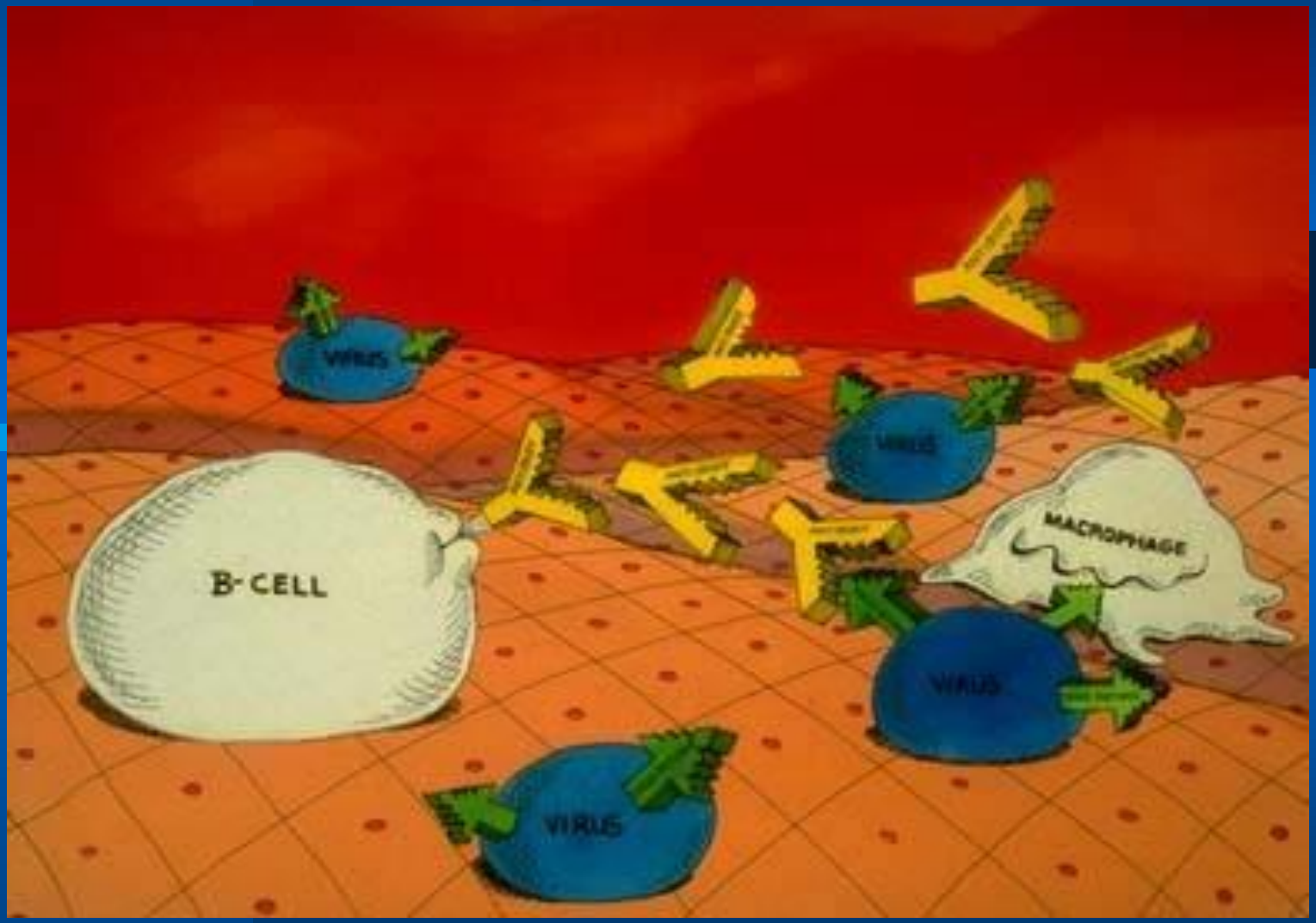
Immune Response



George Ioannidis, Cons Haem

Immunity

"The ability of the body to defend itself from invading microorganisms and damage by foreign substances"

Immunity
First Line of Defense

- Physical Barriers
- Mechanical Barriers
- Normal Bacterial Flora
- Chemical Barriers
- Antimicrobial Substances

Immunity
Second Line of Defense (innate)

- Inflammation
  - Clotting system
  - Complement system
  - Kinin system
  - Phagocytes

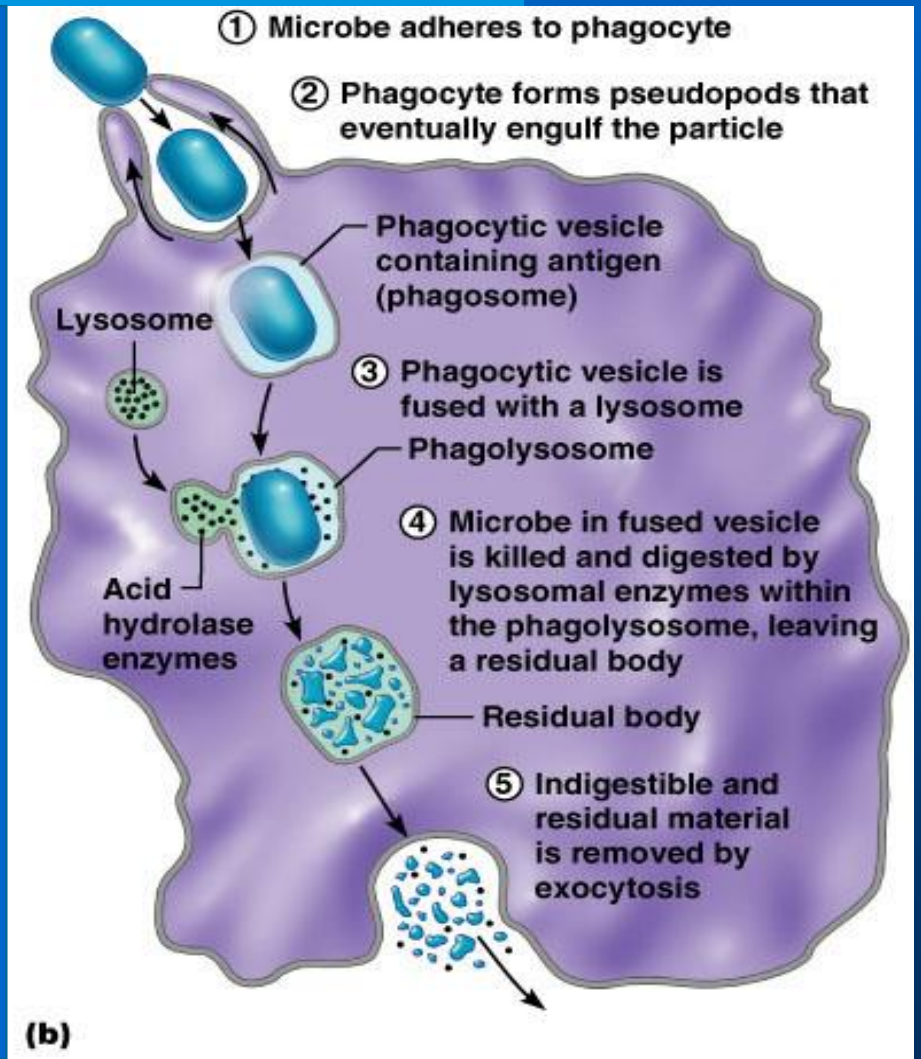
TABLE 1-2 SUMMARY OF NONSPECIFIC HOST DEFENSES

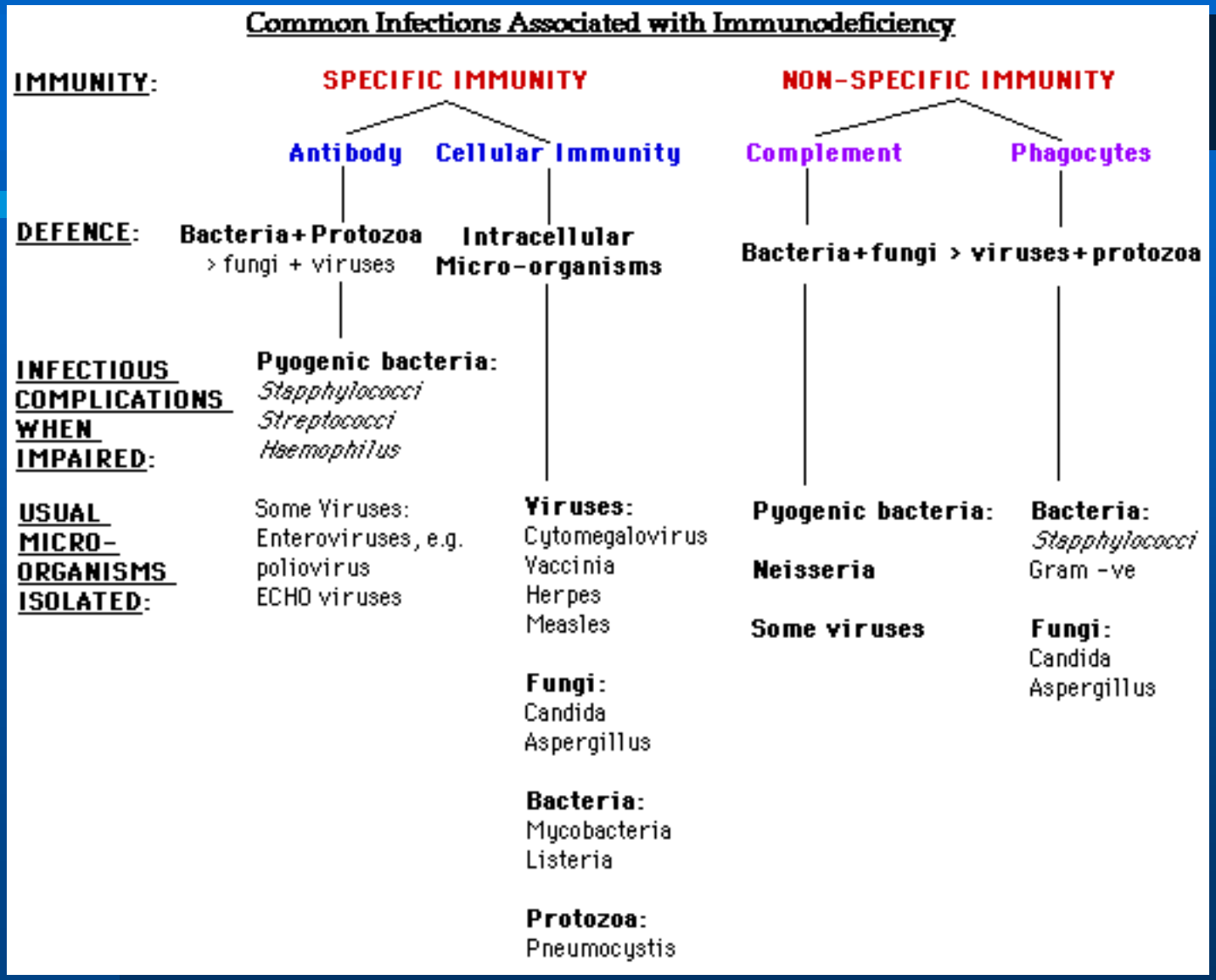
Type	Mechanism
Anatomic barriers	
Skin	Mechanical barrier retards entry of microbes. Acidic environment (pH 3–5) retards growth of microbes.
Mucous membranes	Normal flora compete with microbes for attachment sites and nutrients. Mucus entraps foreign microorganisms. Cilia propel microorganisms out of body.
Physiologic barriers	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.
Low pH	Acidity of stomach contents kills most ingested microorganisms.
Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells. Complement lyses microorganisms or facilitates phagocytosis.
Phagocytic/endocytic barriers	Various cells internalize (endocytose) and break down foreign macromolecules. Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.
Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

Kuby Immunology, page 7

- Neutrophils
  1. Stored in the BM and rapidly released in the bloodstream in response to infection
  2. Surface receptors for IgG,IgA and complement components
  3. Phagocytosis and destruction of bacteria
- Basophils & mast cells
  1. Basophils circulate, mast cells are tissue bound
  2. Surface receptors for C3,C5 and IgE
  3. Produce histamine,PGs,LTR, and proteases
  4. Involved in response to proteases
  5. Interaction of Ag with bound IgE produces immediate hypersensitivity
- Eosinophils
  1. Commonly in pts with allergic disease
  2. Surface receptors for IgG,C3,C5
  3. Also bind IgE but less avidly than mast cells or basophils
  4. Phagocytose Ag-Ab complexes

Mechanism of phagocytosis




Common Infections Associated with Immunodeficiency
IMMUNITY:
SPECIFIC IMMUNITY
NON-SPECIFIC IMMUNITY
Antibody
Cellular Immunity
Complement
Phagocytes
DEFENCE:
Bacteria+Protozoa
> fungi + viruses
Intracellular
Micro-organisms
Bacteria+fungi > viruses+protozoa
INFECTIOUS COMPLICATIONS WHEN IMPAIRED:
Pyogenic bacteria:
Staphylococci
Streptococci
Haemophilus
USUAL MICRO-ORGANISMS ISOLATED:
Some Viruses:
Enteroviruses, e.g.
poliovirus
ECHO viruses
Viruses:
Cytomegalovirus
Vaccinia
Herpes
Measles
Fungi:
Candida
Aspergillus
Bacteria:
Mycobacteria
Listeria
Protozoa:
Pneumocystis
Pyogenic bacteria:
Neisseria
Some viruses
Bacteria:
Staphylococci
Gram -ve
Fungi:
Candida
Aspergillus

Innate and Adaptive Immunity

	Innate	***Adaptive***
Encoding of Receptors	**germline**	**somatically**
Distribution of receptors	**non-clonal**	**clonal**
Repertoire of receptors	**limited**	**very large**
Target	**invariant**	**variable**
Recognition	**perfect**	**imperfect**
Speed	**fast**	**slow**
Long-lasting memory	**no**	**yes**
Specificity	**no/yes**	**yes**

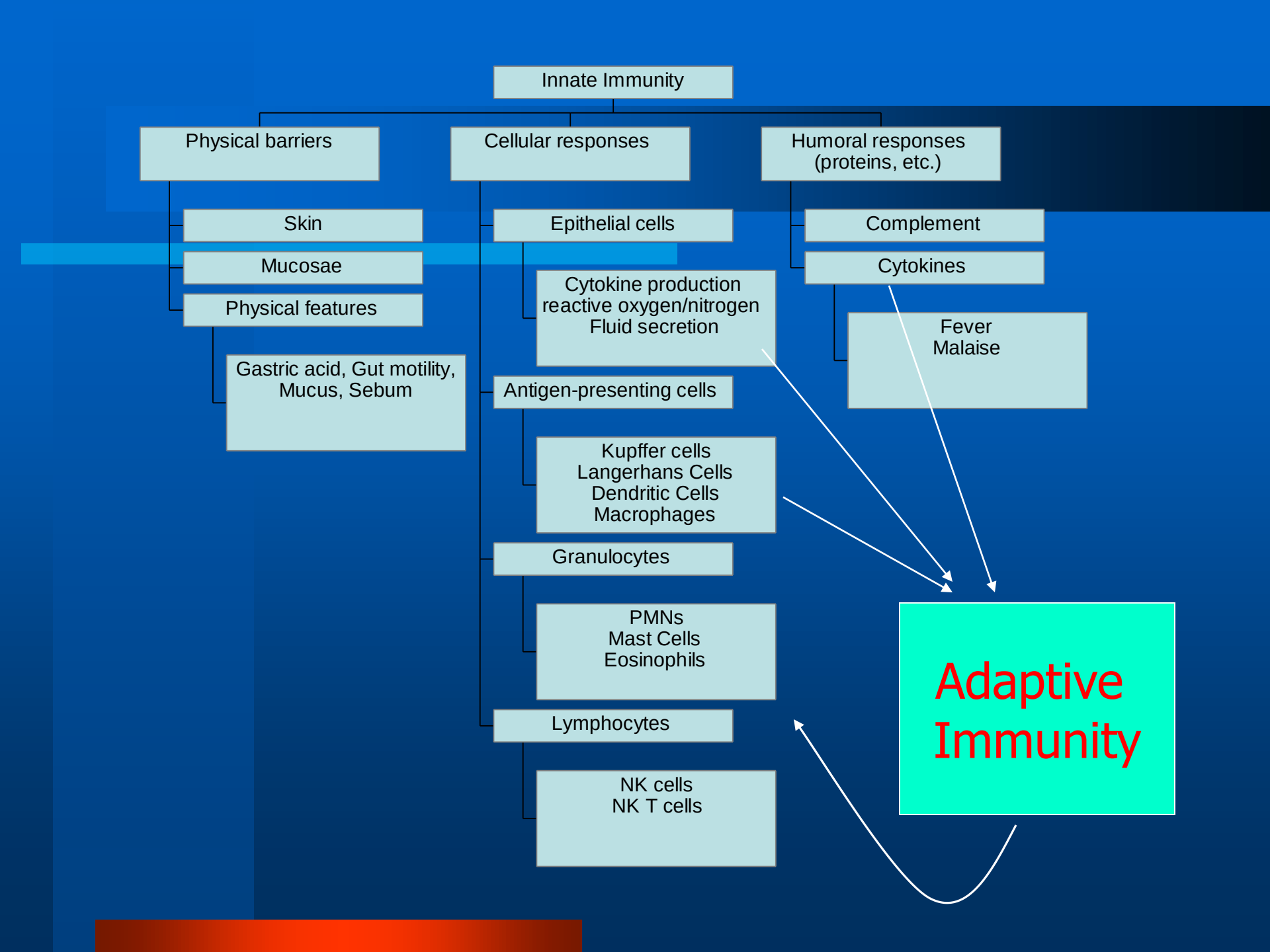
Cross-talk between Innate and Adaptive Immunity

- Induction of an immune response is only appropriate if the antigen recognized is derived from, or belongs to, a pathogen.
- Activation of lymphocytes specific for self antigens, or innocent persistent environmental antigens, is deleterious.

Therefore,

- The adaptive immune response requires signals that provide information about the origin of the antigen and the type of response to be induced.

These signals are provided by the innate immune system.


Innate Immunity
Physical barriers
Cellular responses
Humoral responses (proteins, etc.)
Skin
Mucosae
Physical features
Gastric acid, Gut motility, Mucus, Sebum
Epithelial cells
Cytokine production
reactive oxygen/nitrogen
Fluid secretion
Antigen-presenting cells
Kupffer cells
Langerhans Cells
Dendritic Cells
Macrophages
Granulocytes
PMNs
Mast Cells
Eosinophils
Lymphocytes
NK cells
NK T cells
Complement
Cytokines
Fever
Malaise
Adaptive Immunity

Receptors & Targets of Innate Recognition

- The targets of recognition represent molecular patterns, called PAMPs for pathogen-associated molecular patterns, rather than particular structures.

- Host organisms have developed a set of receptors that can specifically recognize PAMPs and are referred to, therefore, as pattern recognition receptors (PRRs).

Innate Immunity - Basic features

- The ability of the innate immune system to discriminate between self and nonself is perfect.
- None of the compounds recognized by the innate immune system are produced by the host organism, and all of them are essential for the physiology and survival of the respective microbes.

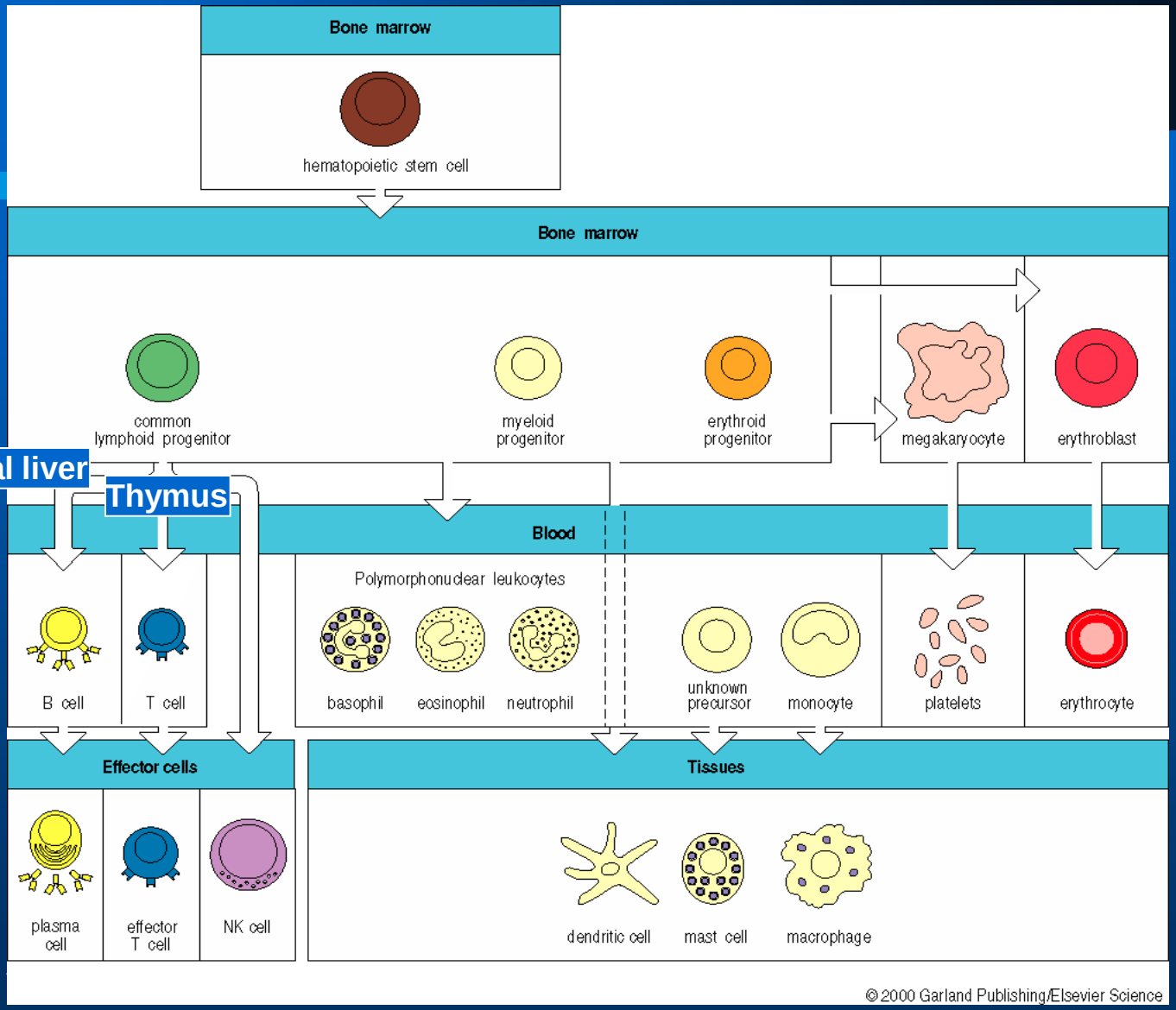
MHC Proteins - Introductory

- Our cells are dotted with protein molecules (self-antigens) that are not antigenic to us but are strongly antigenic to others
- One type of these, MHC proteins, mark a cell as self
- The two classes of MHC proteins are:
  - Class I MHC proteins – found on virtually all body cells
  - Class II MHC proteins – found on certain cells in the immune response

Immunity
Third Line of Defense (adaptive)

- Humoral Immunity (B cells)
  - production of specific antibodies
- Cell Mediated Immunity (T cells)
  - direct cell destruction

Cells of the immune system



Bone marrow/fetal liver

Thymus

Naïve, resting lymphocytes in lymph nodes, spleen

Adaptive

Innate

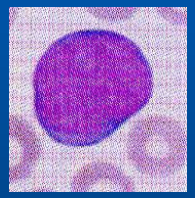
Lymphocytes

- T-lymphocytes
- B-lymphocytes
- Natural killer cells
- LGL?

Lymphocyte Development

Common Lymphoid Progenitor (CLP)

B-Lymphoblast



T-Lymphoblast

Prolymphocyte

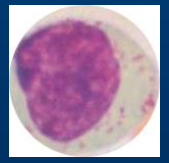
Thymocyte

T-Helper T-Killer

B-Lymphocytes

T-Lymphocytes

NK-Cells

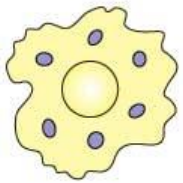
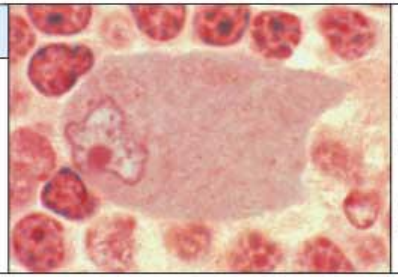
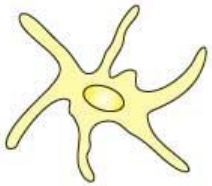
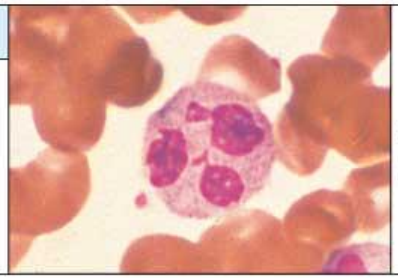


Plasma cell

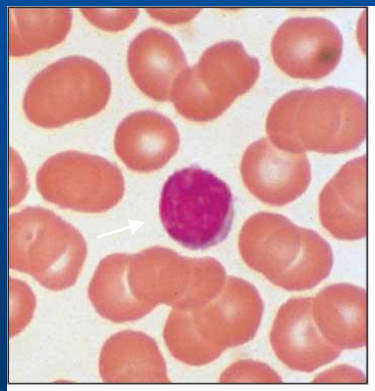
T_H-Ly

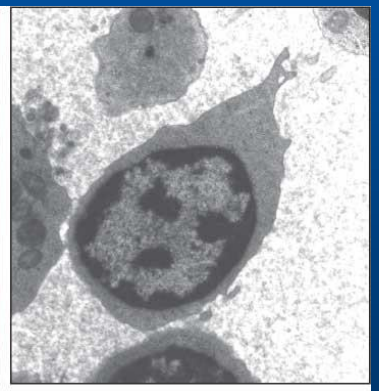
T_C-Ly (CTL)

Unlike other leucocytes, lymphocytes are morphologically nondescript

Cell		Activated function
Macrophage 		Phagocytosis and activation of bactericidal mechanisms Antigen presentation
Dendritic cell 		Antigen uptake in peripheral sites Antigen presentation in lymph nodes
Neutrophil 		Phagocytosis and of activation bactericidal mechanisms

Resting lymphocyte





LGL

- 5-15% of blood lympocytes
- Morphologically defined cells containing large amounts of cytoplasm with azurophilic granules.
- Both NK cells an $\gamma\delta$T cells have this morphology

T-lymphocytes

- 70-80% of total population
- Intracellular infections,tumour surveillance and graft rejection
- CD3 (part of the T-cell receptor) is present on all T cells
- CD4 is a Gp,recognizes MHC-II Ags on APCs*.Present on the cell surface of Th and monocytes
- CD8 is a Gp on CTL,recognizes class I Ags on target cells
- Orchestrate the immune response

APCs:DCs,macrophages,B_a cells

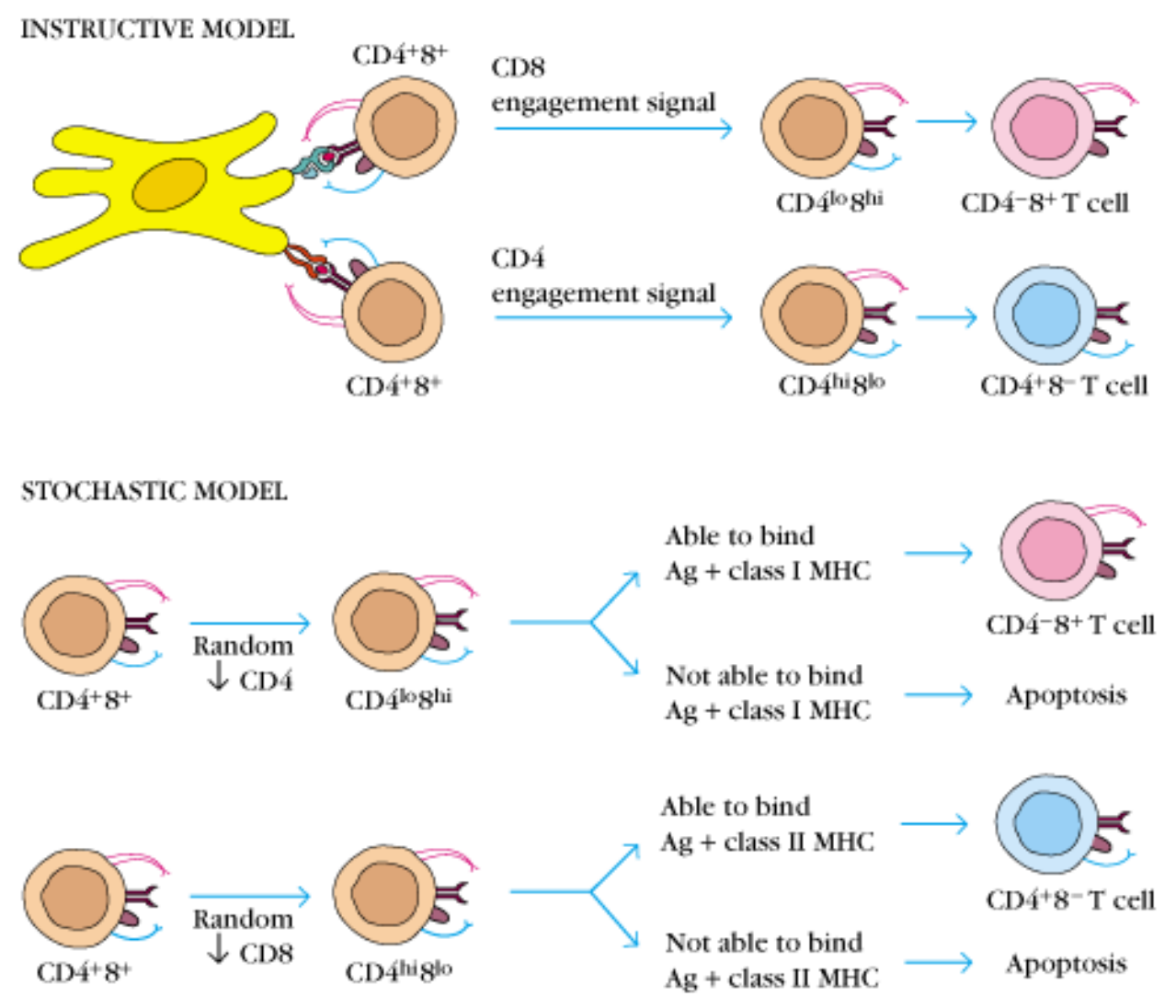
CD4

- 60% of T-cell population
- Help B-cell differentiation and type IV hypersensitivity
- Th_1: recognize Ags presented by macrophages. Activated secrete IL-2 & IFN.Produce type IV CM immunity. Suppressed by IL-10
- Th_2: recognize Ags presented by B-cells. Activated secrete IL-4,5,6,10 causing B differentiation, proliferation and secretion IgG,IgA,IgE contributing to type II & II immunity. Suppressed by IFN

CD8

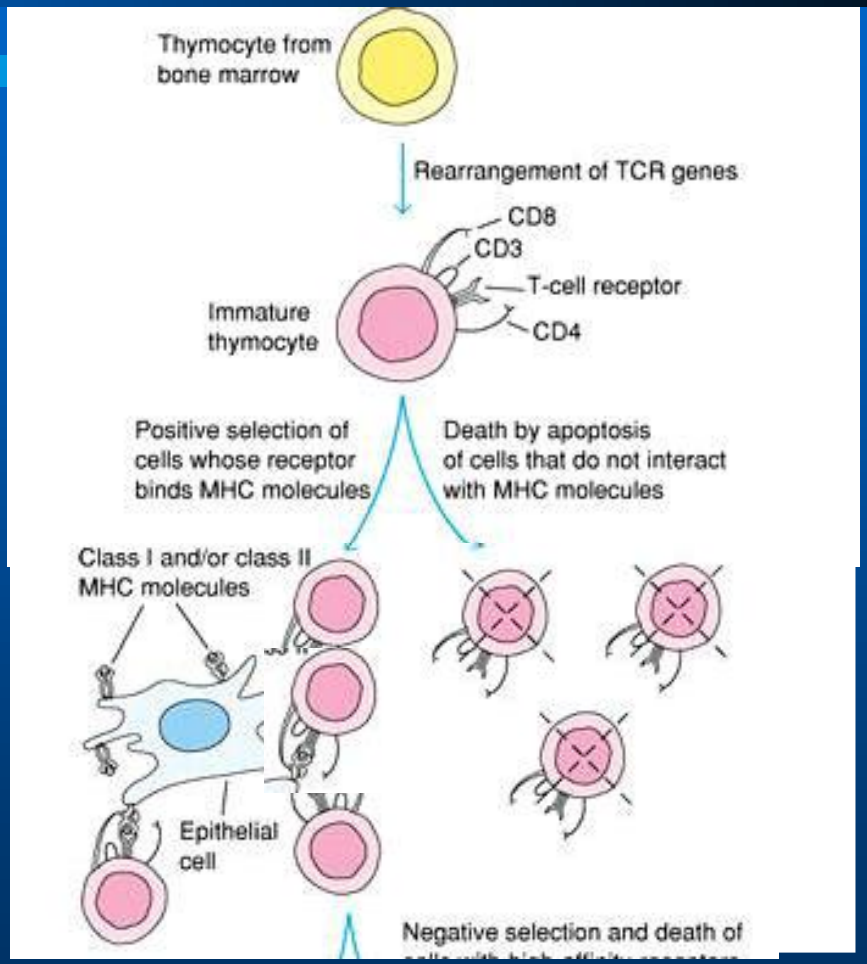
- Cytotoxic cells,recognize Ag presented with class I MHC Ag
- 35% of circulating T population
- Important in eliminating cells infected by viruses

How do double-positive thymocytes decide to become CD4+8- or CD4-8+ ? The instructional model and the stochastic model



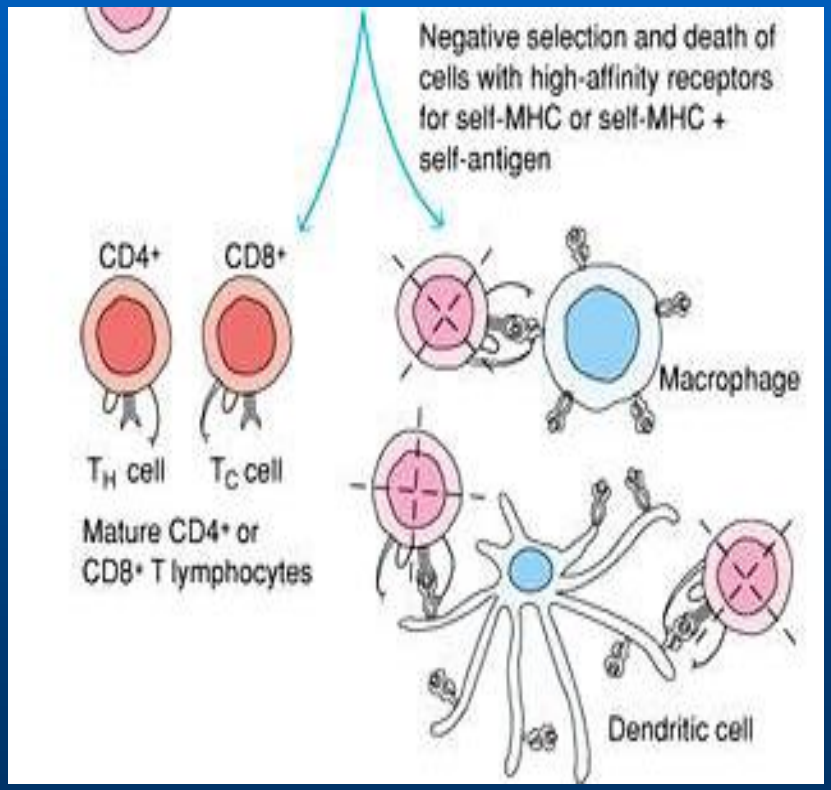
Steps in T cell development

Step 1. Positive selection
occurs in the thymic cortex

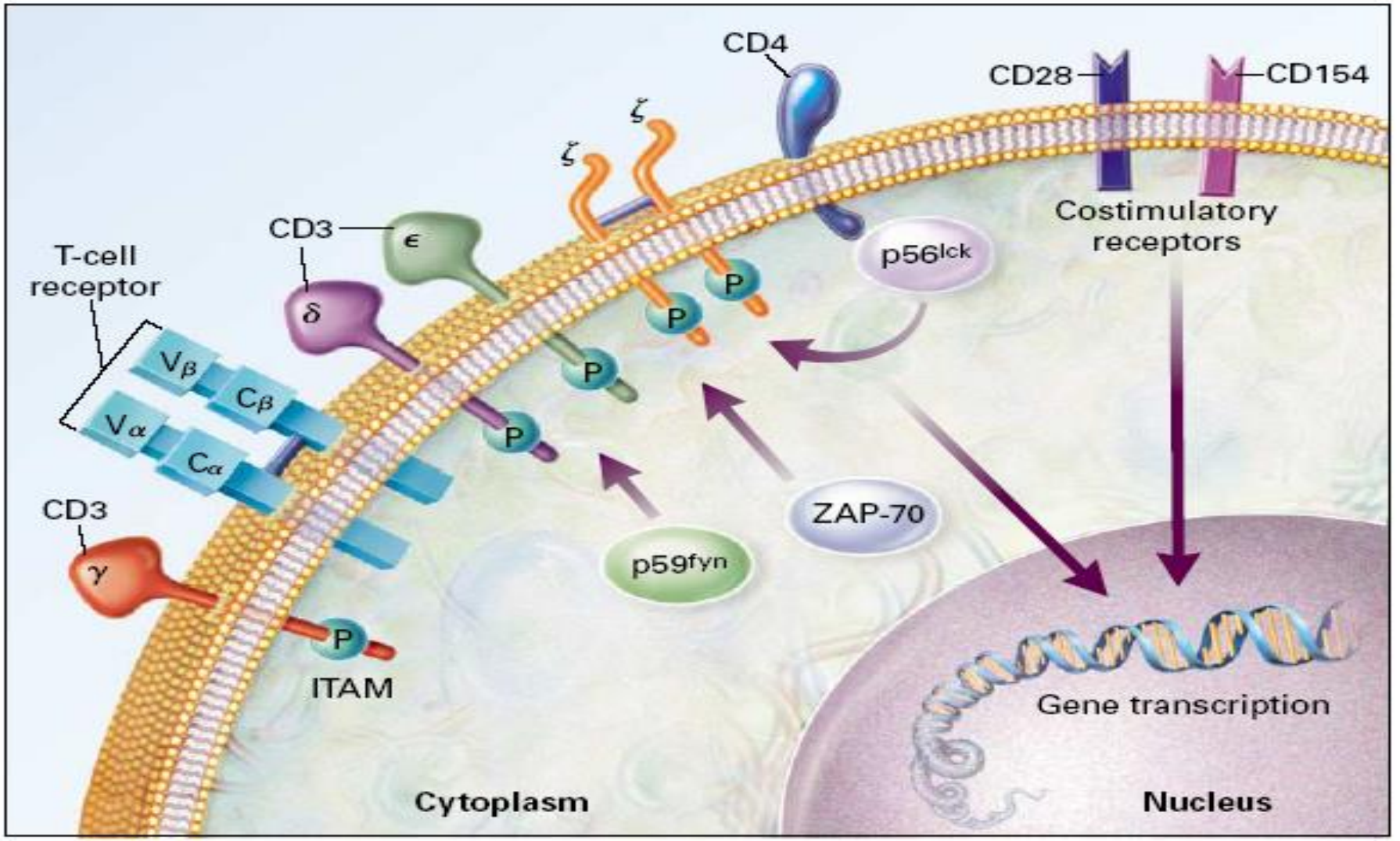


Steps in T cell development-2

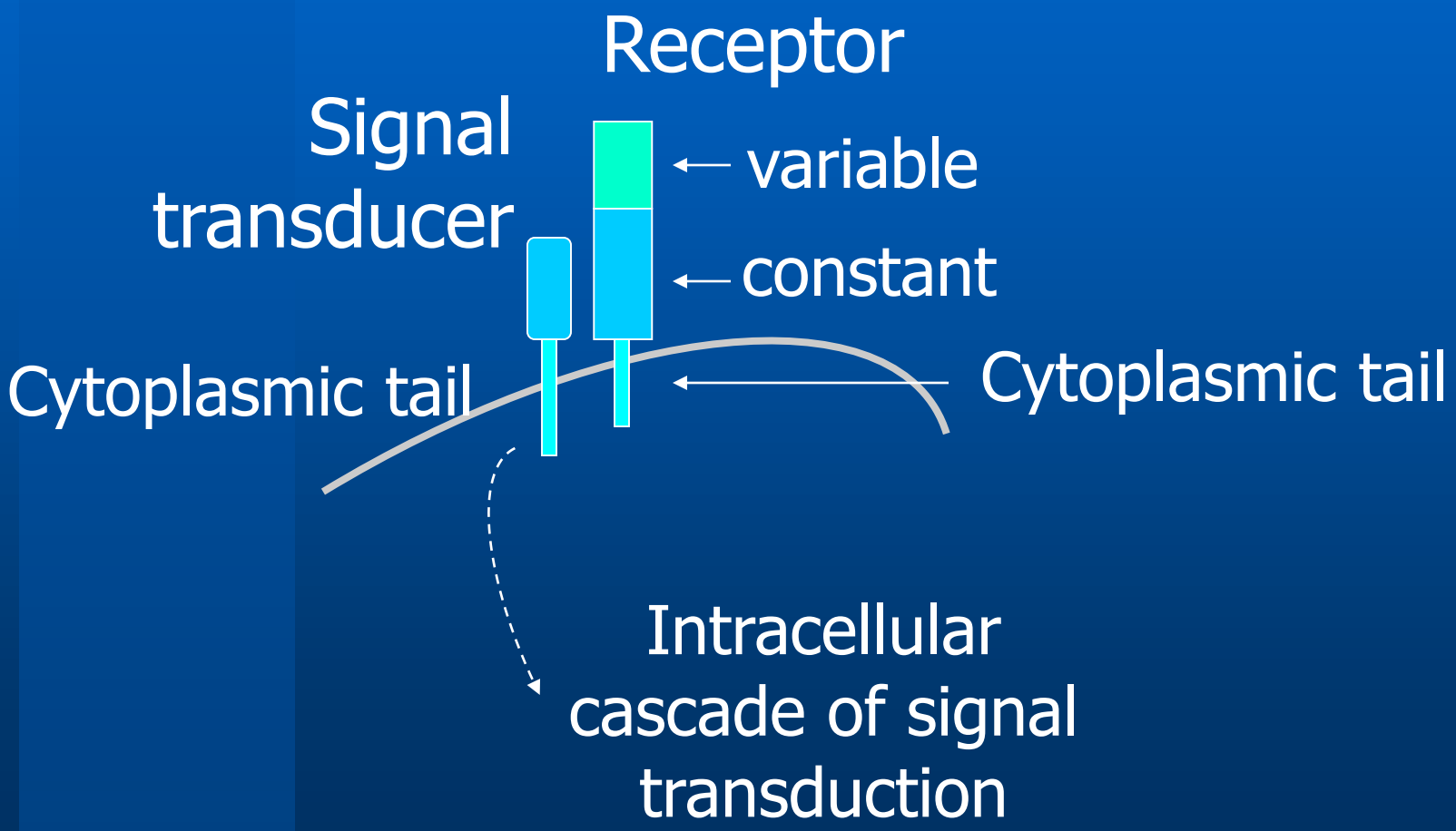
Step 2. Negative selection occurs in the thymic medulla.



T Cell Activation



The General Principle of an Antigen Receptor



Receptor Clustering Results in Phosphorylation Events

- Conformation changes
- Cytoplasmic domains can now associate with intracellular molecules that provide initial phosphorylation

Protein Phosphorylation

- Addition of phosphate group to an amino acid by kinases
  - Tyrosine in early activation events
  - Serine and threonine in later events
- Enzyme activation
- Creation of binding sites for other proteins (adaptor proteins)
- Quick
- Reversible through phosphatases

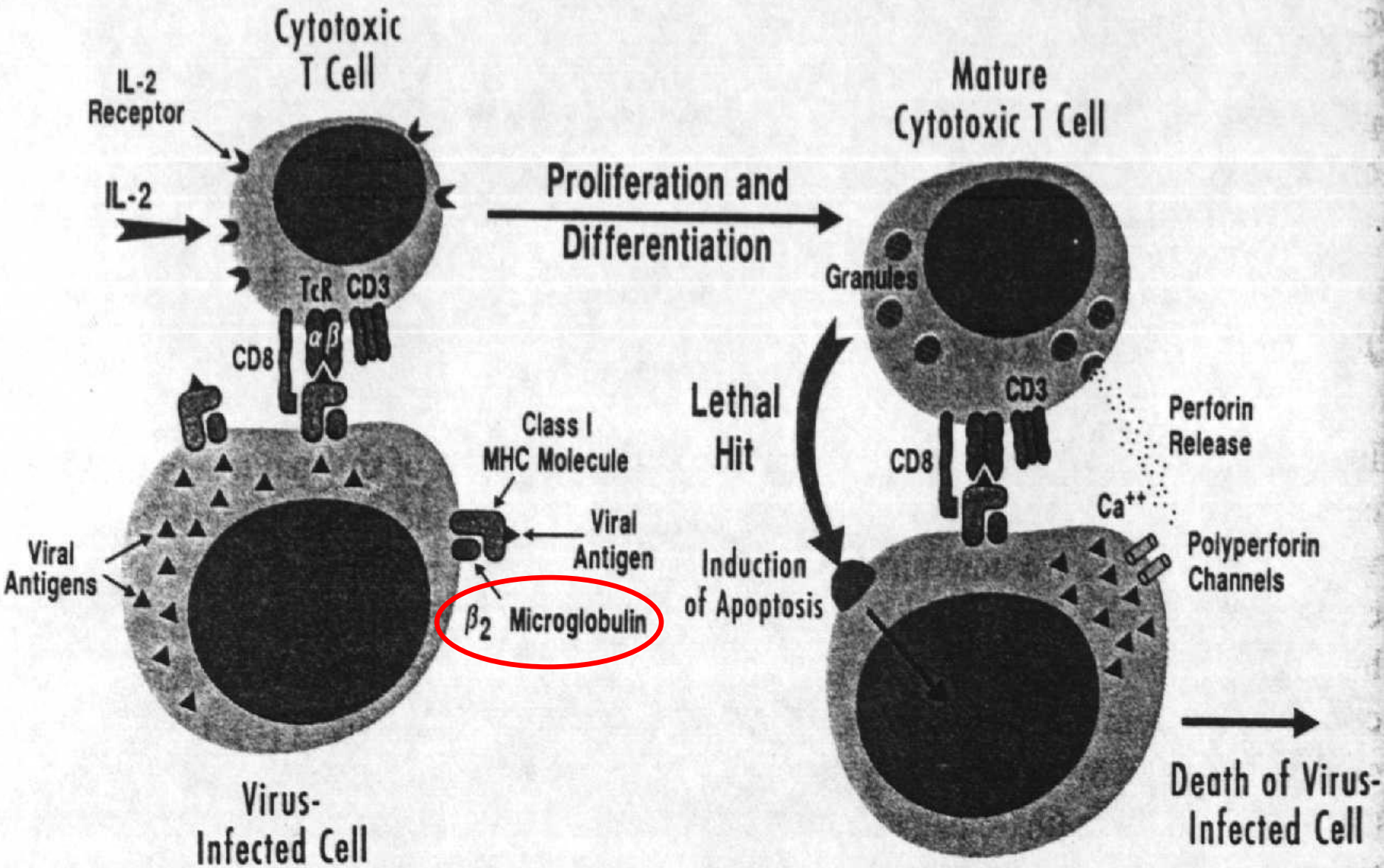


Figure 11.4

Recognition and killing by CD8+ cytotoxic T cells.

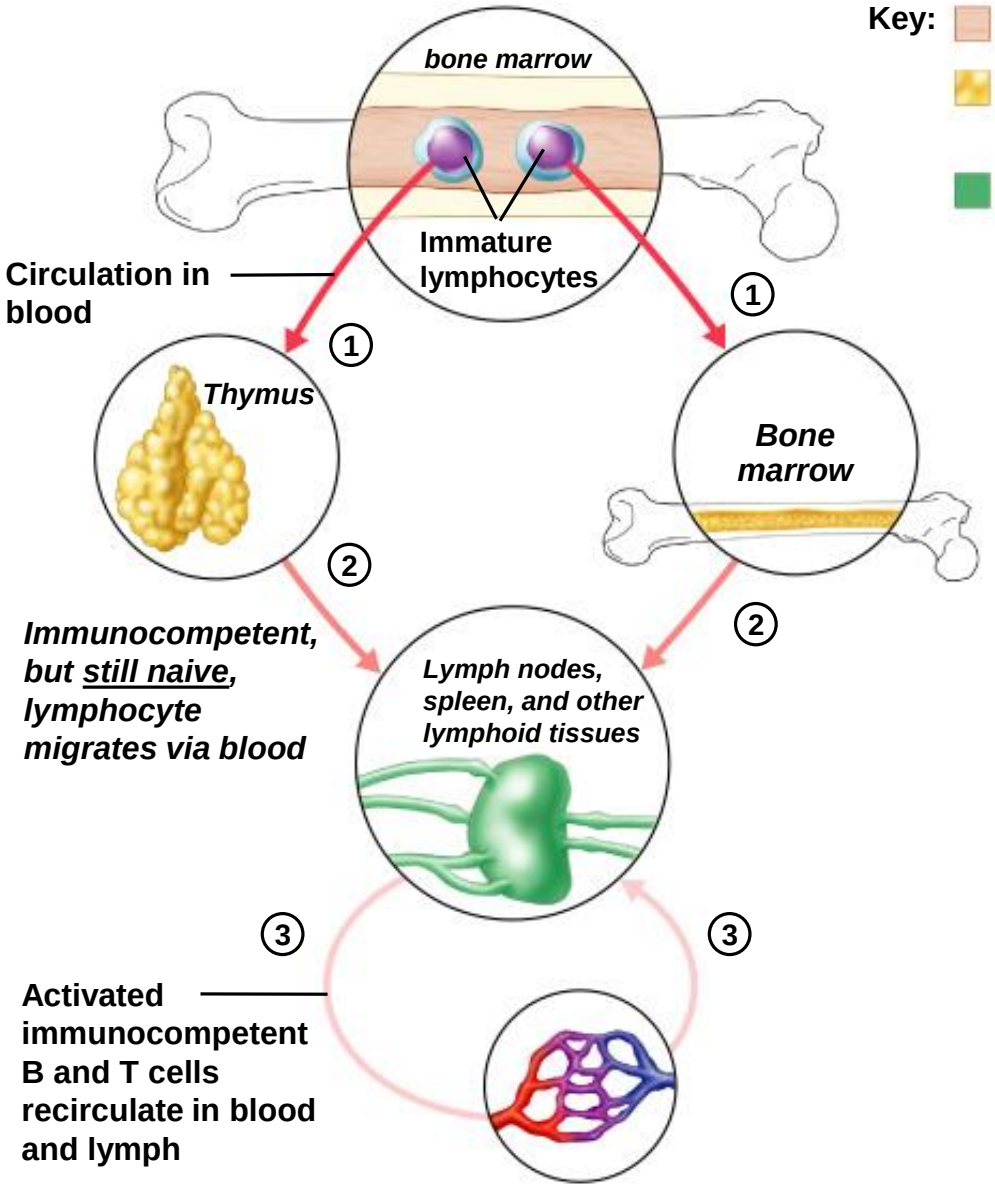
B lymphocytes

- Develop in BM with final maturation in spleen and LNs
- Ig is expressed on cell surface. Immature cells cannot secrete Ab
- Activation requires both Ag and Th2. Some Ags induce activation without the need for Th2,but without collaboration, low affinity Abs are produced and memory is poor

B lymphocytes

- B cells become immunocompetent and self-tolerant in bone marrow
- Some self-reactive B cells are inactivated (anergy) while others are killed
- Other B cells undergo receptor editing in which there is a rearrangement of their receptors

Immunocompetent B or T cells



Key:

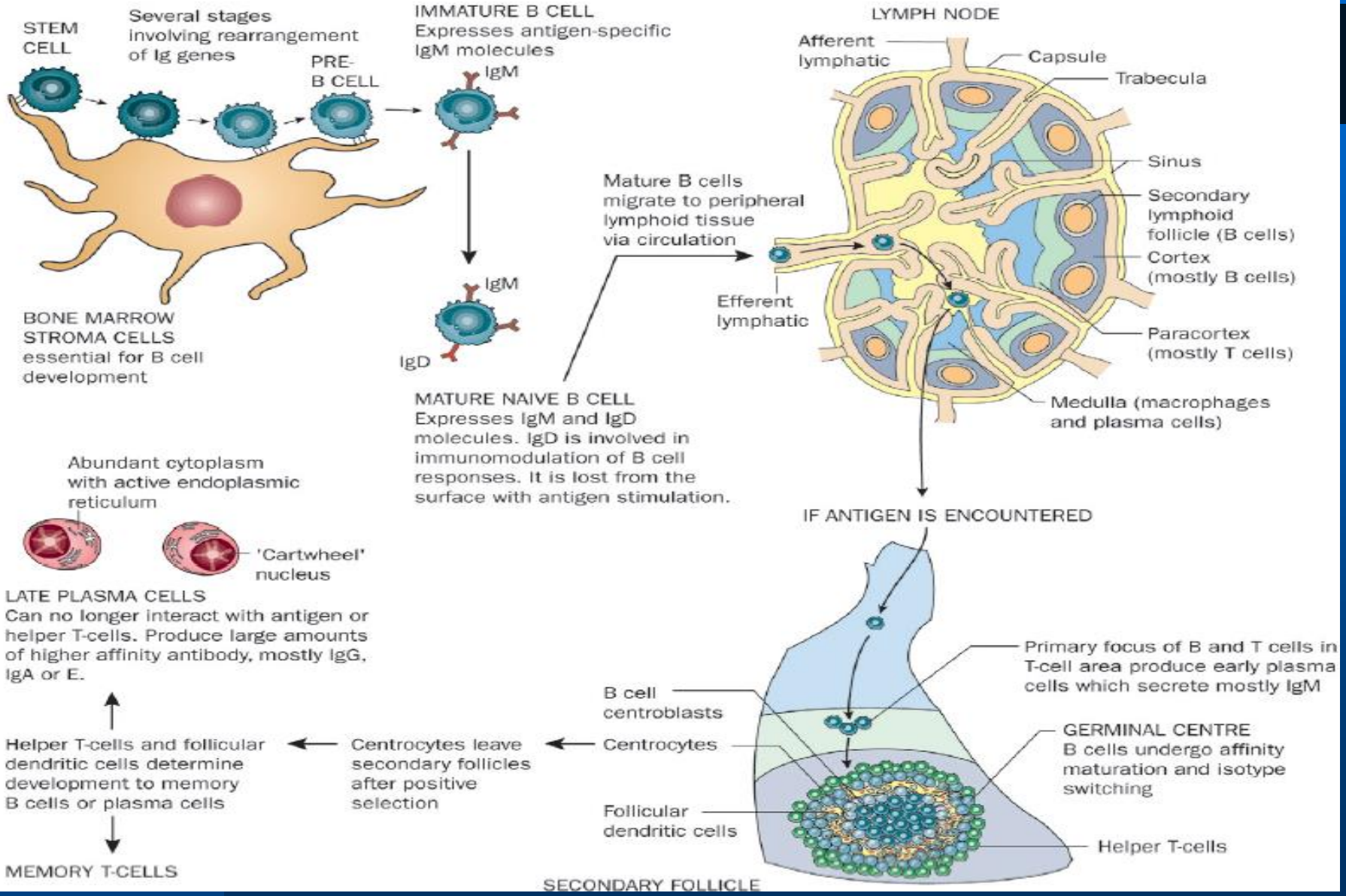
- = Site of lymphocyte origin
- = Site of development of immunocompetence as B or T cells; primary lymphoid organs
- = Site of antigen challenge and final differentiation to activated B and T cells

1. Lymphocytes destined to become T cells migrate to the thymus and develop immunocompetence there. B cells develop immunocompetence in red bone marrow.

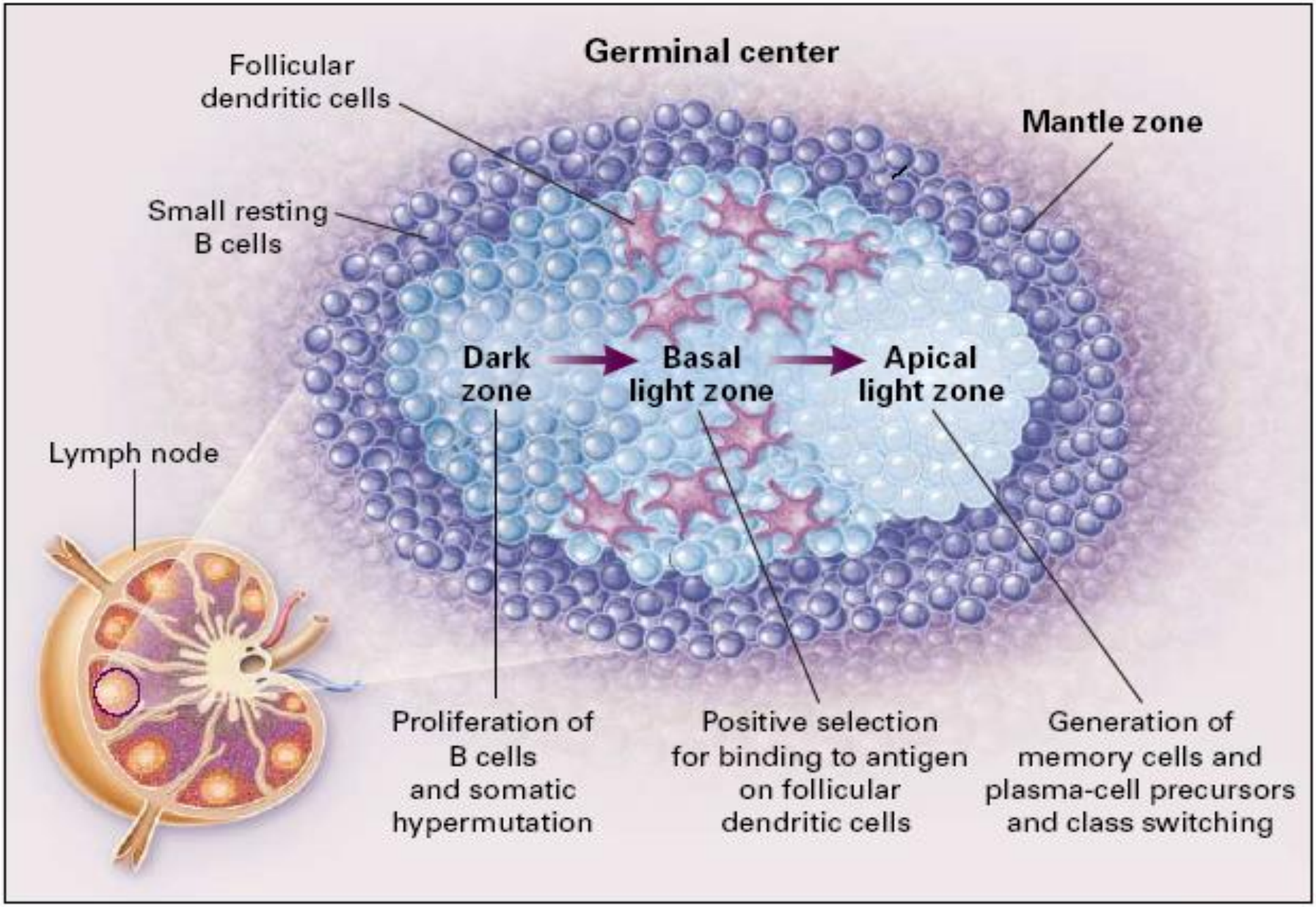
2. After leaving the thymus or bone marrow as naive immunocompetent cells, lymphocytes "seed" the lymph nodes, spleen, and other lymphoid tissues where the antigen challenge occurs.

3. Mature (antigen-activated) immunocompetent lymphocytes circulate continuously in the bloodstream and lymph and throughout the lymphoid organs of the body.

The pathway of B lymphocyte development

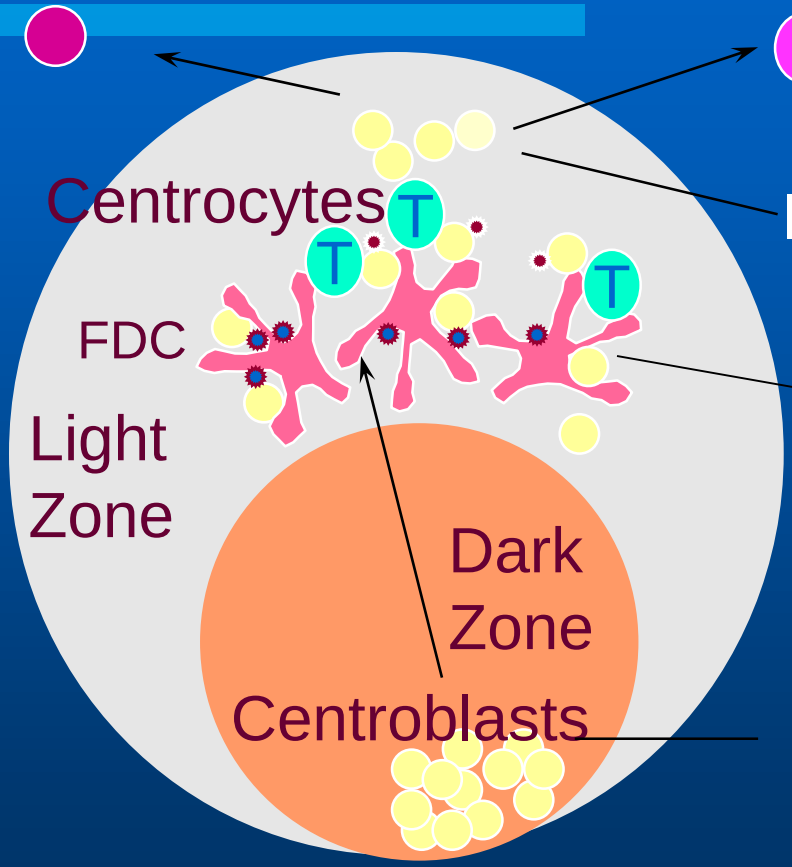


Parkin J. Lancet 2001; 357: 1777–89


Germinal center
Follicular dendritic cells
Mantle zone
Small resting B cells
Dark zone
Basal light zone
Apical light zone
Lymph node
Proliferation of B cells and somatic hypermutation
Positive selection for binding to antigen on follicular dendritic cells
Generation of memory cells and plasma-cell precursors and class switching

B Cell Development in Germinal Centers

Plasma Cell
(long lived)



Memory B Cell

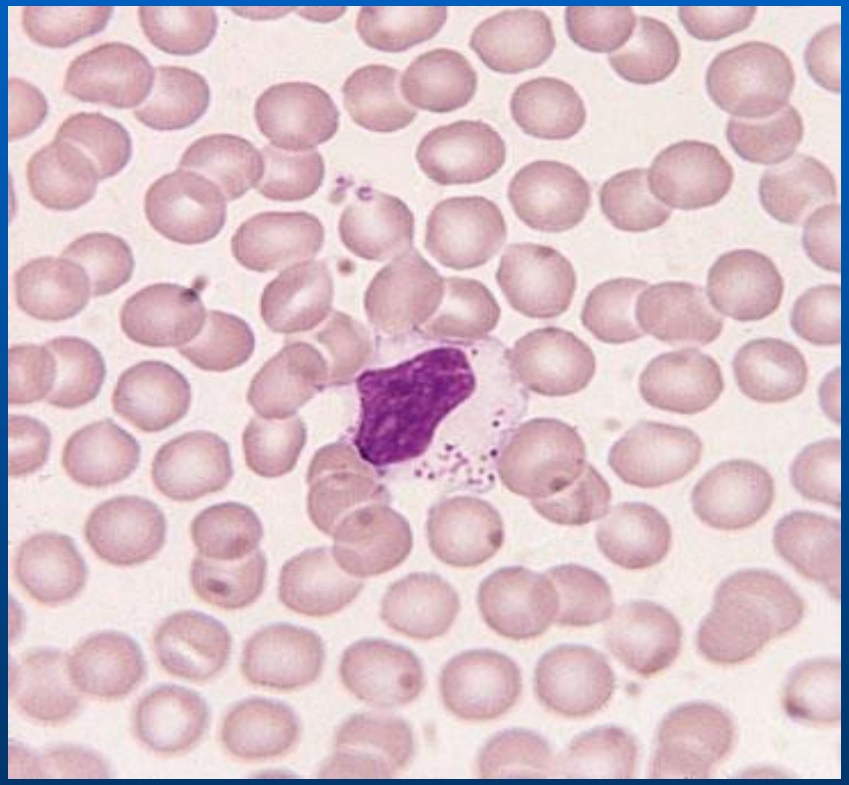
Isotype Switching

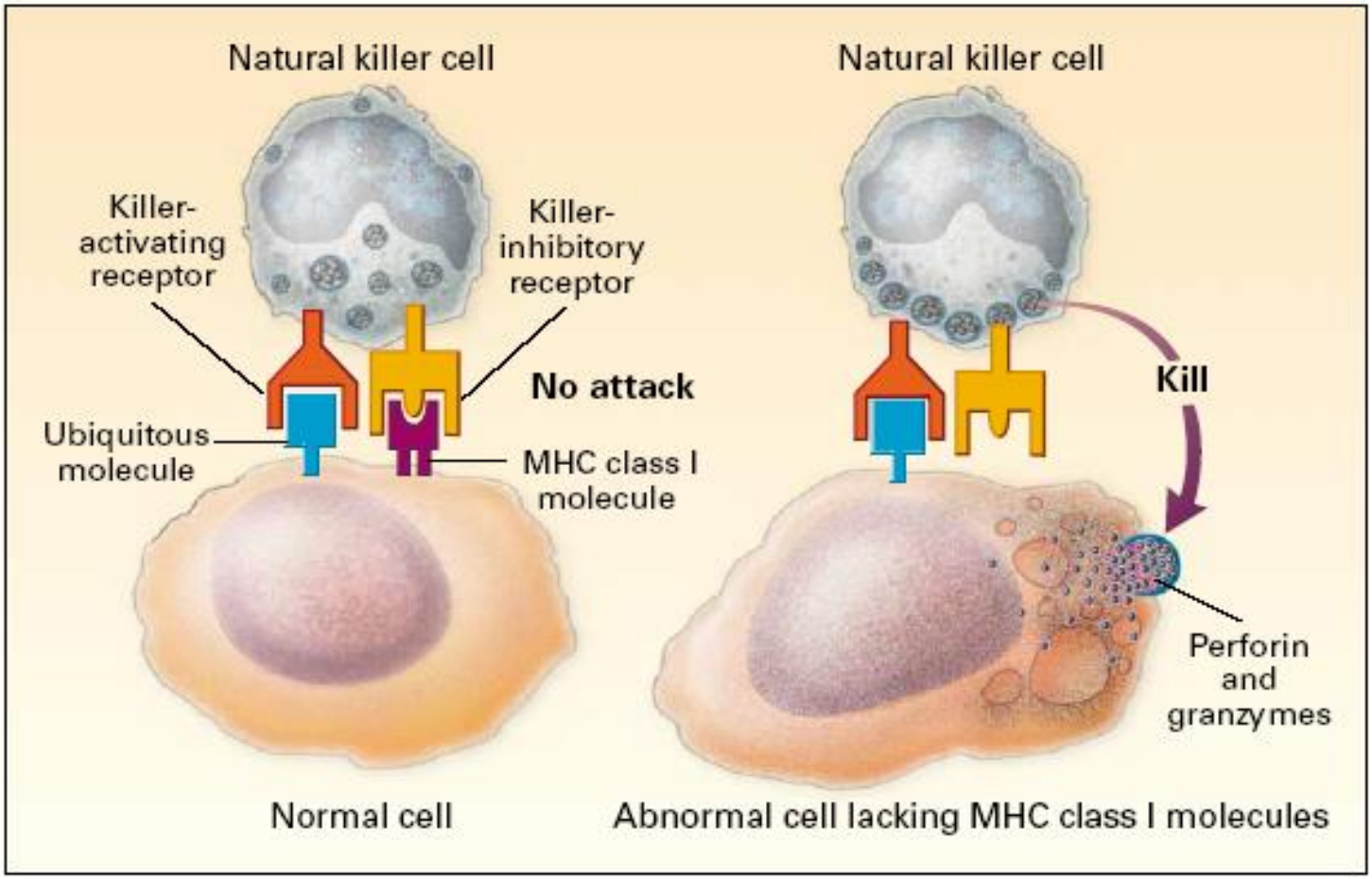
Affinity Maturation
Selection/Apoptosis

Cell Proliferation
Telomere Maintenance
V Gene Hypermutation

Natural Killer (NK) cells

- Large granular lymphocytes
- Recognize and lyse cells bearing viral or tumour surface markers



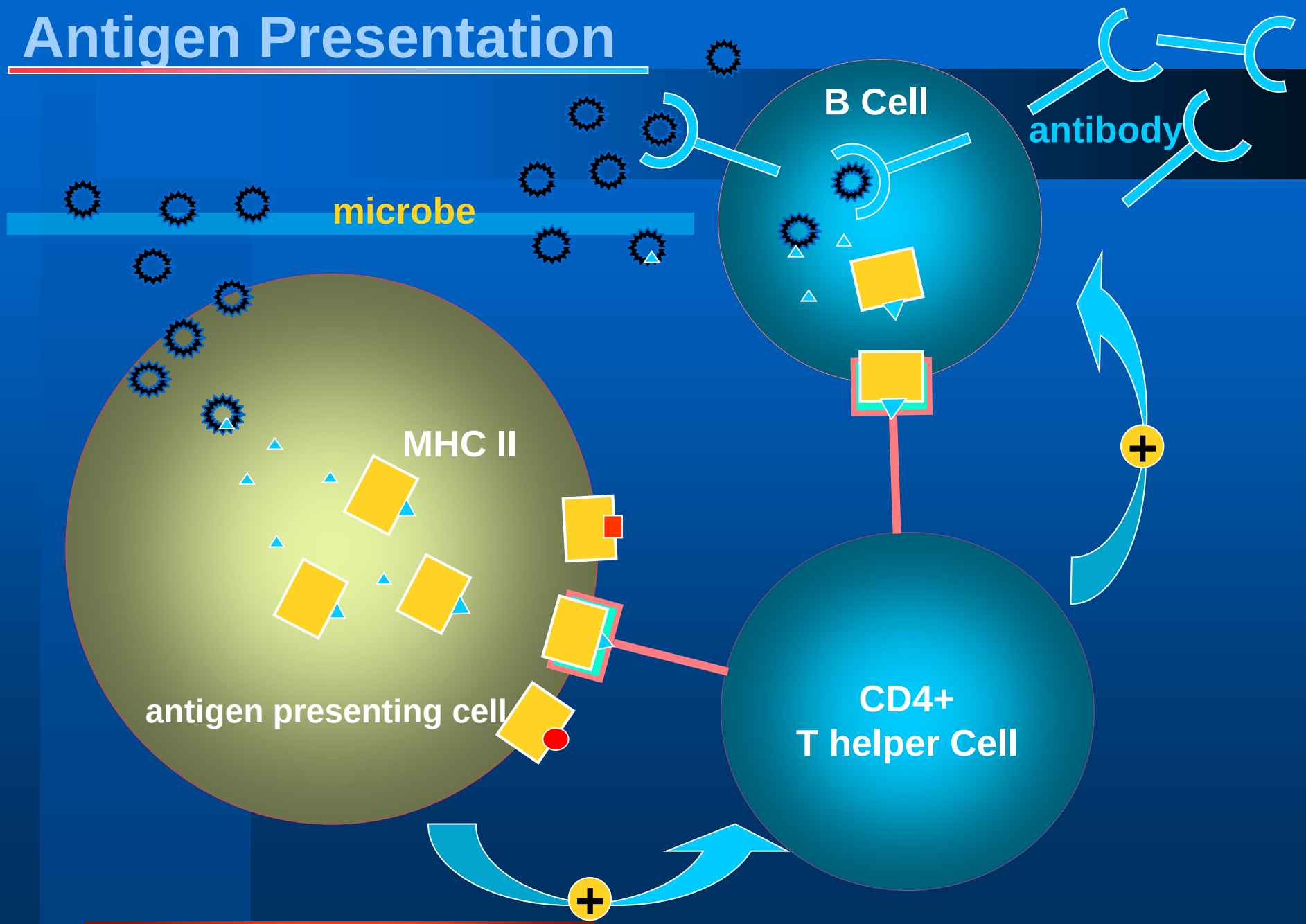


A System Used by Natural Killer Cells to Recognize Normal Cells and Cells That Lack Major-Histocompatibility-Class I Surface Molecules.

Killer-activating receptors recognize a number of molecules present on the surface of normal, nucleated cells, and in the absence of an inhibitory signal from killer-inhibitory receptors, which recognize major-histocompatibility-complex (MHC) class I molecules, the receptors issue an order to the natural killer cells to attack and kill the other cell. The cytotoxic granules of the natural killer cells, which contain perforin and granzymes, become polarized at the interface with the target cell and are then released into the cell.

Delves PJ. NEJM 2000;343:37

Antigen Presentation



Dendritic Cells Initiate Adaptive Immune Responses

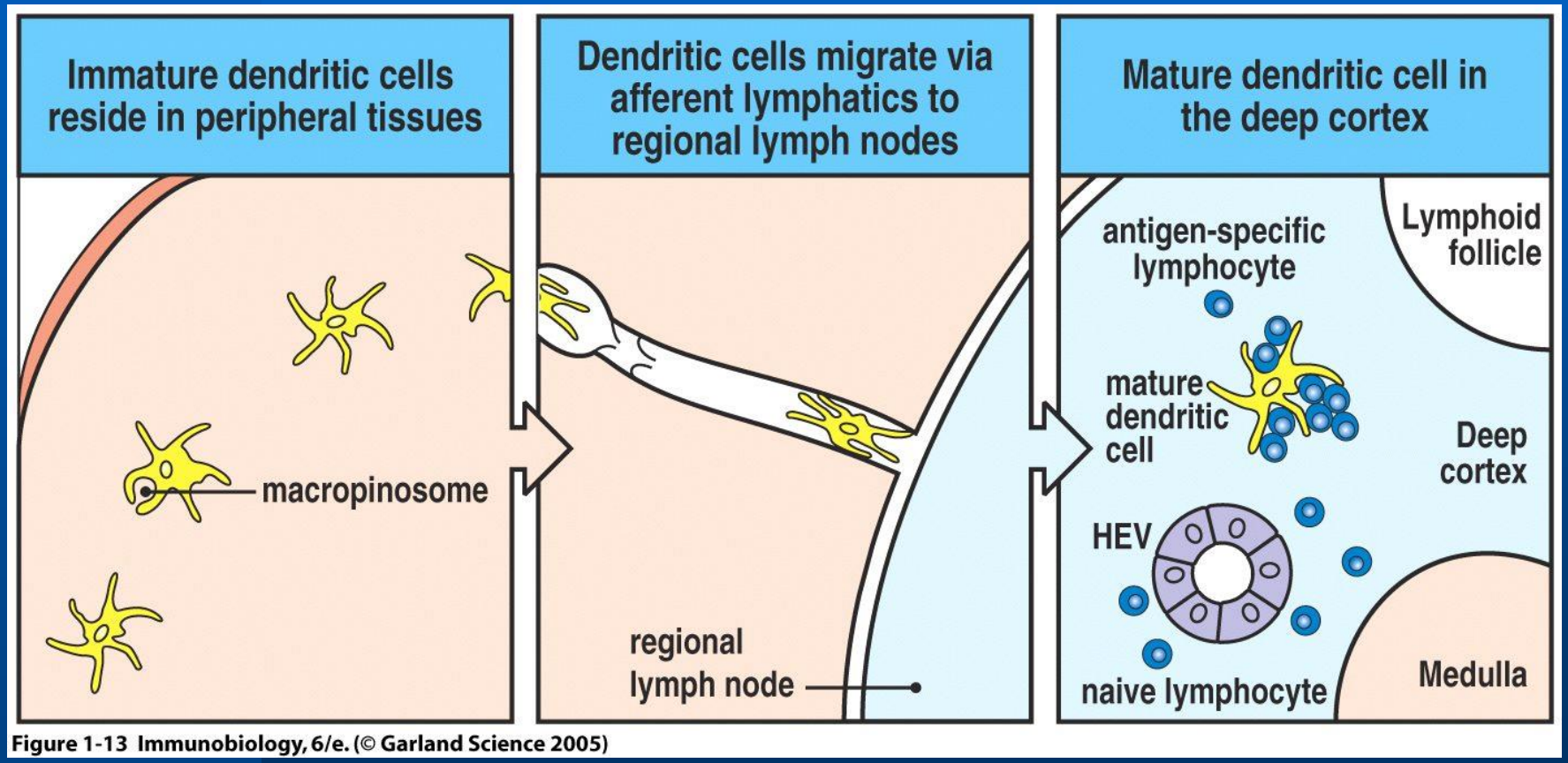
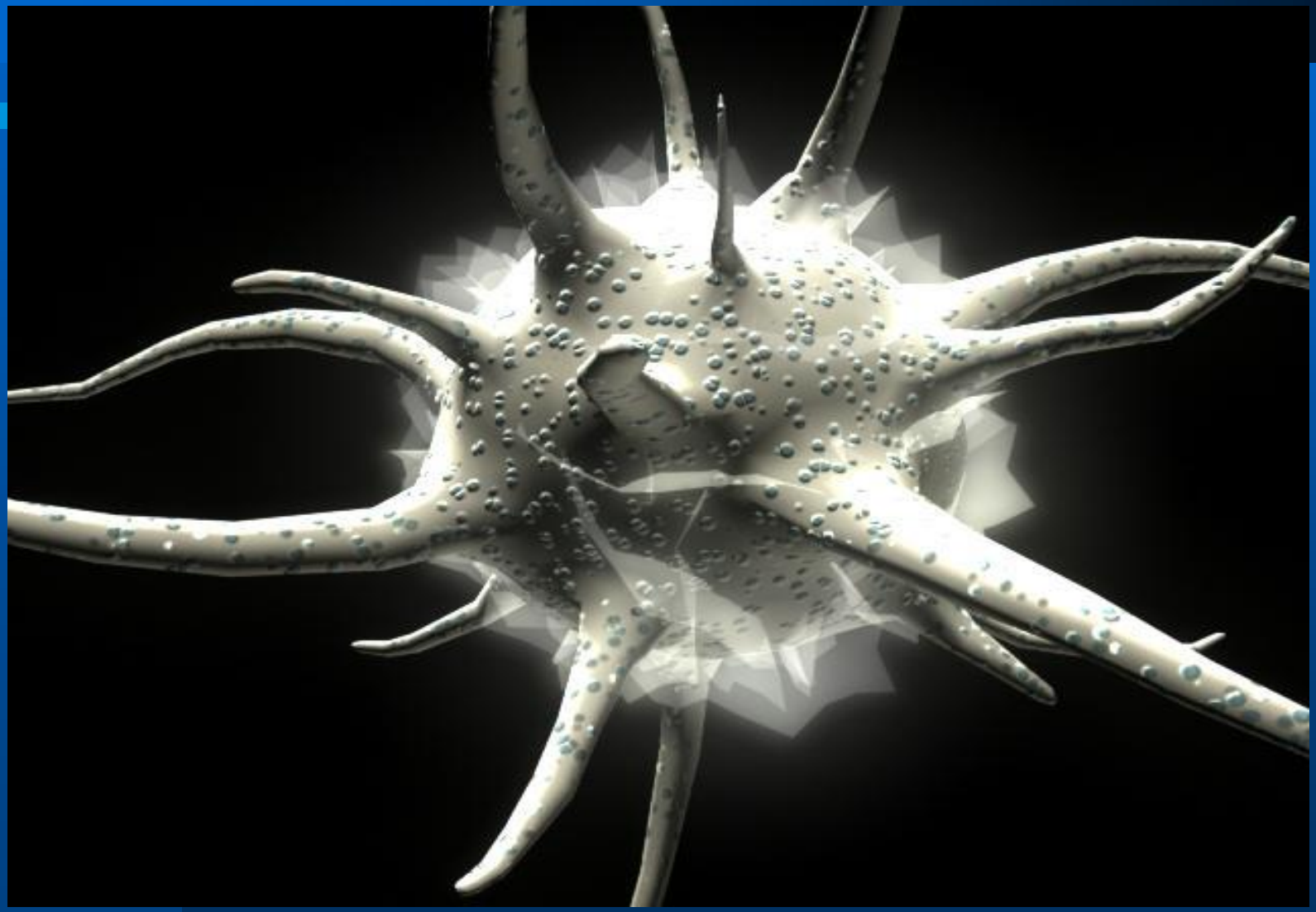
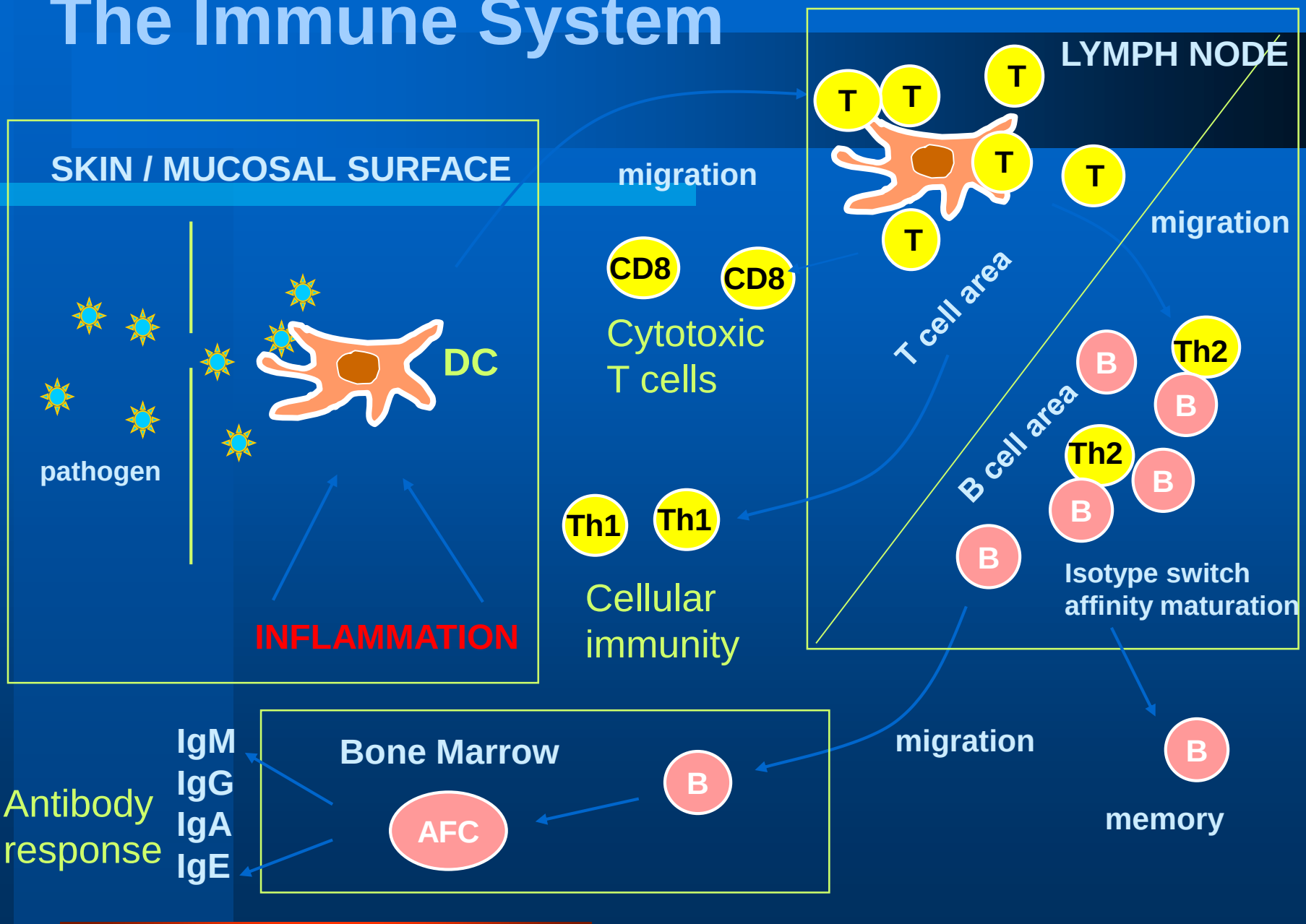


Figure 1-13 Immunobiology, 6/e. (© Garland Science 2005)

Dendritic cells are the link between innate and adaptive immunity. Moreover, they are superb phagocytes.



The Immune System



Overview of lymphocyte development

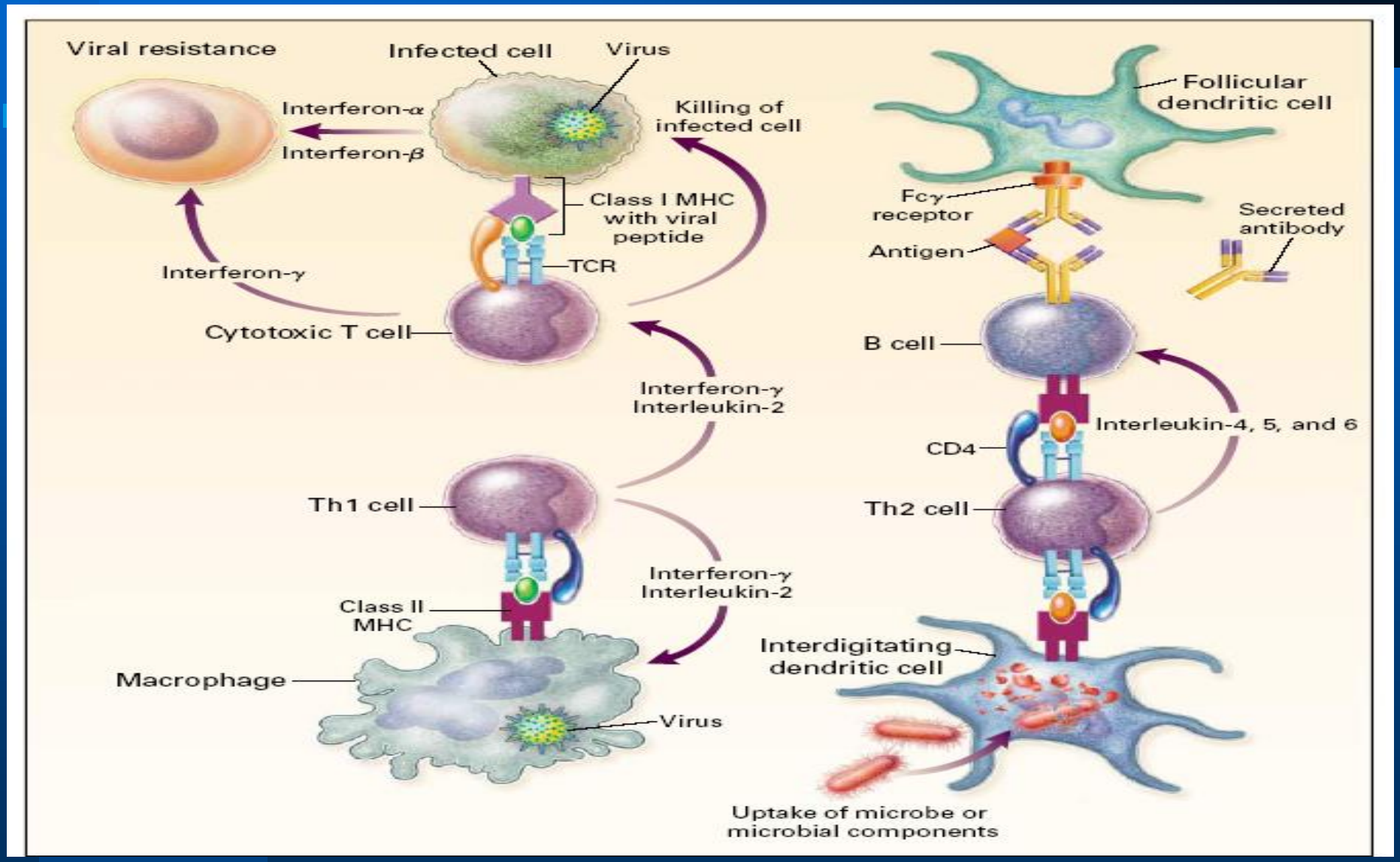
- Develop from a common lymphoid progenitor cell
- B & T cell maturation occurs primarily in BM and thymus respectively
- Process and signals of maturation differ, but they rely on similar genetic events to generate specific Abs or cell surface receptors
- These gene rearrangements are critical for the development of a broad immune repertoire and also provide molecular markers of clonality that can be used to diagnose lymphoid malignancies

TIME COURSE OF GENE EXPRESSION BY T_H CELLS FOLLOWING INTERACTION WITH ANTIGEN

Gene product	Function	Time mRNA expression begins	Location	Ratio of activated to nonactivated cells
		Immediate		
c-Fos	Protooncogene; nuclear-binding protein	15 min	Nucleus	> 100
c-Jun	Cellular oncogene; transcription factor	15–20 min	Nucleus	?
NF-AT	Transcription factor	20 min	Nucleus	50
c-Myc	Cellular oncogene	30 min	Nucleus	20
NF-κB	Transcription factor	30 min	Nucleus	> 10
		Early		
IFN-γ	Cytokine	30 min	Secreted	> 100
IL-2	Cytokine	45 min	Secreted	> 1000
Insulin receptor	Hormone receptor	1 h	Cell membrane	3
IL-3	Cytokine	1–2 h	Secreted	> 100
TGF-β	Cytokine	<2 h	Secreted	> 10
IL-2 receptor (p55)	Cytokine receptor	2 h	Cell membrane	> 50
TNF-β	Cytokine	1–3 h	Secreted	> 100
Cyclin	Cell-cycle protein	4–6 h	Cytoplasmic	> 10
IL-4	Cytokine	<6 h	Secreted	> 100
IL-5	Cytokine	<6 h	Secreted	> 100
IL-6	Cytokine	<6 h	Secreted	> 100
c-Myb	Protooncogene	16 h	Nucleus	100
GM-CSF	Cytokine	20 h	Secreted	?
		Late		
HLA-DR	Class II MHC molecule	3–5 days	Cell membrane	10
VLA-4	Adhesion molecule	4 days	Cell membrane	> 100
VLA-1, VLA-2, VLA-3, VLA-5	Adhesion molecules	7–14 days	Cell membrane	> 100, ?, ?, ?

SOURCE: Adapted from G Crabtree, *Science* **243**:357.

An Overview of Lymphocyte Responses



Hallmarks of the Immune Response

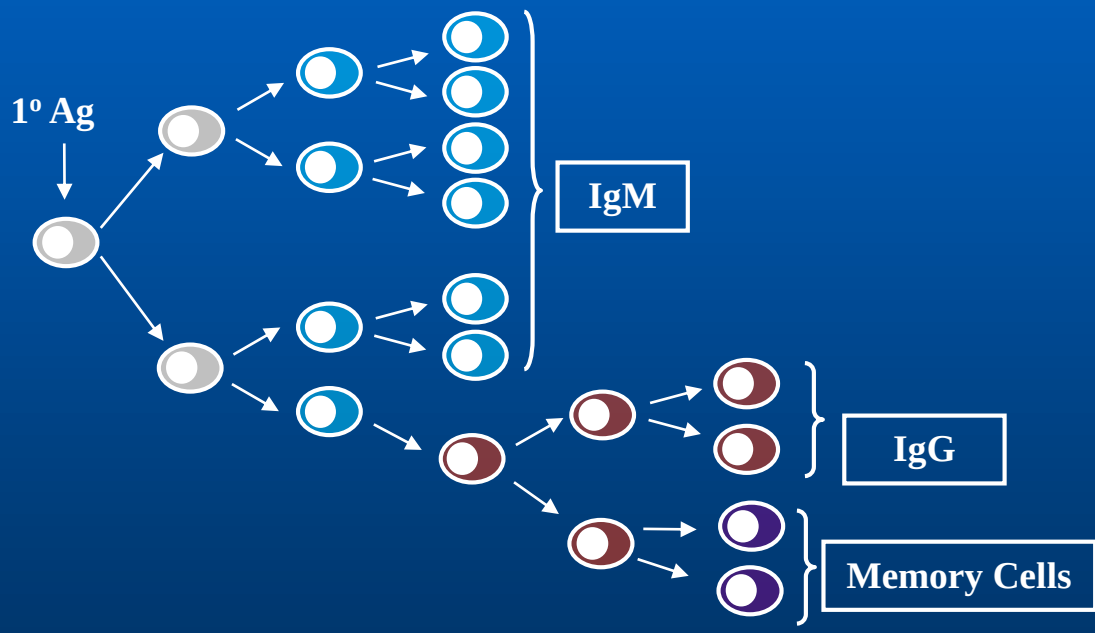
- Self/Non-self Discrimination
- Memory
- Specificity (*immune response to single foreign antigen*)

Primary Response of Humoral Immunity

- About 6 days after an antigen is introduced, antibodies of the IgM class can be detected.
- IgG appears at about the 10 day, peaks at several weeks and lasts much longer.

Cellular Events in 1º Response to T-dependent Ags

- Lag
  - Clonal selection
- Log
  - IgM
  - Class switching
- Stationary
- Decline
- Memory Cell Pool



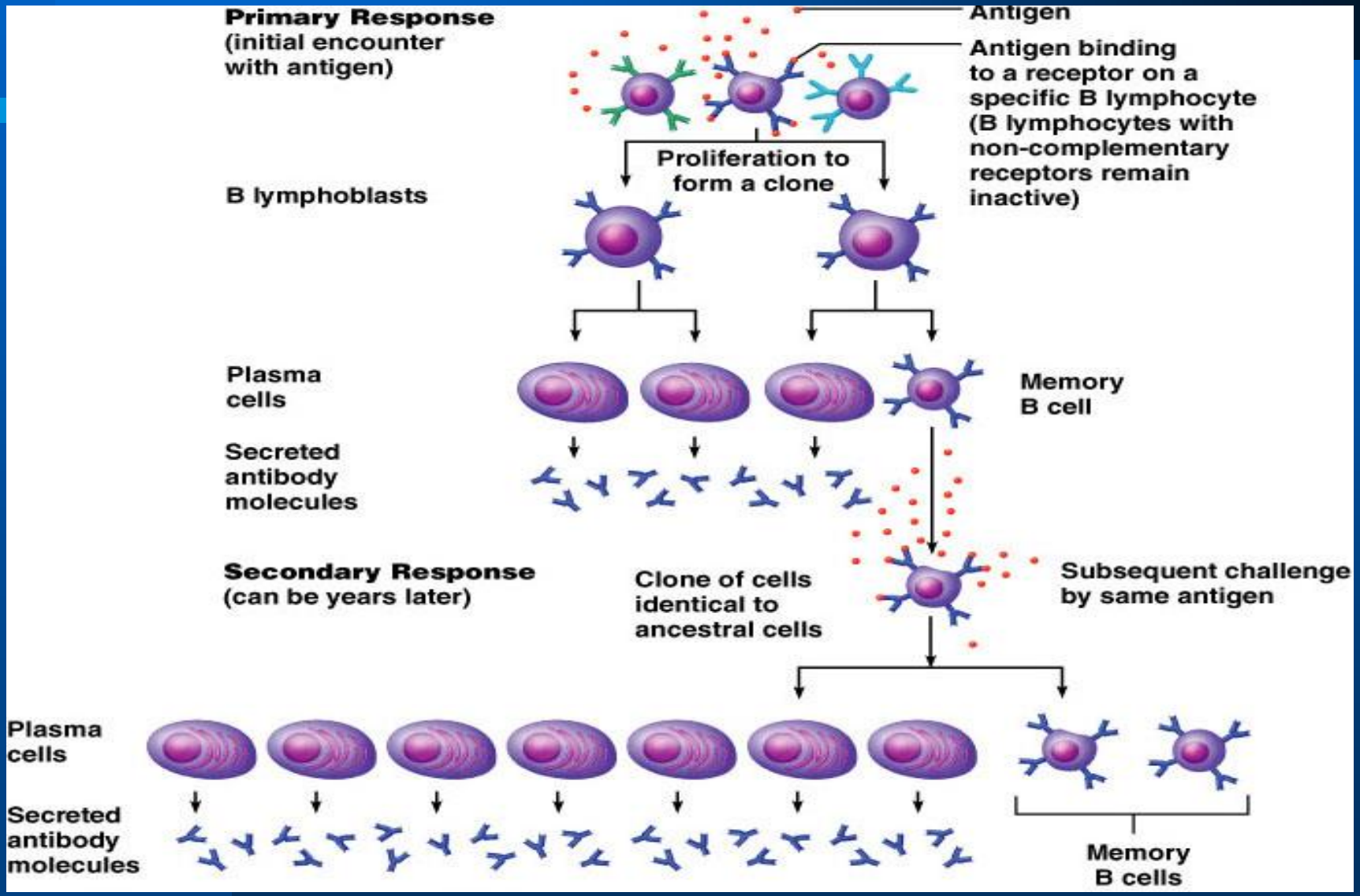
Immunological Memory

- Primary immune response – cellular differentiation and proliferation, which occurs on the first exposure to a specific antigen
  - Lag period: 3 to 6 days after antigen challenge
  - Peak levels of plasma antibody are achieved in 10 days
  - Antibody levels then decline

Clonal Selection

- Stimulated B cell growth forms clones bearing the same antigen-specific receptors
- A naive, immunocompetent B cell is activated when antigens bind to its surface receptors and cross-link adjacent receptors
- Antigen binding is followed by receptor-mediated endocytosis of the cross-linked antigen-receptor complexes
- These activating events, plus T cell interactions, trigger clonal selection

Clonal Selection

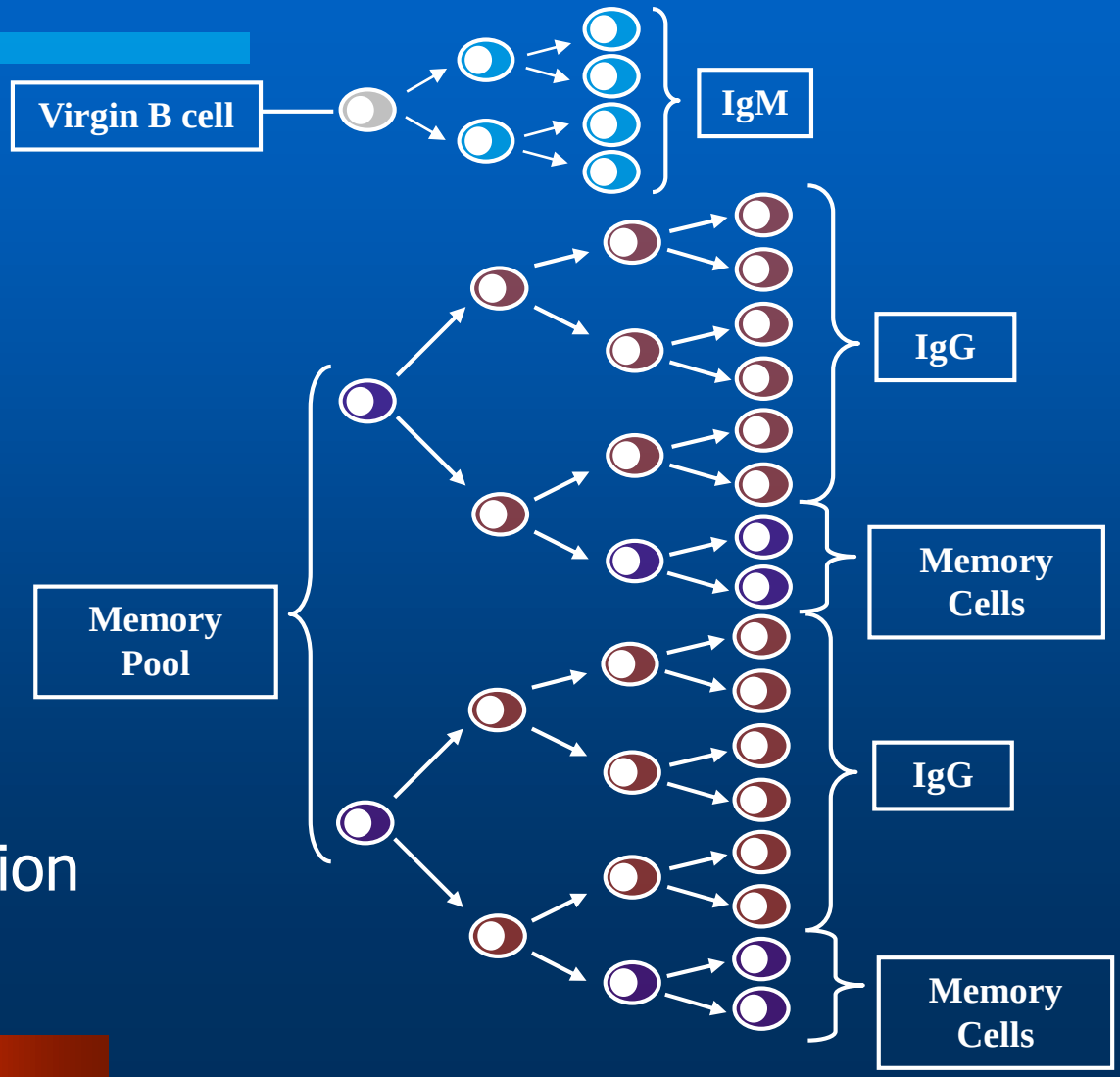


Fate of the Clones

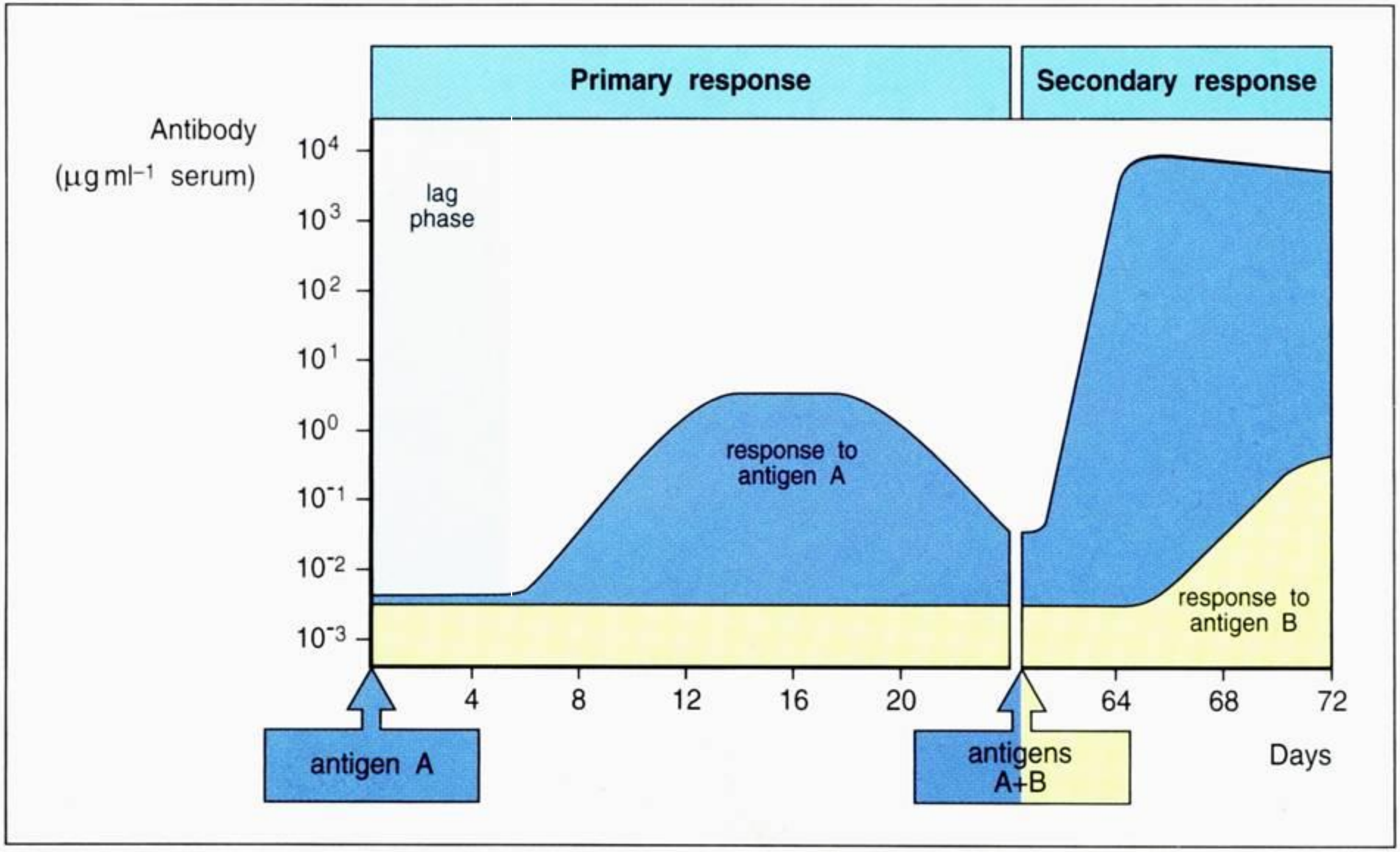
- Most clone cells become antibody-secreting plasma cells
- Plasma cells secrete specific antibody at the rate of 2000 molecules per second

Cellular Events in 2º Response to T-dependent Ags

- Lag phase
  - Virgin cells
  - Memory cells
- Log phase
  - Pool size
  - IgG, IgA or IgE
- Stationary
- Decline
  - Sustained production



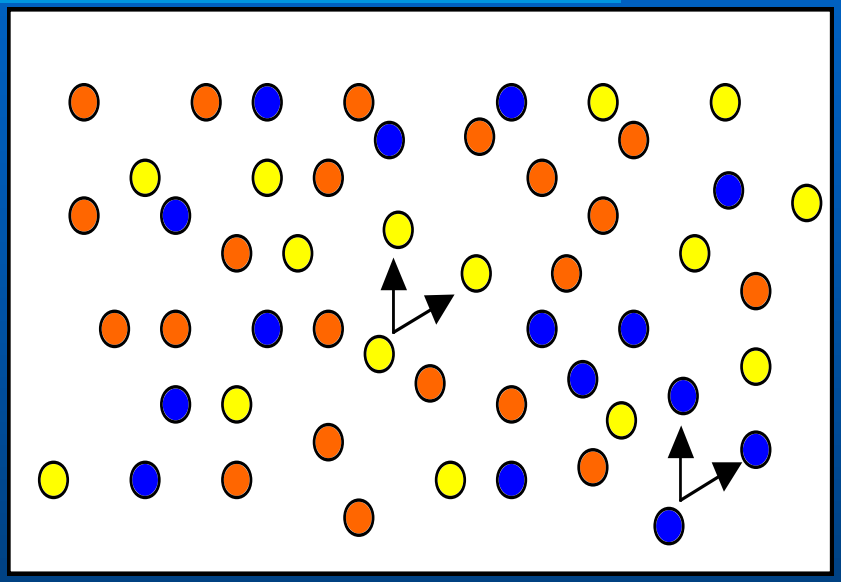
Memory & specificity – key features of the adaptive immunity



Homeostasis in the memory T cell pool

input

Antigen activation of naïve and memory T cell



output

Death

and/or differentiation into

effector

cells

Homeostatic proliferation of memory T cell

The Number Dilemma

- **You have about a trillion different antibodies able to react with millions of different types of Ag**
- **but you only have about 30,000 genes which code for all the proteins you need in your entire body, most of which are not Ab**
- **so there cannot be one gene for one antibody to code for these – we wouldn't have enough antibodies!**

So how can your body produce Ab to so many antigens, even those it's never seen?

Antibody Variability

There are several reasons why there is an enormous number of different antibodies:

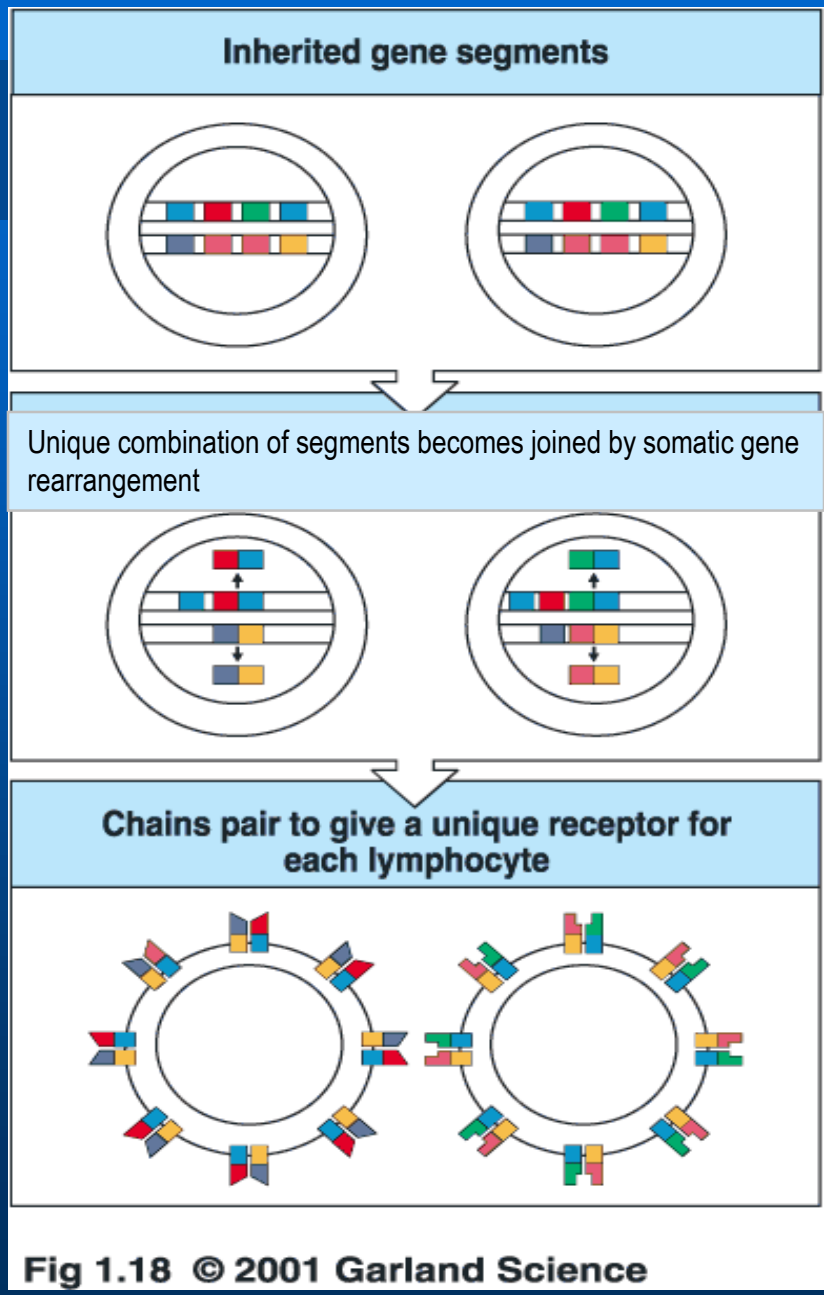
- **different combinations of heavy and light chains which are encoded by different genes**
- **recombination**
- **others**

Formation of Specific Antibodies

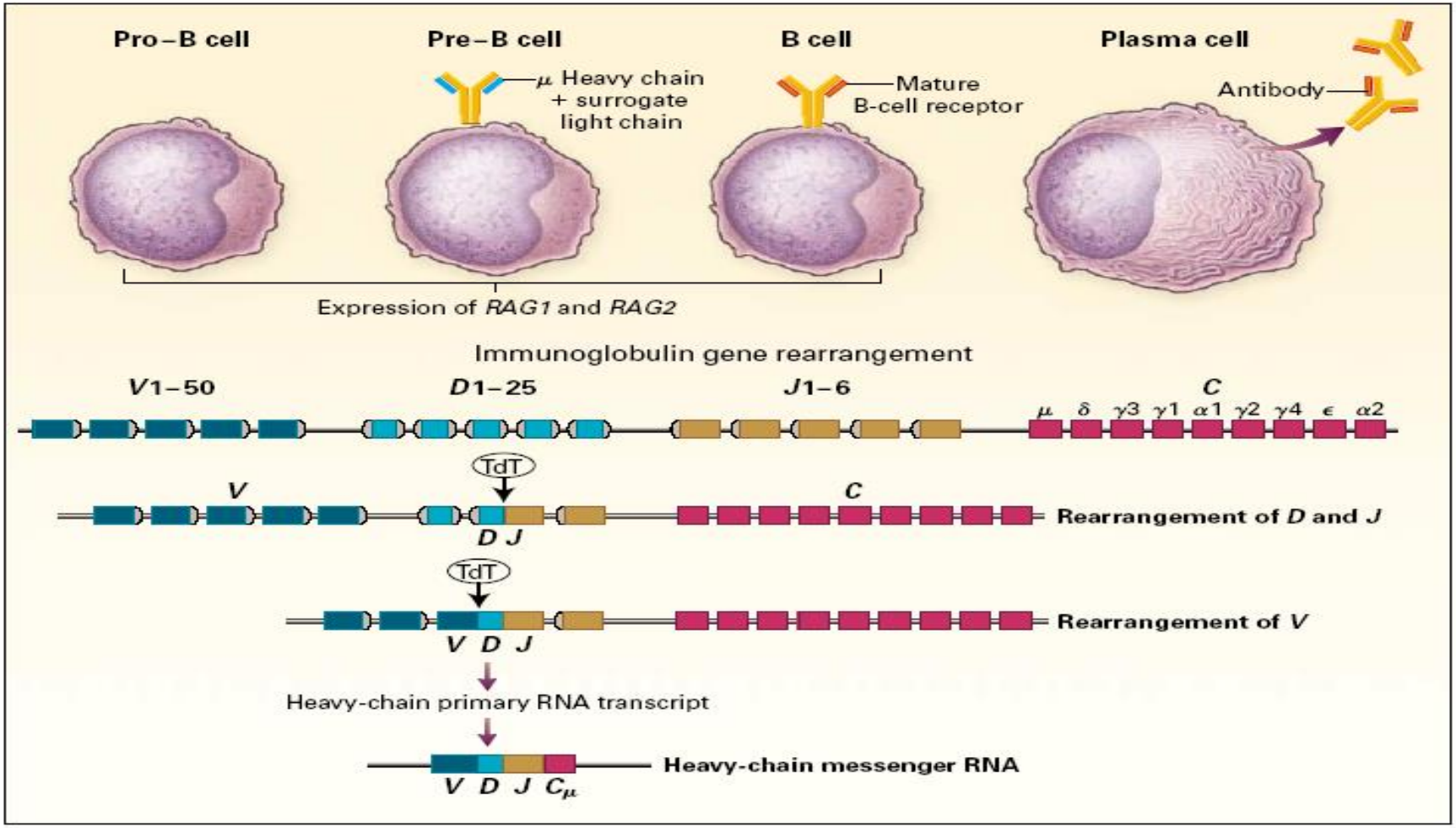
- Antibody genes consist of 4 regions
  - Constant segment
  - Variable segment
  - Diversity segment
  - Joining segment
- Intervening segments of DNA are cut out
- Selected pieces are spliced together.

A unique recombination occurs in each B cell

- each B cell combines these gene segments to make an Ab chain like shuffling a deck of cards
  - V, D, and J for the heavy chain, V and J for the light chain
- since there are multiple types of each gene segment, there are many thousands of possible V-D-J combinations so that each B cell gets a unique combination of segments!

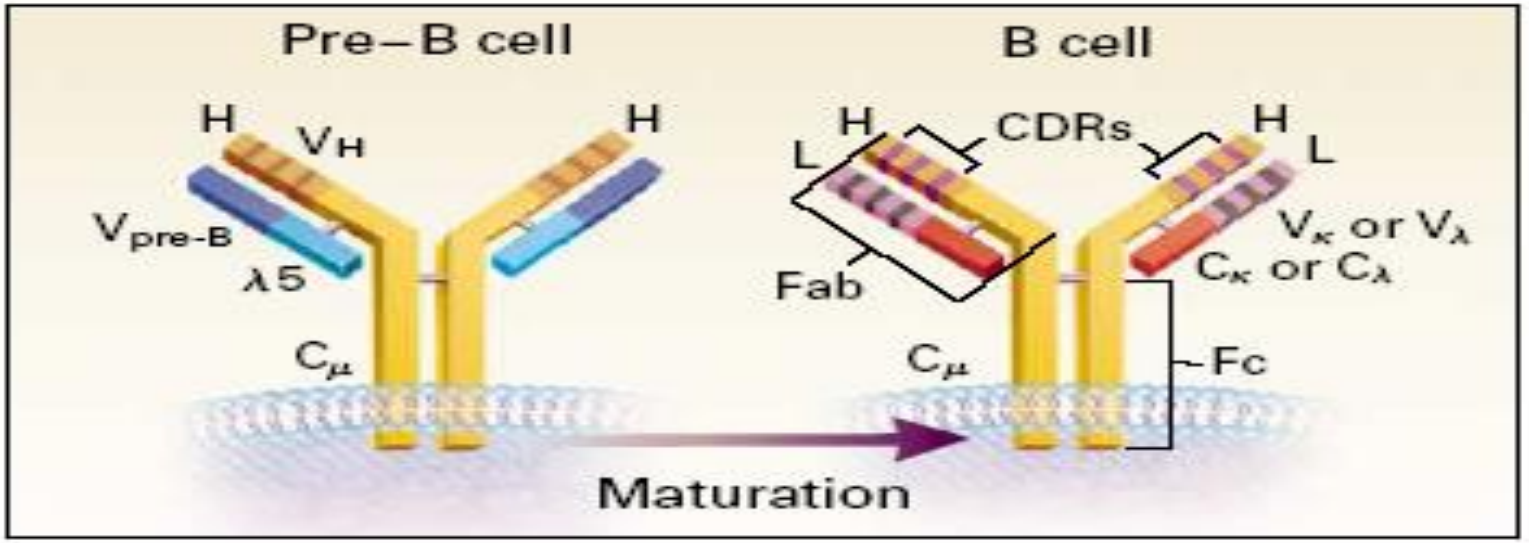


Diversity of Antigen Receptors



…plus splicing inaccuracies and incorporation of nucleotides mediated by terminal nucleotidyltransferase (TdT) !

Structure:Immature & Mature B- and T-Cell Ag Receptors



Immune Response
Time scale

1. Antigen appears
   T lymphocytes / B lymphocytes interact
   recognize it as foreign

2. Thymus
   lymph nodes release T lymph
   T cells attack antigen directly

3. B cells
   release
   -IgM- initial response
   then
   IgG- long term immunity
   have specific sites that attach to antigens

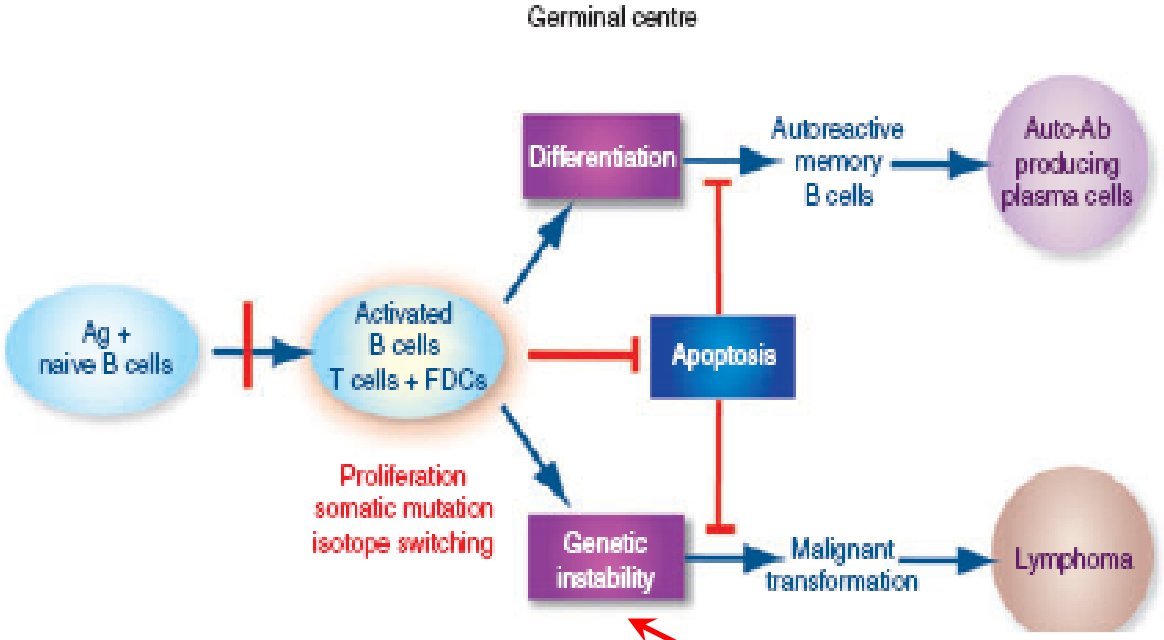
4. Change in Antibody shape
   complement cascade
   circulating proteins

5. Serum complement
   stimulates release of histamine
   ↑capillary permeability
   promote vasodilation
   stimulate release of macrophages

6. Complement roughens antigen's surface
   attracts macrophages and neutrophils- which engulf & attack the cell

7. Lysosomes attach to cell
   and release enzyme hydrolase-
   breaks down cell's protein and lipids

8. Some IgG- "remember"



Initiation event in lymphomagenesis?

Figure 2. Fine tuning of B-cell apoptosis by follicular dendritic cells (FDC). In order to produce high-affinity protective antibody (Ab), antigen (Ag)-activated B cells proliferate vigorously and undergo somatic hypermutation and isotype switching in the presence of FDC and T cells in the germinal centre (GC). These GC reactions are prone to produce genetic instability, such as mutation and translocation of critical genes. Failure to eliminate these aberrant B cells by apoptosis in the GC would be a critical component of autoimmune diseases and lymphomagenesis. The apoptotic mechanism in the GC needs to be precisely regulated to maintain normal B-cell homeostasis in the peripheral lymphoid tissues. In the absence of regulation of B-cell apoptosis, elimination of self-reactive B cells is impaired, resulting in autoimmune diseases. FDCs are the main source of anti-apoptotic factors such as BAFF/BLys and IL-15 in the GC. The physiological interaction between FDCs and B cells may regulate the appropriate concentrations of these cytokines in the GC microenvironment. We suggest that blockade of the survival signals provided by FDCs is one way to treat autoimmune diseases and B-cell lymphomas, in addition to the elimination of pathological B cells.

i.e. NF-kB inhibitors ?

 Immunology, **114**, 2–10

Conclusions - 1

- To establish an infection, a pathogen must first overcome numerous surface barriers such as enzymes and mucous that either are directly antimicrobial or inhibit attachment of the microbe
- Microbes must rupture the ectoderm. Any organism that breaks through the first barrier encounters the 2 further levels of defence, the innate and the adaptive (acquired)

Innate Immunity provides signal for the Adaptive Response