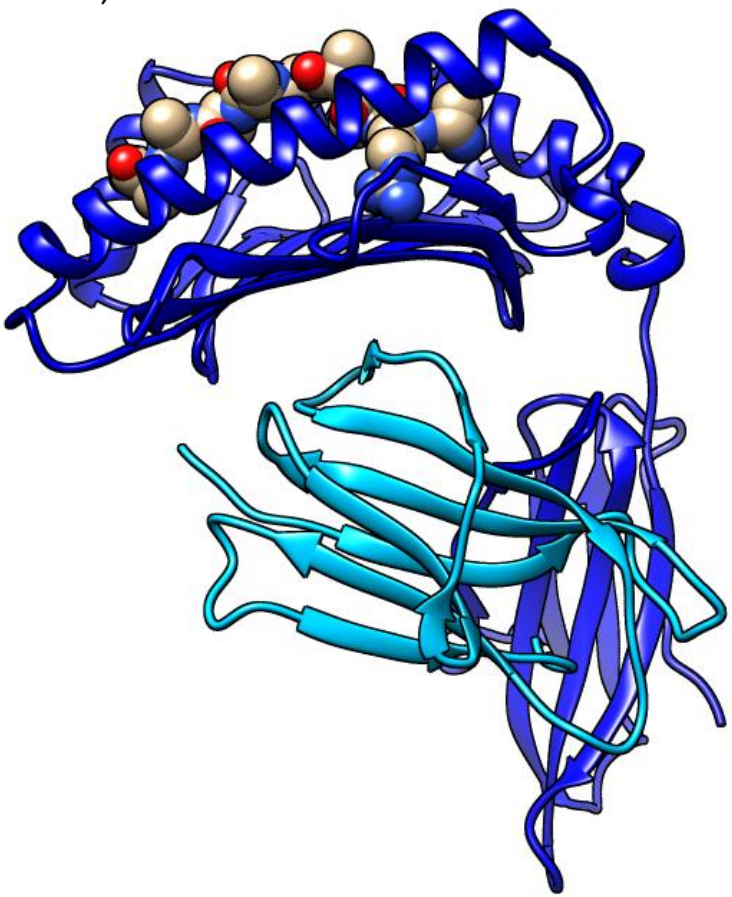
MHC II, PDB ID 1DLH



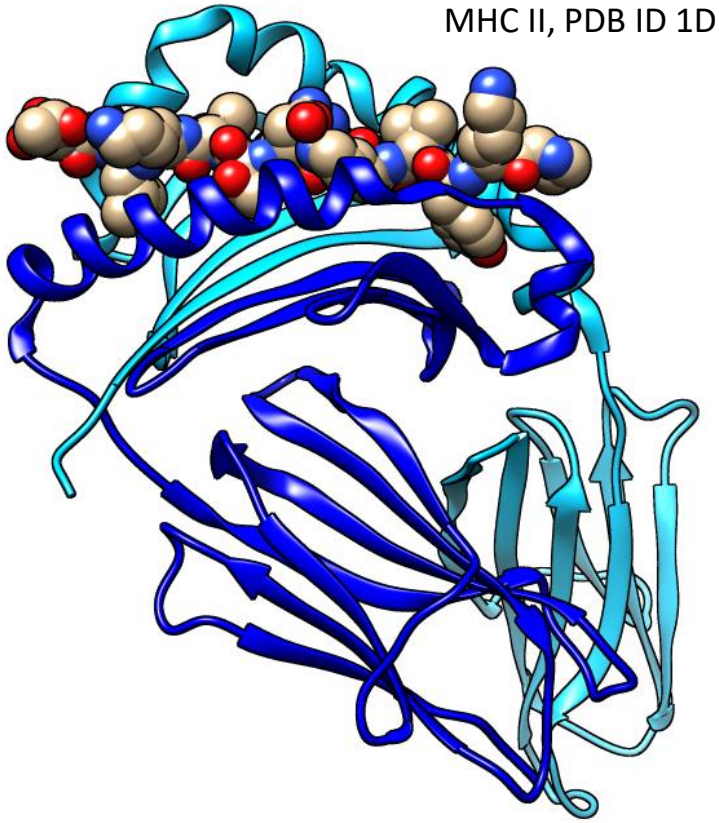
To examine these structures further go to www.rcsb.org
Type in the PDB ID in the top search box and explore

# MHC proteins

MHC I, PDB ID 1HSA



MHC II, PDB ID 1DLH



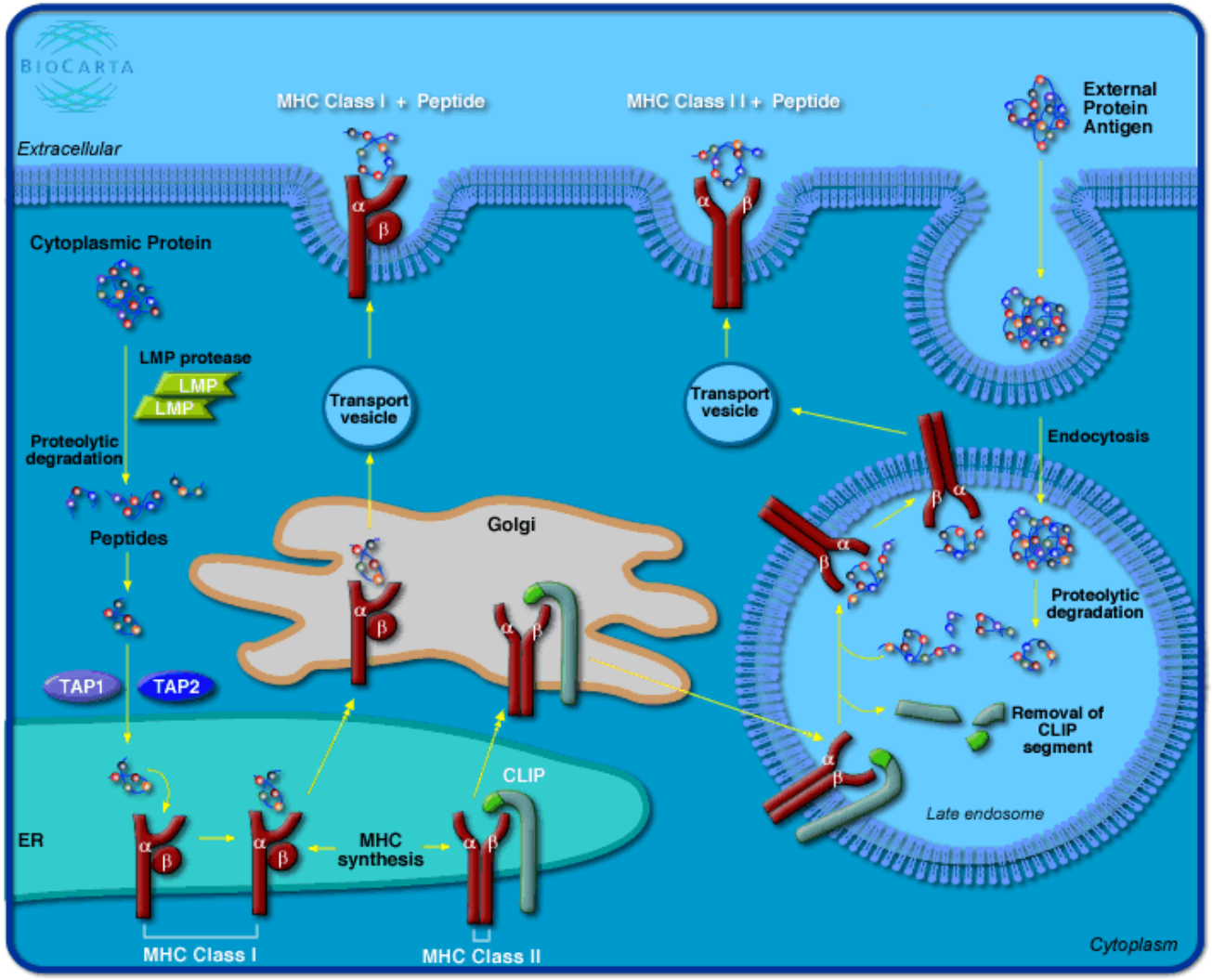
To examine these structures further go to www.rcsb.org
Type in the PDB ID in the top search box and explore

# A broad peptide repertoire

***Class I and Class II molecules acquire peptides degraded in the cytosol or in lysosomes. This allows for a broad peptide repertoire presented at the cell surface for T cells to recognize***

(viruses)

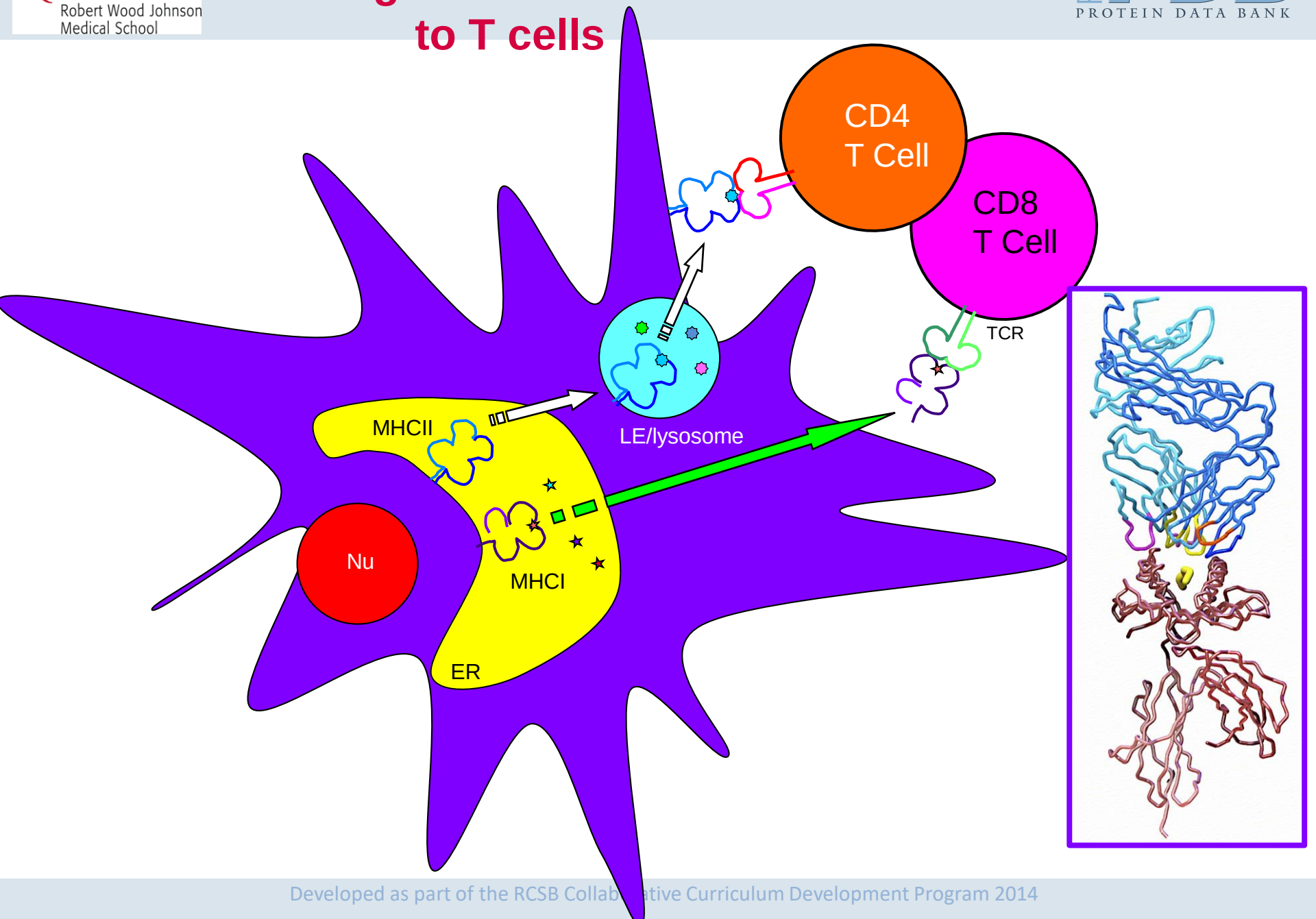
***Class 1 pathway binds and presents endogenous peptides found in the cytosol (in ER)***



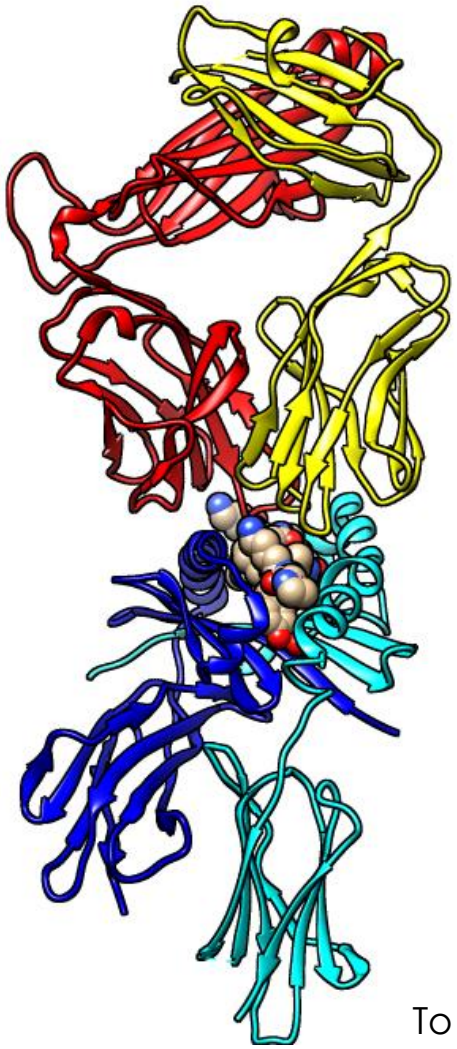

(bacteria)

***Class 2 pathway binds and presents exogenous peptides brought into the cell (in endosomes and lysosomes)***

# Antigen Presentation to T cells



# Interaction: T cell receptor with MHC II



TCR alpha chain

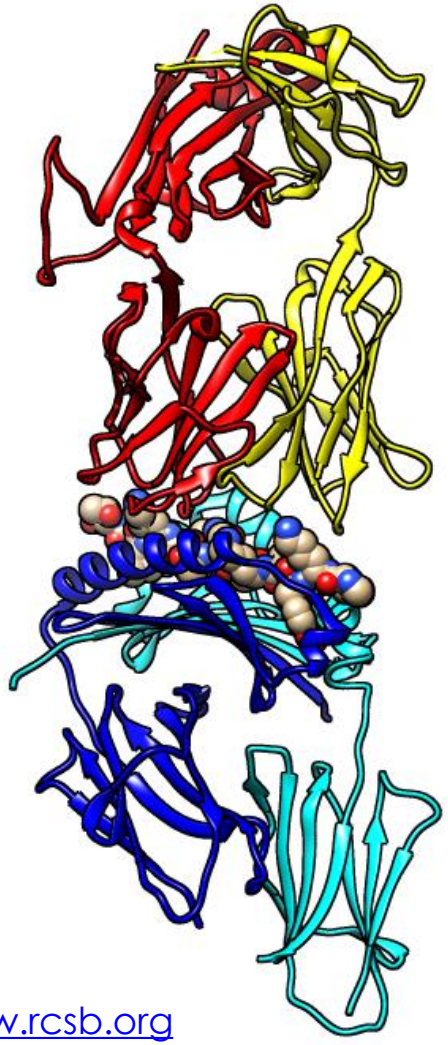
TCR beta chain

Rotate about vertical axis

Influenza hemagglutinin peptide

MHC II alpha chain

MHC II beta chain

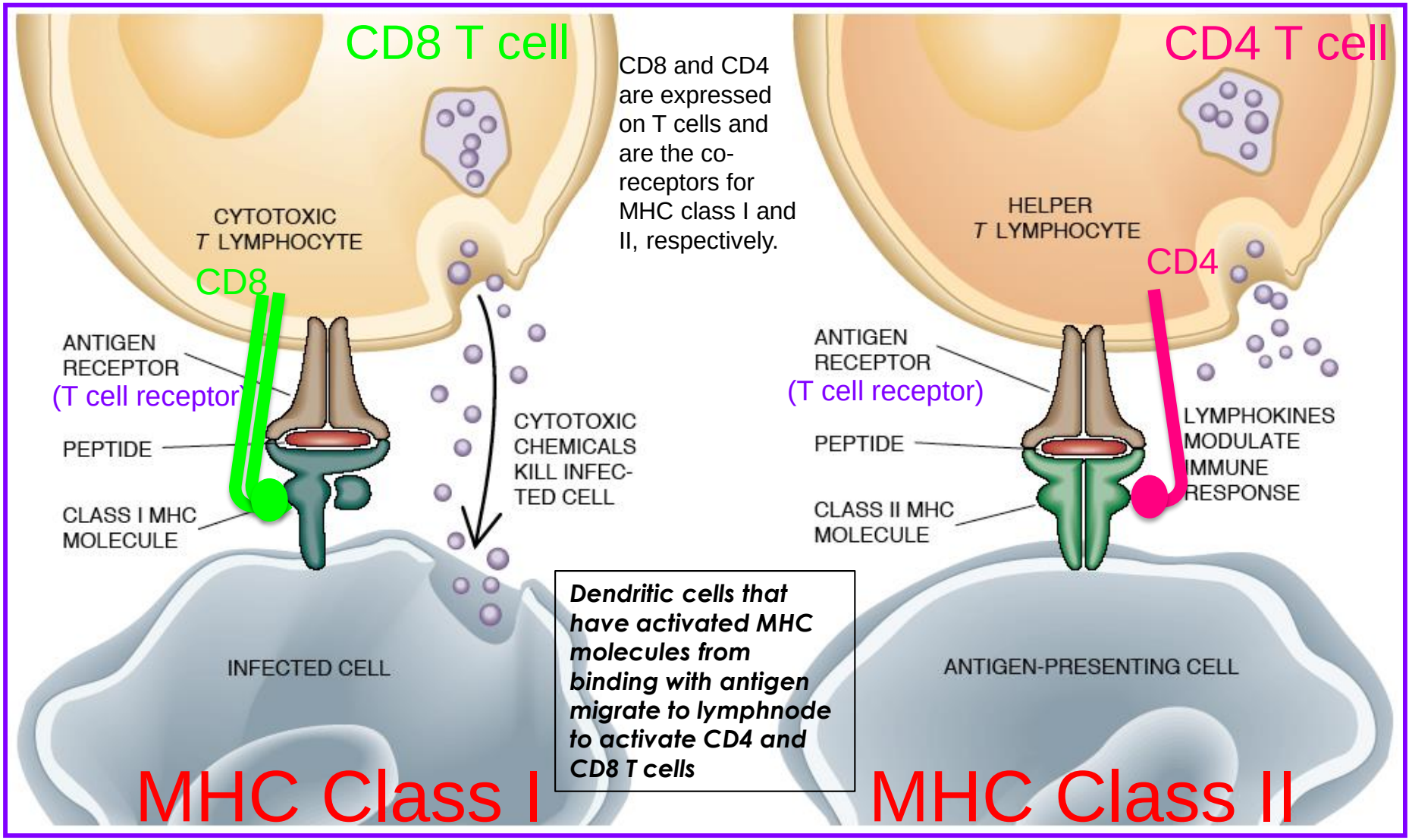


PDB ID: 1FYT

To examine this structure further go to www.rcsb.org
Type in the PDB ID in the top search box and explore

# Two types of MHC molecules –

## class I and class II activate different types of T cells – CD8 and CD4

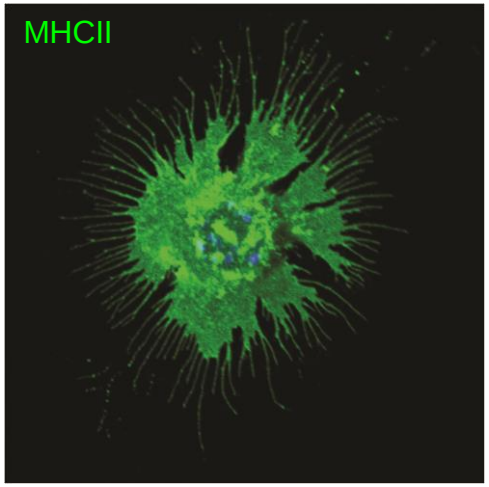


# Antigen Presenting Cells (APCs)

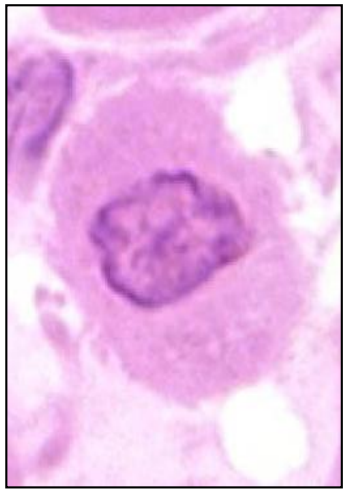
## Process and Presentation of Peptide-MHC complexes to CD4 and CD8 T cells

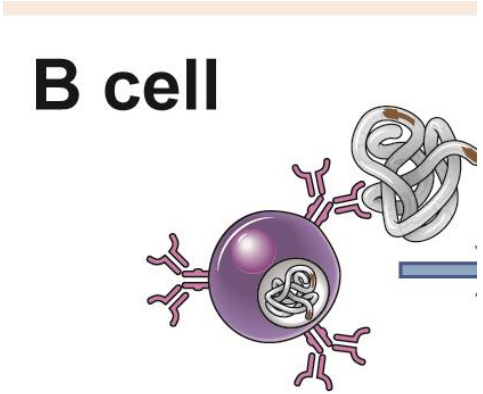
- Only some cells can present peptide to T cells for initial T cell activation
- These cells are called professional antigen presenting cells
  - dendritic cells
  - macrophages
  - B cells

dendritic cells




macrophage

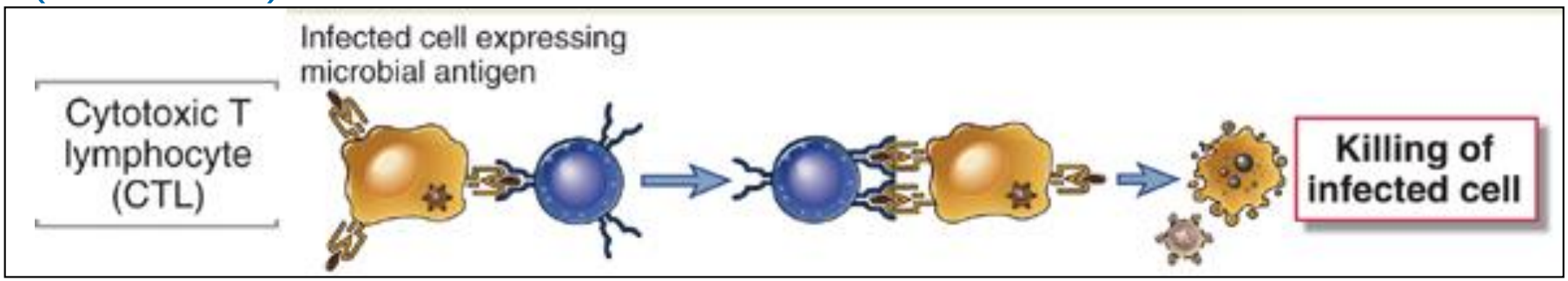





- Other cells can present, but they can not initiate T cell responses

# The Function of CD8 T cells in the Immune Response (How do they clear infection?)

(CD8; Killer)

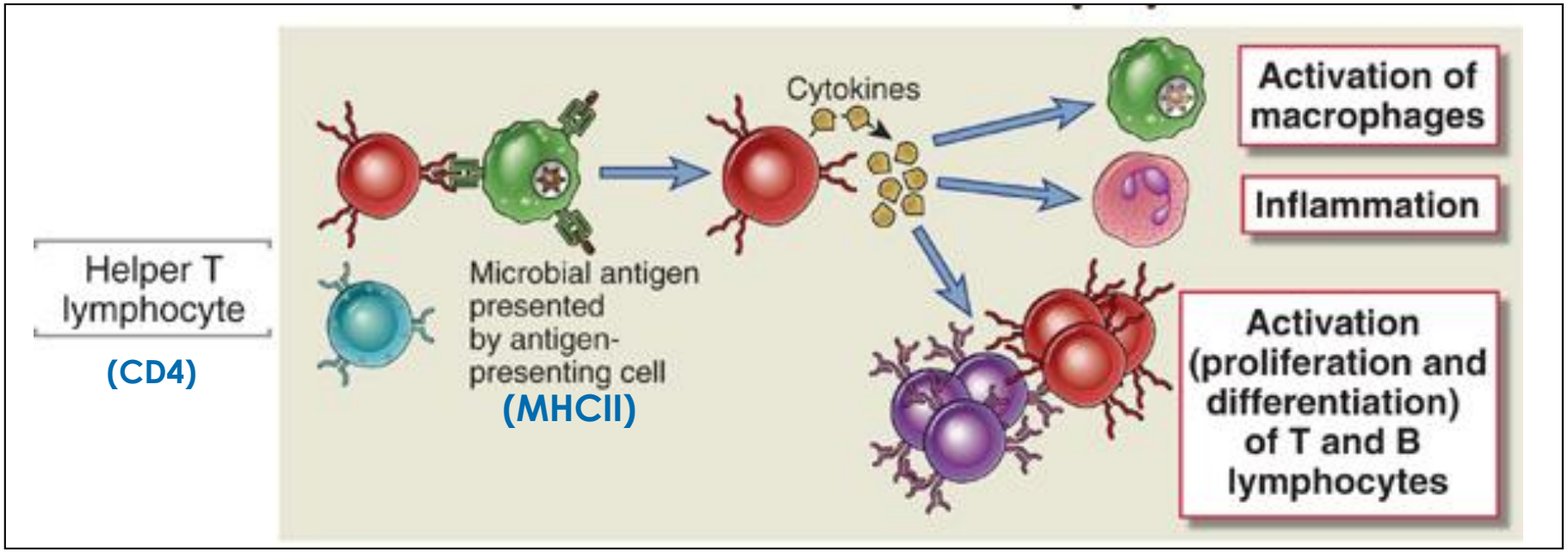



(MHCI)

***CD8 T cells bind infected cell and kill.***

Important mediators of immune responses especially important for killing virally infected cells

# The Function of CD4 T cells in the Immune Response (How do they clear infection?)



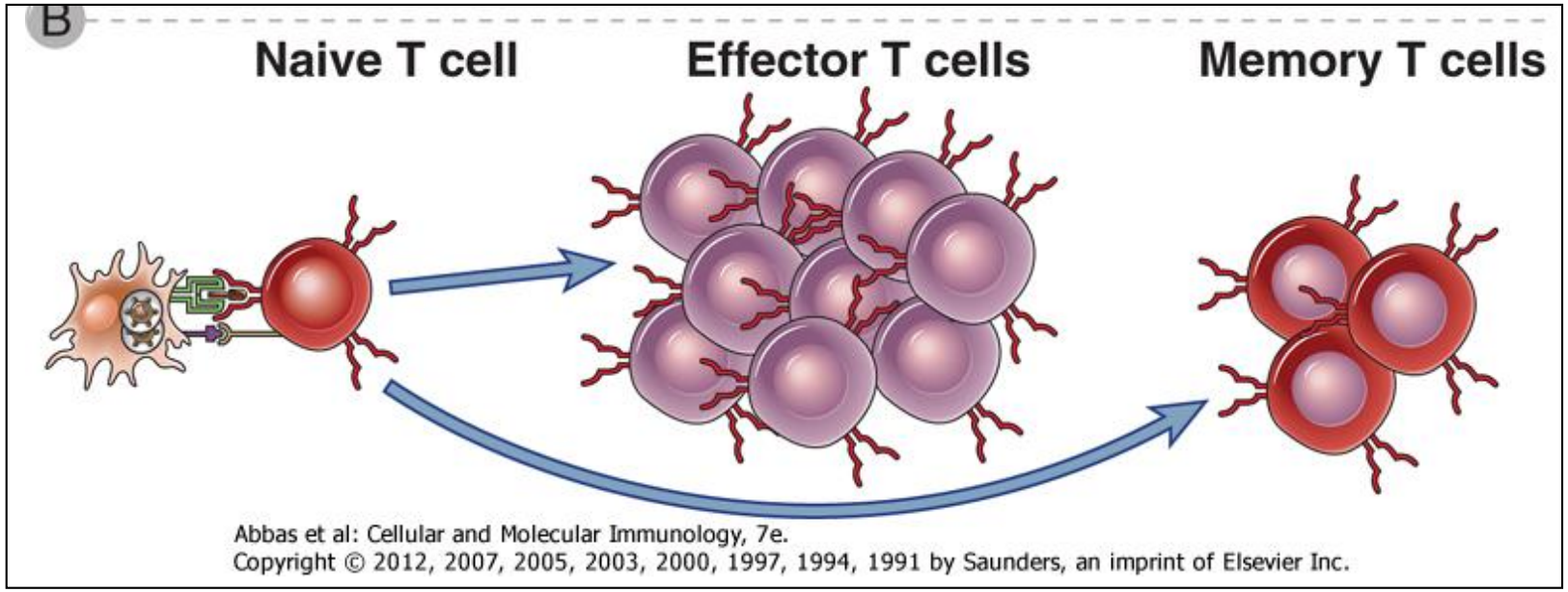

***Once activated, CD4 cells secrete cytokines to promote macrophages to kill cells that they ingest; promote inflammation; activate T and B cells***

# Memory cells



Defined:

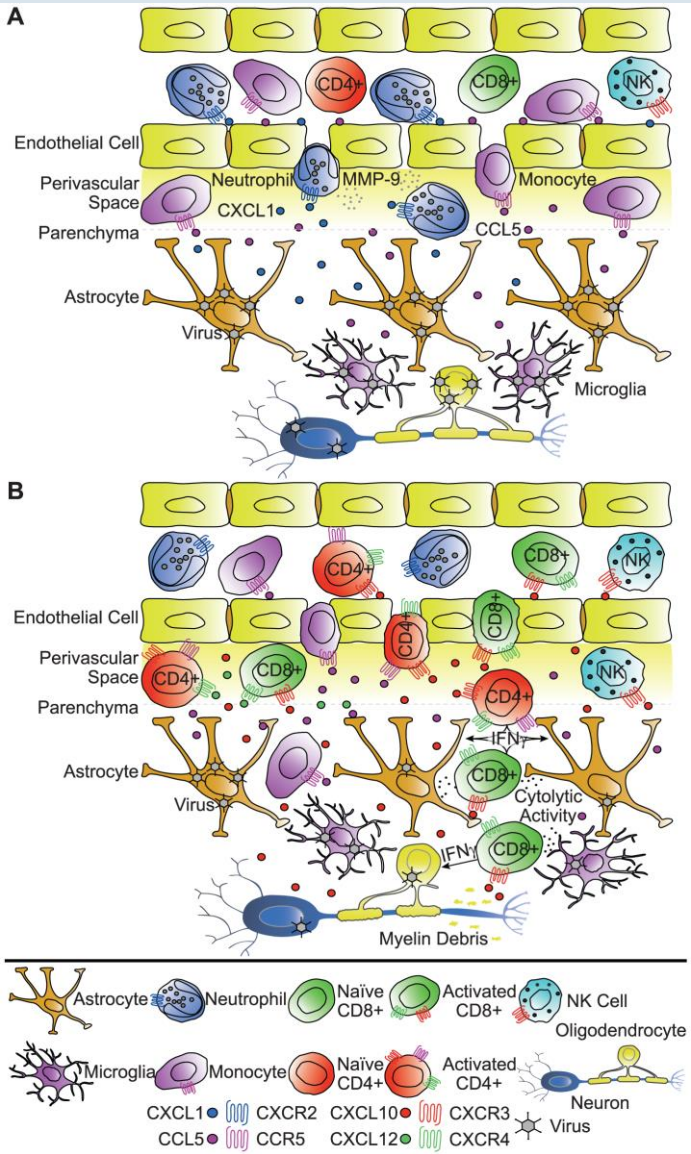
- memory T and B cells are produced by antigen stimulation of naïve lymphocytes and survive in a functionally quiescent state for many years after the antigen is eliminated
- mediate a rapid and enhanced (recall) response to second and subsequent exposures to antigens ***(this is what you are aiming for when you vaccinate someone)***






***In some cases immunological memory doesn't last forever – this is why we must get booster vaccinations (e.g. tetanus)***

# Chemokines and Chemokine Receptors control immune cell trafficking in the body




***In some cases immunological memory doesn't last forever – this is why we must get booster vaccinations (e.g. tetanus)***

# Overview of Adaptive Immune Response

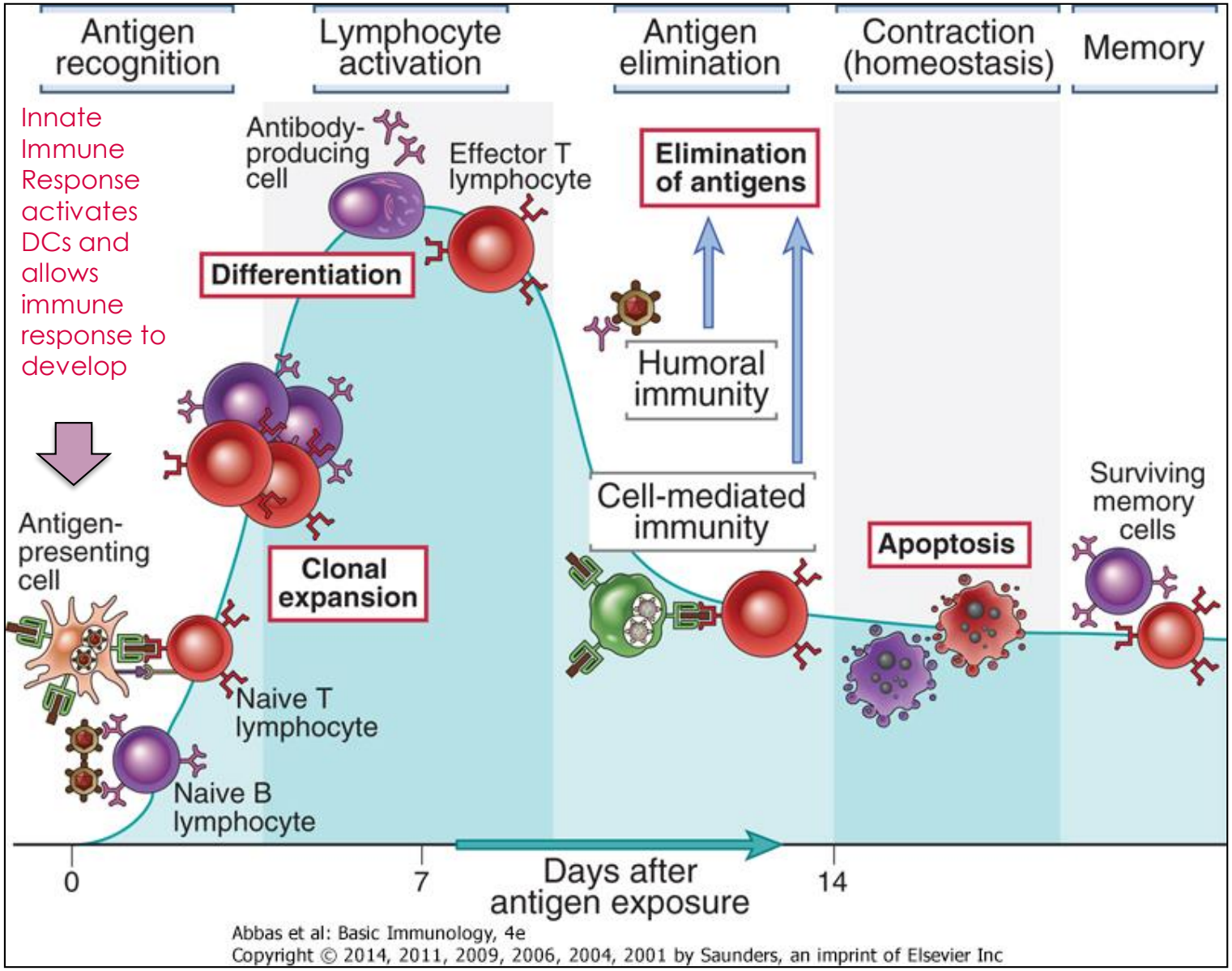



Figure 1-17 Phases of adaptive immune response. An adaptive immune response consists of distinct phases; the first three are recognition of antigen, activation of lymphocytes, and elimination of antigen (effector phase). The response declines as antigen-stimulated lymphocytes die by apoptosis, restoring the baseline steady state called homeostasis, and the antigen-specific cells that survive are responsible for memory. The duration of each phase may vary in different immune responses. These principles apply to both humoral immunity (mediated by B lymphocytes) and cell-mediated immunity (mediated by T lymphocytes).

***Save some cells that produce specific antibodies so immune response is faster the second time around.***

# Immune Regulation:



## Balance Between Immune Activation and Control

**IMMUNITY** **CONTROL**

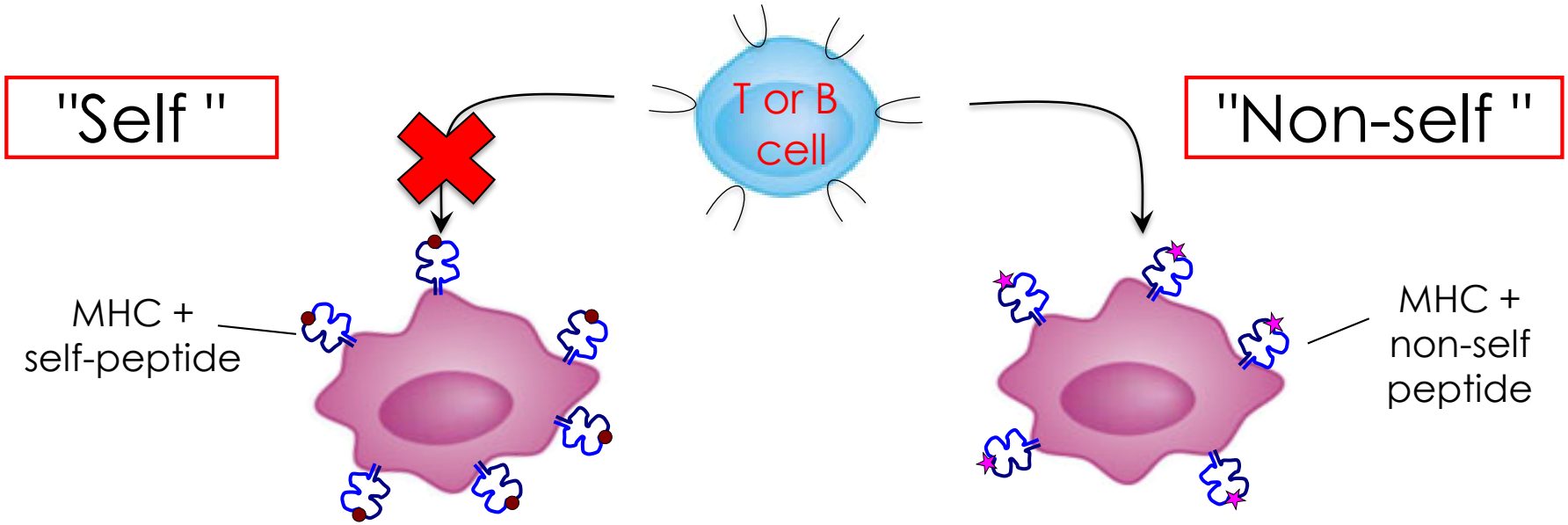

**T and B cell activation:**
NORMAL response against pathogens

CD4 & CD8 T cells
B cells
DCs & macrophages, etc

**Regulatory Cells:**
Regulatory T cells
Regulatory B cells
Regulatory dendritic cells

# The fundamental question for the immune system

## Is this cell/tissue/protein "self" or is it "non-self"?




| | |
|---|---|
| Normal, healthy cell | Abnormal, unhealthy cells e.g. infected or cancer cells |
| If healthy cells are attacked by the immune system, normal tissues are destroyed potentially causing an autoimmune disease | If unhealthy cells are not recognized by the immune system, infections/diseases (cancer) will not be eliminated. |

Autoimmunity - the destruction of healthy tissues leading to very nasty disease states

# Diversity of T cell receptors: A perspective



6,000

Visible stars in the night sky

1,000,000,000 (billion)

Grains of sand on a beach

100,000,000,000,000 (100 trillion)

number of cells in the human body

1,000,000,000,000,000,000 (quintrillion)

Possible different TCRs

***1 quintrillion TCRs can be generated! (made possible by gene shuffling), but are not all generated***

# Why so many T cell receptors?????



because your immune system needs to pick out which receptors recognize self and get rid of them.

this leaves receptors behind that can recognize non-self (pathogens)

this is the basis of self-non-self recognition in the immune system

this is fundamental for understanding how the immune system works

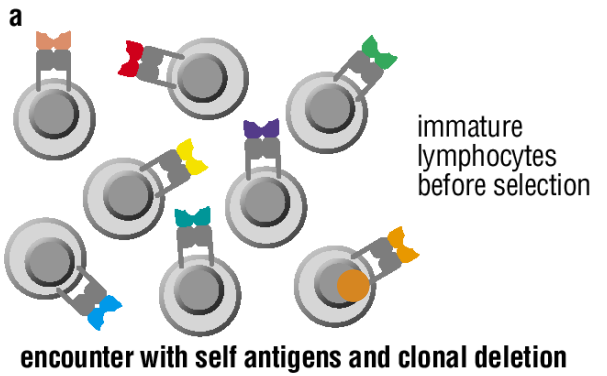
did I explain this???

***Purging of self-recognizing cells (aka self-tolerance) occurs in the thymus. (prevents autoimmunity)***
***Millions of T cells are born then die everyday due to this process.***

# The Clonal Selection Hypothesis

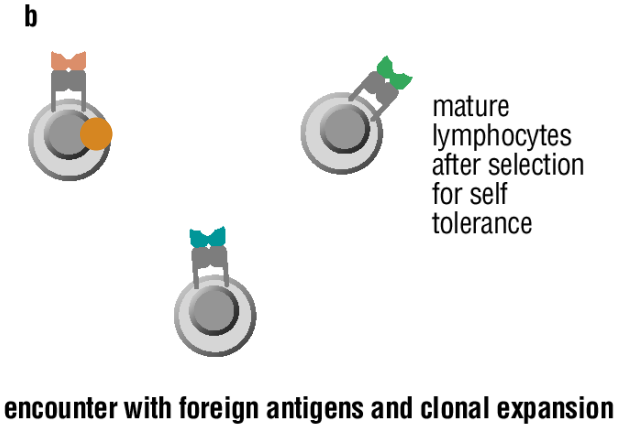


Generation of lymphocytes of many specificities (that are there PRIOR to antigen encounter)



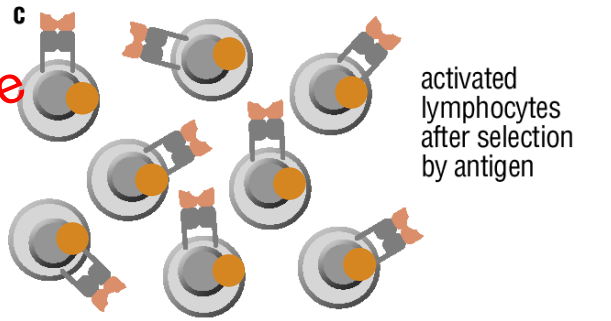

Clonal deletion to remove self-reactive lymphocytes

Self-tolerance




Clonal selection to expand pathogen-reactive lymphocytes during an immune response

Adaptive immune response

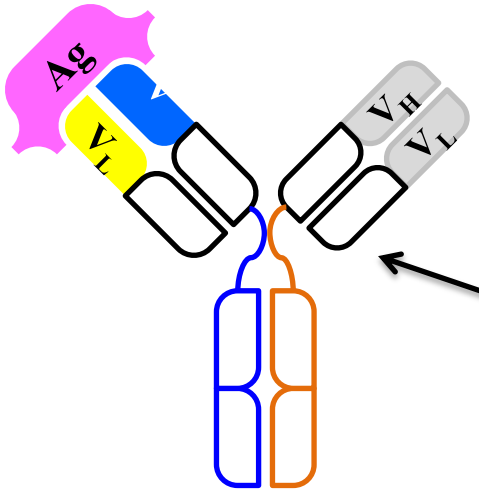


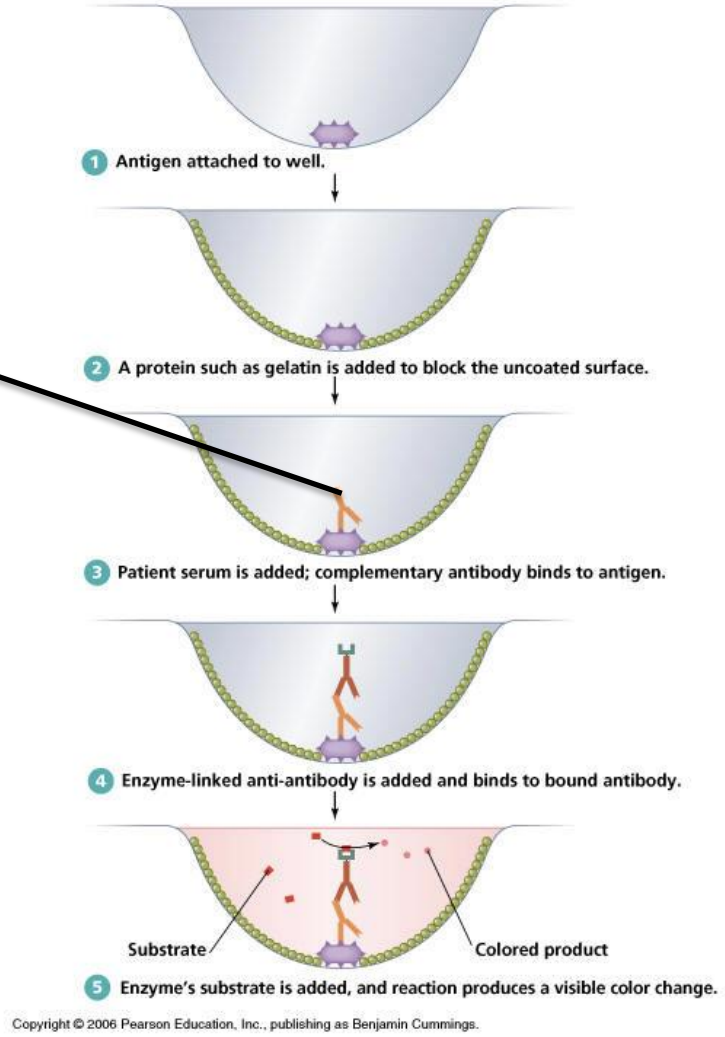

true for both B and T cells

# Immunologists tool box

## ELISA-

**E**nzyme
**L**inked
**I**mmuno-
**S**orbent
**A**ssay






- ***ELISA takes advantage of the specificity of antibodies.***

- ***Antibody-antigen complex detected by adding enzyme-linked antibody to bind the antigen specific antibody. Following this substrate is added and a color change indicates presence of antibody. If the test was using an HIV antigen and a patient's serum - color change means person has HIV (because they have antibodies for the virus in their system).***

# Immunologists Tool Box – Flow cytometry



## FLOW CYTOMETRY:

- Flow = cells in motion
- Cyto=cell
- Metry=measure
- Measuring properties of cells while in a fluid stream

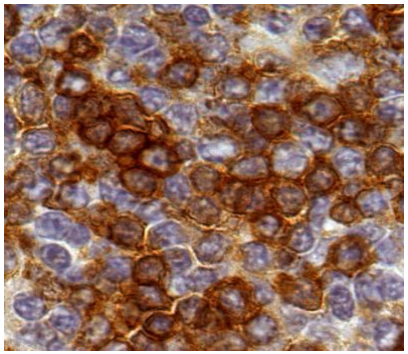
# Cytometry vs. Flow Cytometry

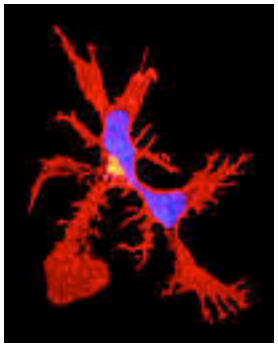
Cytometry (or immunofluorescence):

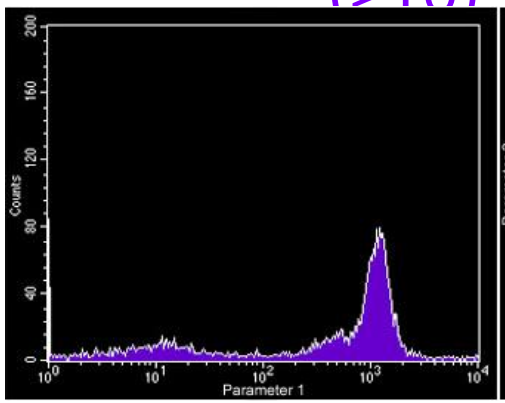
- Localization of antigen is possible
- Poor enumeration of cell subtypes
- Limiting number of simultaneous measurements (3 is easy, more harder but not 10)

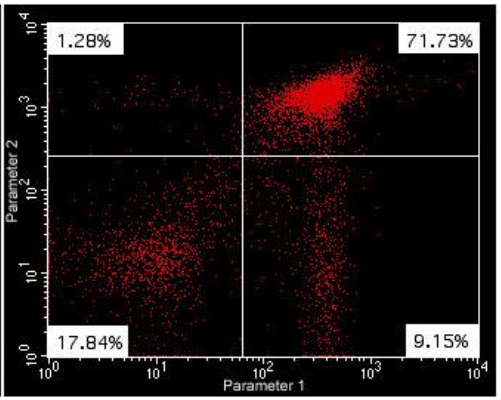
Flow Cytometry:

- Cannot tell you where antigen is.
- Can analyze many cells in a short time frame.
- Can look at numerous parameters at once (>10)

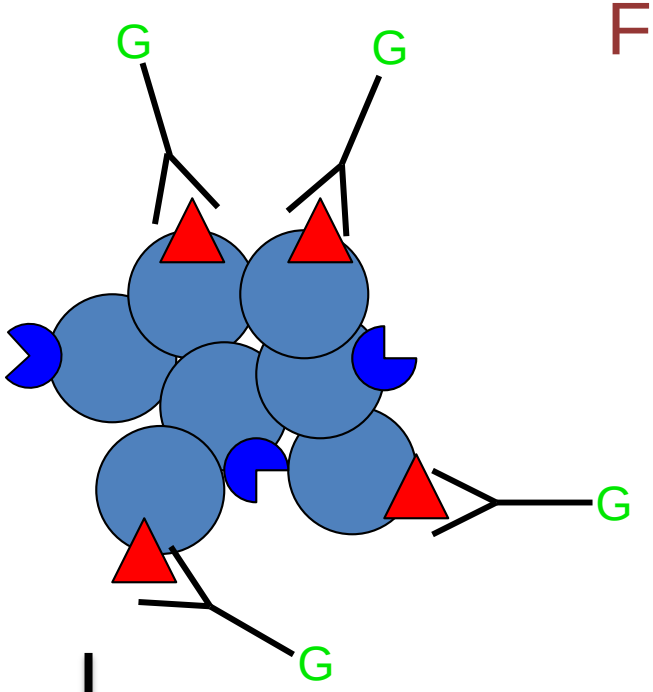


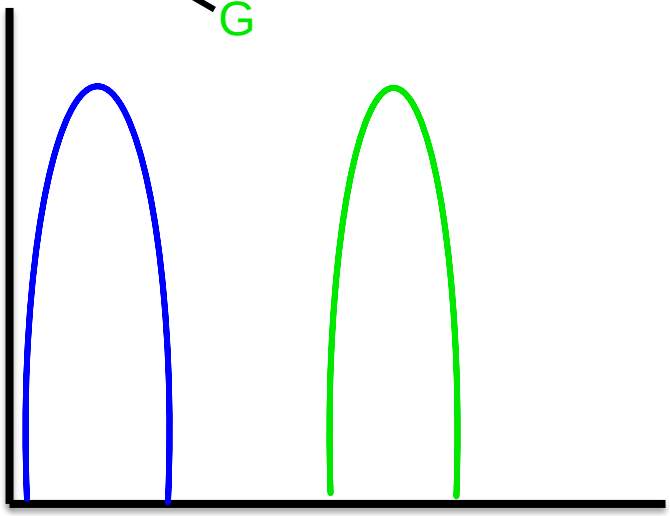




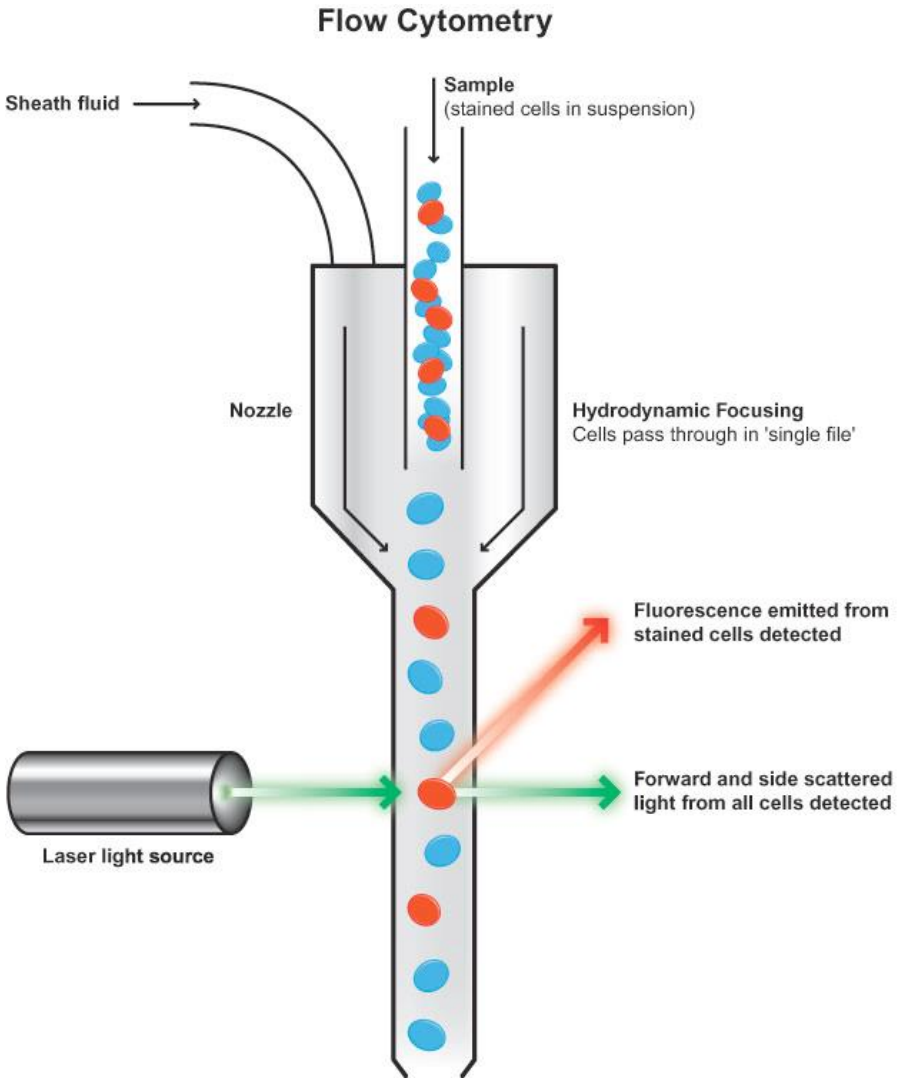



# Immunologists Tool Box – Flow cytometry



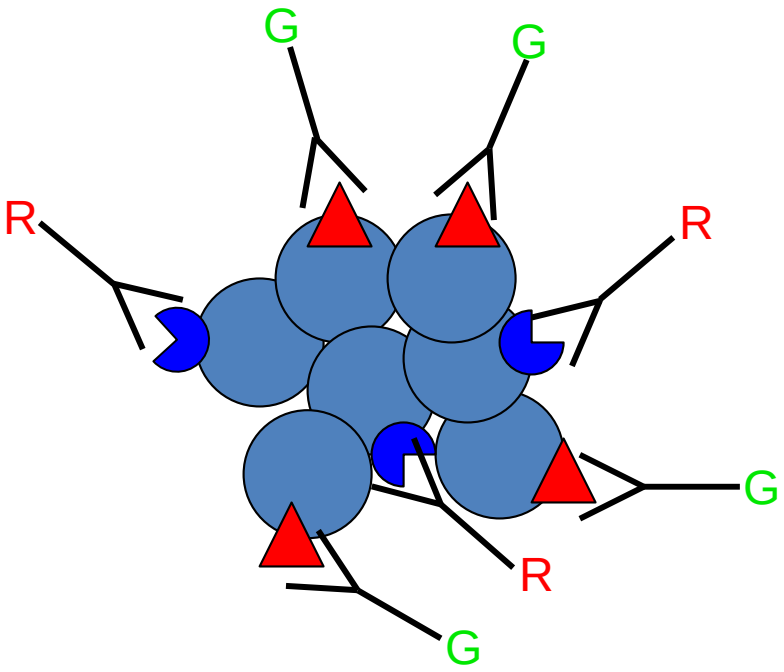



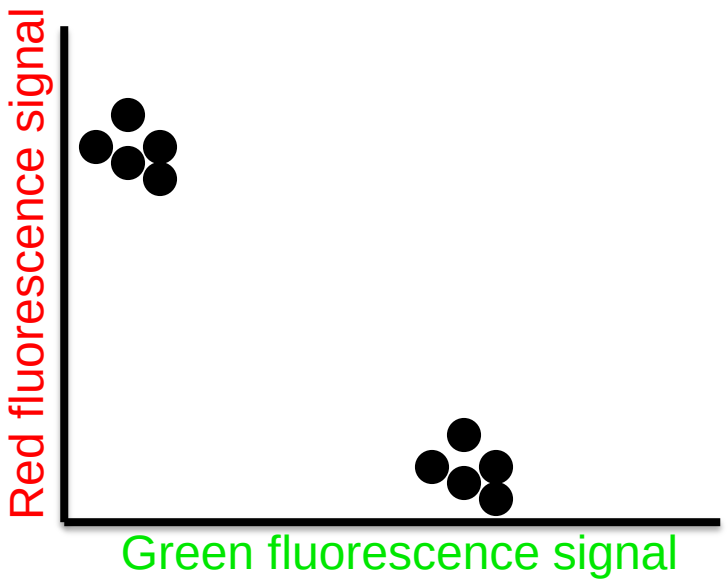
Green fluorescence signal



# Immunologists Tool Box – Flow cytometry Two colors

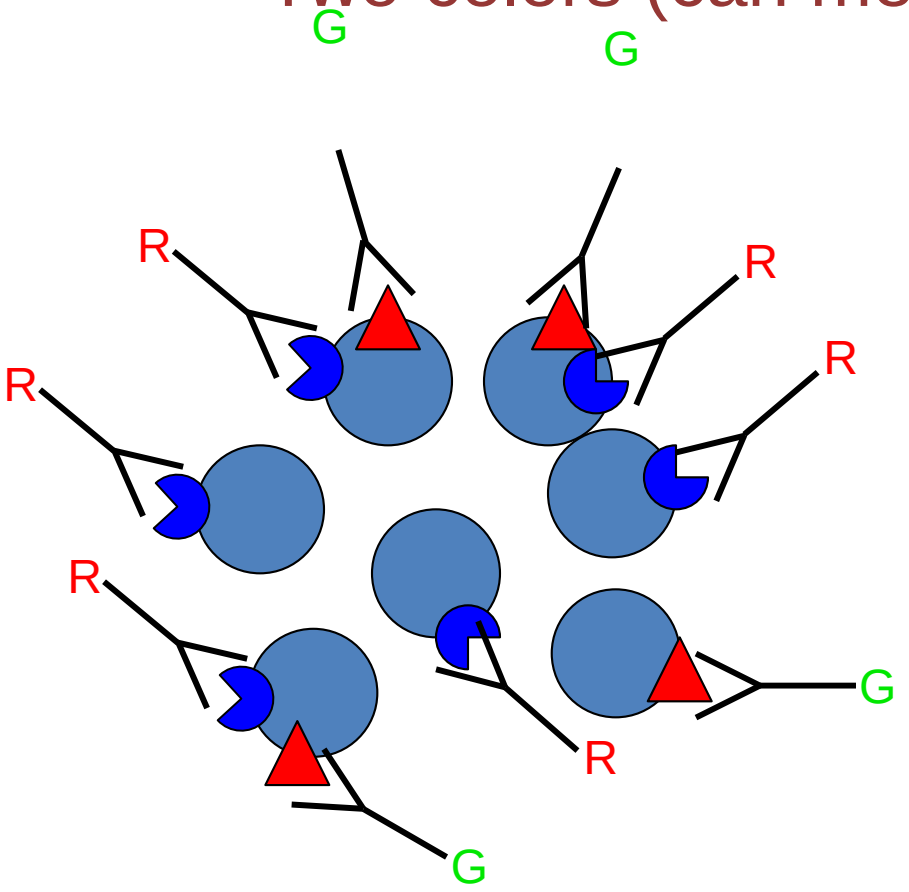


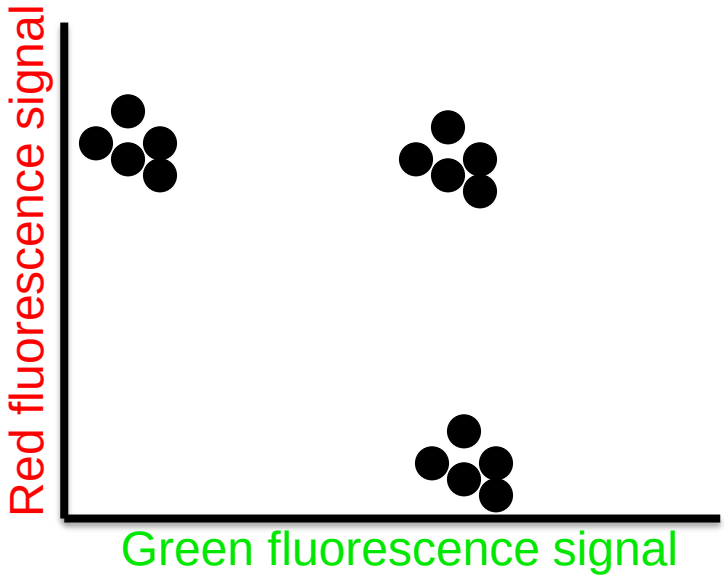


# Immunologists Tool Box – Flow cytometry

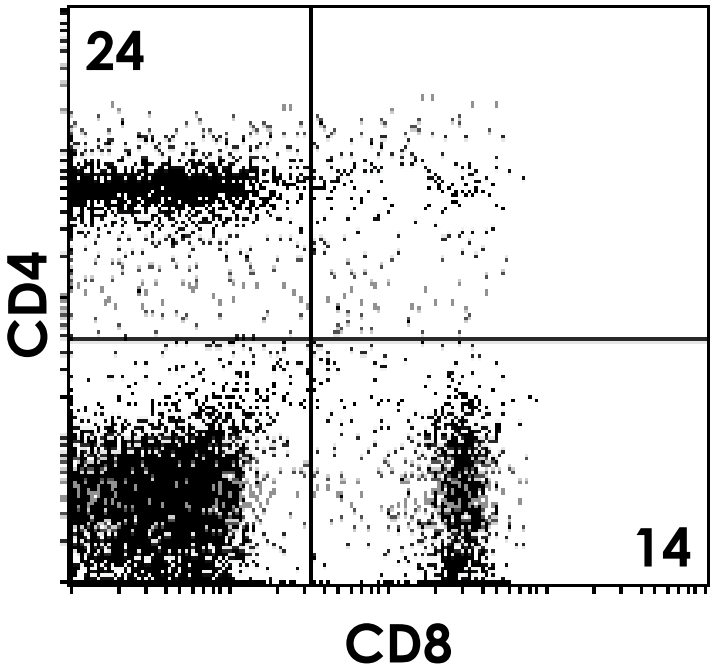
## Two colors (can measure >10 colors)



# Example FACS plot:

## $CD4^+$ and $CD8^+$ T cells from a mouse spleen

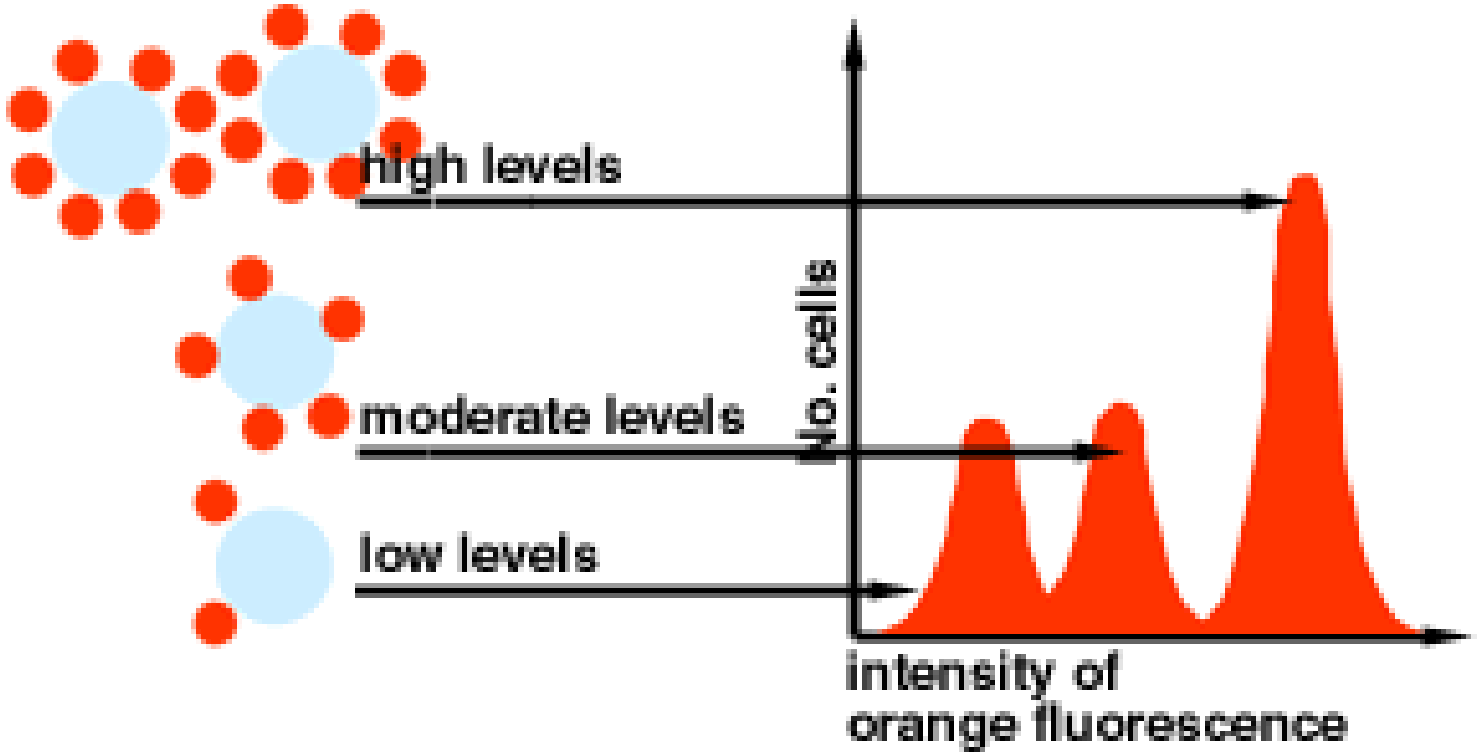



| | |
|---|---|
| CD4pos<br>CD8neg | CD4pos<br>CD8pos |
| CD4neg<br>CD8neg | CD4neg<br>CD8pos |

# Fluorescence Activated Cell Sorting



## FACS



# A Hematopoeitic Stem Cell Generates All Cells of the Immune System