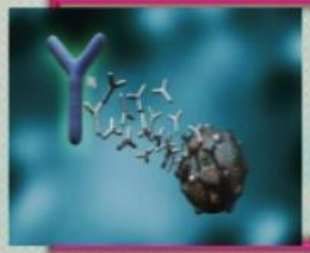
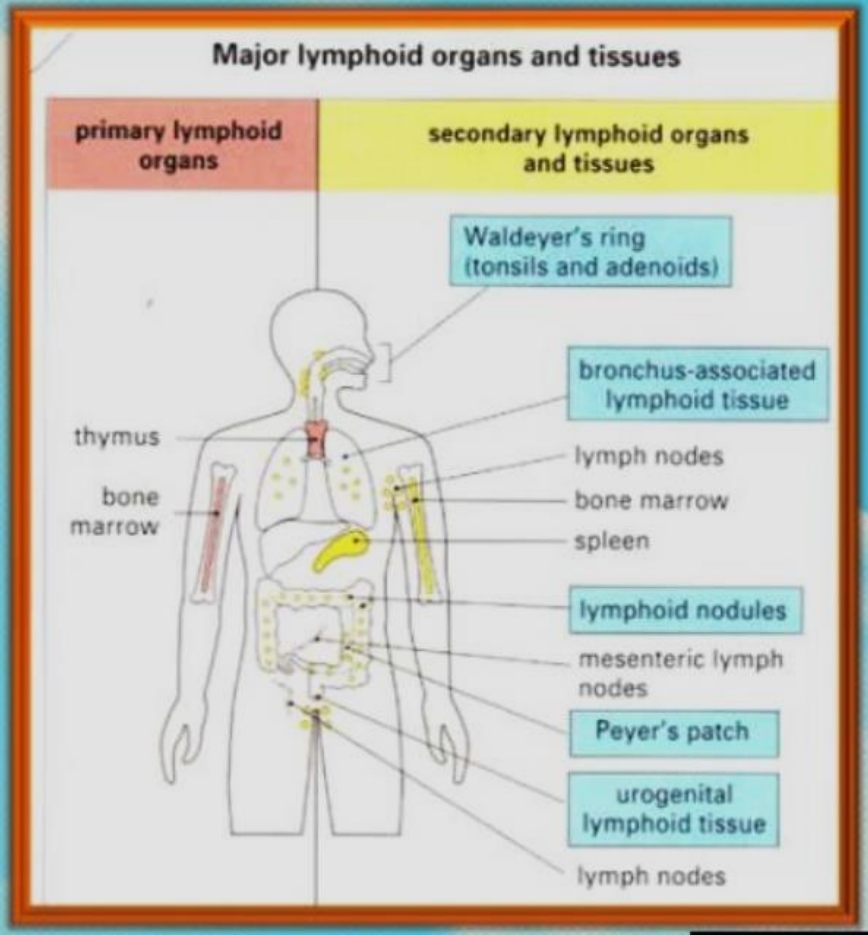
Basic tasks of clinical immunology. Principles of functioning of the immune system, clinical and laboratory evaluation of its disorders.

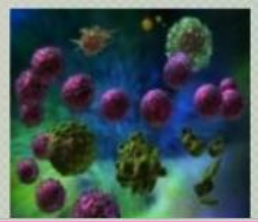
Professor Vladimir Babadzhan

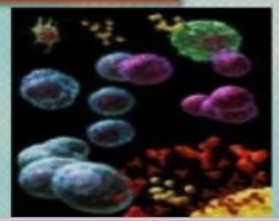


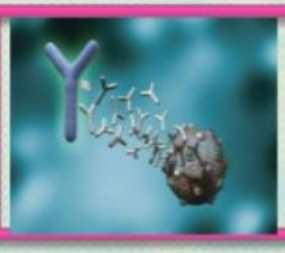
Organs of Immune System

- Primary lymphoid organs-maturation of lymphocytes.
- Secondary lymphoid organs- trap antigens & provide sites for mature lymphocytes to interact with that antigen.
- Tertiary lymphoid tissues- cutaneous associated lymphoid tissues.









Lymphoid Organs

Central or Primary

- Provide the appropriate microenvironment for development and maturation of lymphocytes

Thymus

Bone Marrow

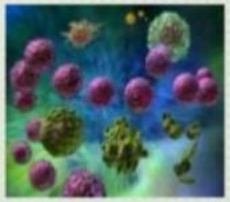
Peripheral or Secondary

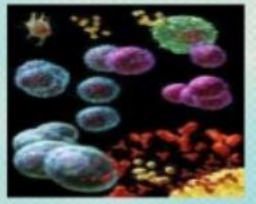
- Sites where mature lymphocytes interact effectively with the antigens.

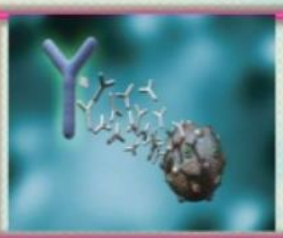
Spleen

Lymph nodes

MALT



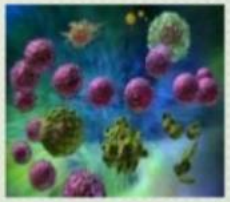


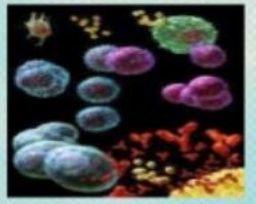


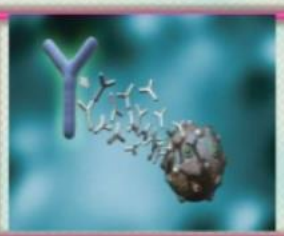
Primary Lymphoid Organs

- Immature lymphocytes generated in bone marrow mature, becomes specific for a particular antigen in central lymphoid organs.

- Such educated cells are immunocompetent cells.
  - T cells arise in thymus .
  - B cells in bone marrow.



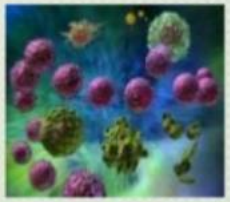


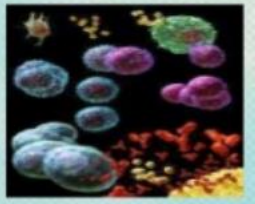


Bone Marrow

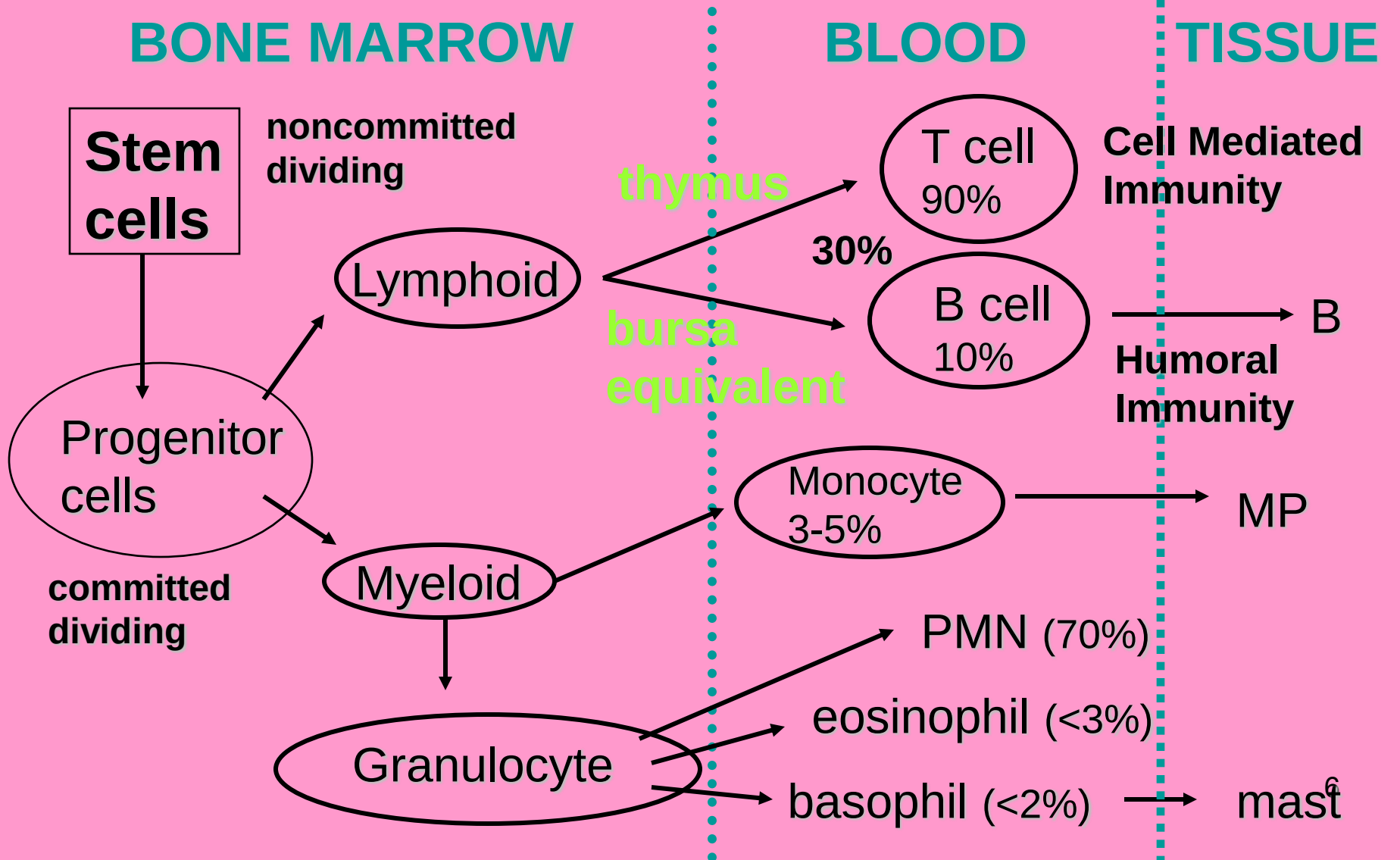
- All lymphocytes originate in bone marrow.
- T lymphocytes develop in thymus.
- B lymphocytes develop in bone marrow itself.

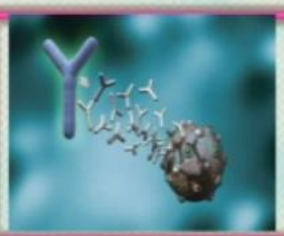
(In birds, bursa of Fabricius – primary site associated with B cell maturation)





Origin and Development of Immune Cells



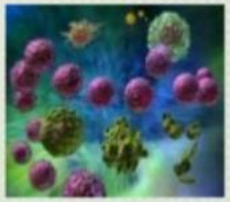


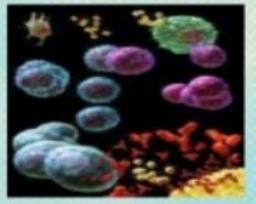
Thymus

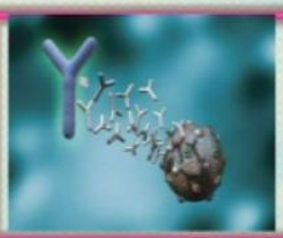
- **Functions:**
  - Production of thymic lymphocytes.
  - Major site of lymphocyte proliferation.
  - Thymus (T) dépendent lymphocytes or T cells – immunocompetent.
  - Important in development of CMI.

- **Thymus dependent areas of peripheral lymphoid organs**
  - White pulp of spleen.
  - Paracortical areas of lymph node.

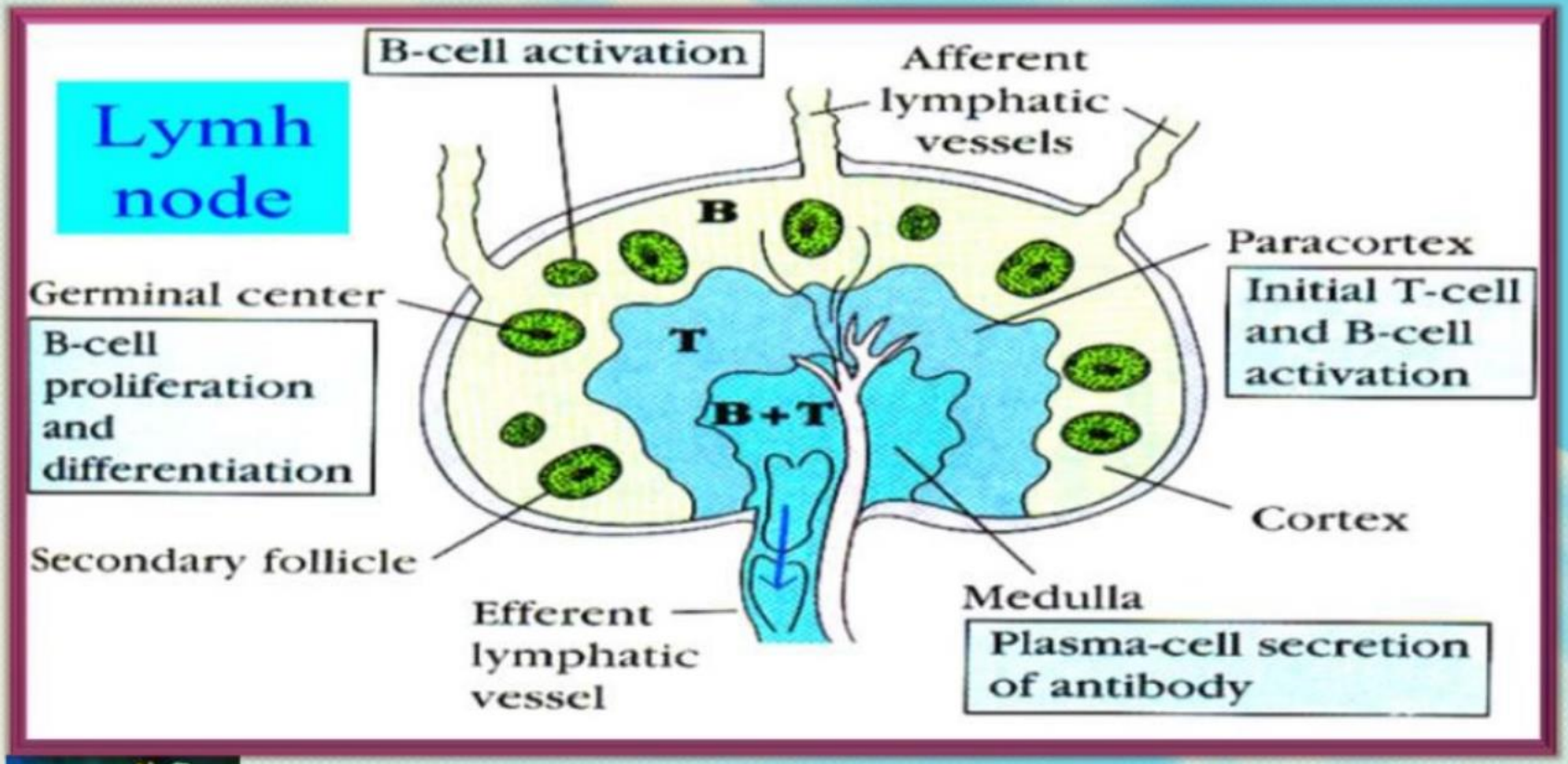
- **Effects of thymectomy or congenital thymic aplasia:**
  - deficient CMI (Di George syndrome)
  - increased chance of getting infectious diseases
  - diminish antibody response towards thymus dependent antigens.

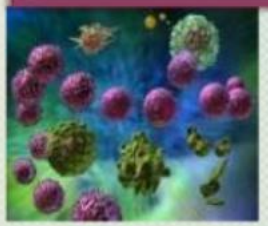


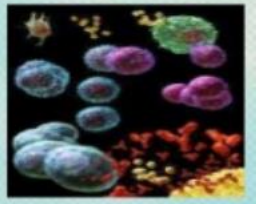


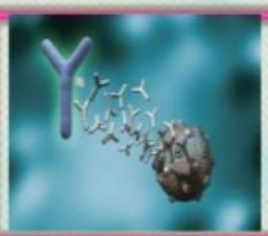


Peripheral Lymphoid Organs





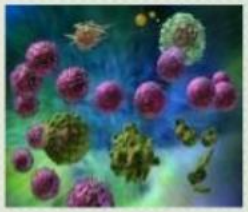


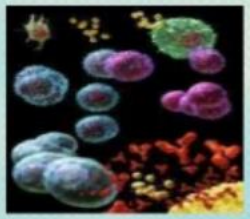


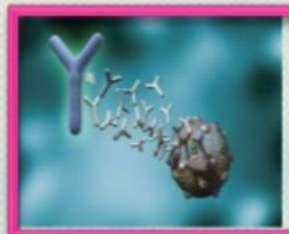
Lymph Node

- Placed along the course of lymphatic vessels.
- Phagocytose foreign materials including microorganisms especially from local tissues.
- Help in the proliferation and circulation of T & B cells.

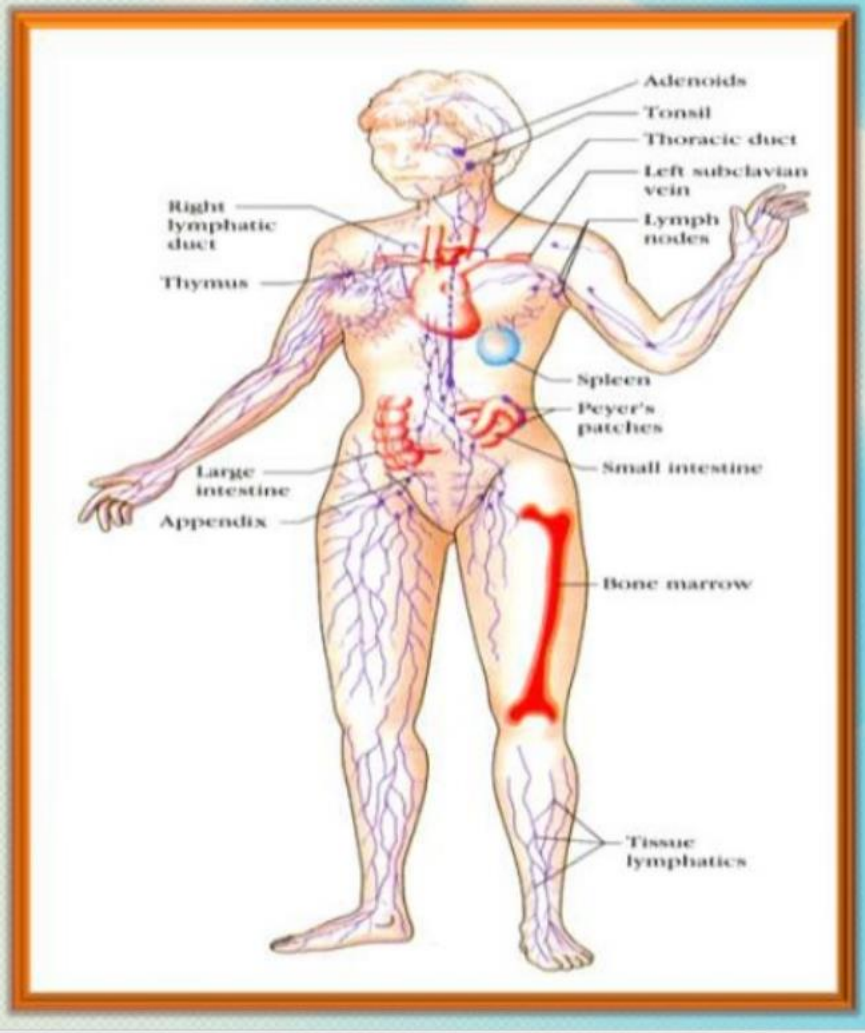
- Histology:
  - Macrophages & dendritic cells–
  - cortex & paracortex
  - T helper cells- paracortex
  - B cells– cortex
  - Plasma cells– medulla





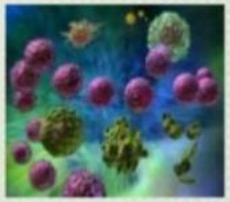


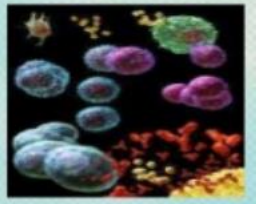
Lymphoid Organs



Functions : -

- Defense
- Homeostasis
- Surveillance





The normal individual has two levels of defence against foreign agents. The first type is named **natural** or **innate** immunity. This type of immunity is sometimes referred to as non-specific but broadly specific. The second type of immunity is **adaptive** or **acquired** immunity and is confined to vertebrates.

INNATE IMMUNITY

PHYSICAL BARRIERS

Skin, mucous membrane

CELLS

granulocytes, monocytes, macrophages

CHEMICAL BARRIERS

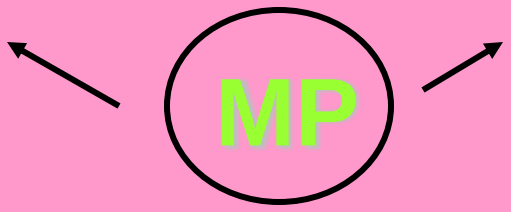
pH, lipids, enzymes

ACQUIRED IMMUNITY

HUMORAL

B cells

antibodies



CELL MEDIATED

T cells

interleukines

Physical barriers are the first line of defense against infection. The skin and mucous membranes provide a continous surface which must be breached and back this up with mechanical protection through cilia and mucous.

Physiological factors such as pH, temperature and oxygen tension limit microbial growth. The acid environment of the stomach combined with microbial competion from the commensal flora inhibits gut infection.

Protein secretions into external body fluids such as lysozyme also help resist invasion. Soluble factors within the body such as **complement**, **interferons** and collectins and other "broadly specific" molecules such as C-reactive protein are of considerable importance in protection against infection.

Phagocytic cells are critical in the defense against bacterial and simple eukaryotic pathogens.

Macrophages and **Polymorphonuclear leucocytes (PMN)** can recognise bacterial and yeast cell walls through broadly specific receptors (usually for carbohydrate structures) and this recognition is greatly enhanced by activated complement (opsonin).

Nonspecific Immunity

- **Granulocytes**
  - Polymorphonuclear leukocytes (PMN, neutrophils)
  - Eosinophils
  - Basophils (blood)
  - Mast Cells (tissues)

  ] allergic and hypersensitivity reactions
- **Mononuclear Phagocytes**
  - Monocytes (blood)
  - Macrophages (tissue)

Functions of Phagocytic Leukocytes (mono, MP and PMN)

- **Localization and Removal of Foreign Substances** (Inflammation and wound healing)

  -**Chemotaxis**: directed migration to site of injury; chemical mediators (chemotactic factors)

  -**Phagocytosis**: ingestion of foreign substances

  -**Metabolic destruction**: digestion and killing

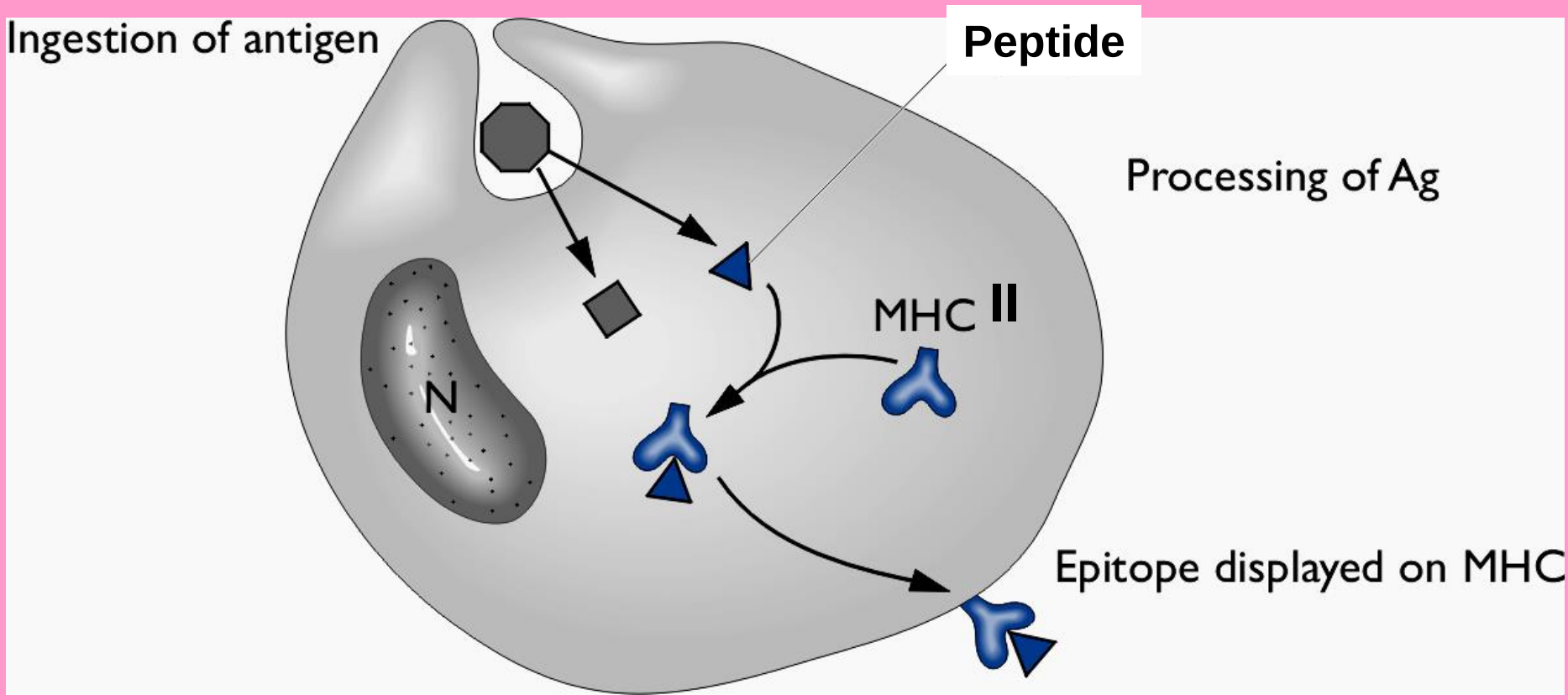
  Oxygen-dependent (MPO, ROI, RNI)

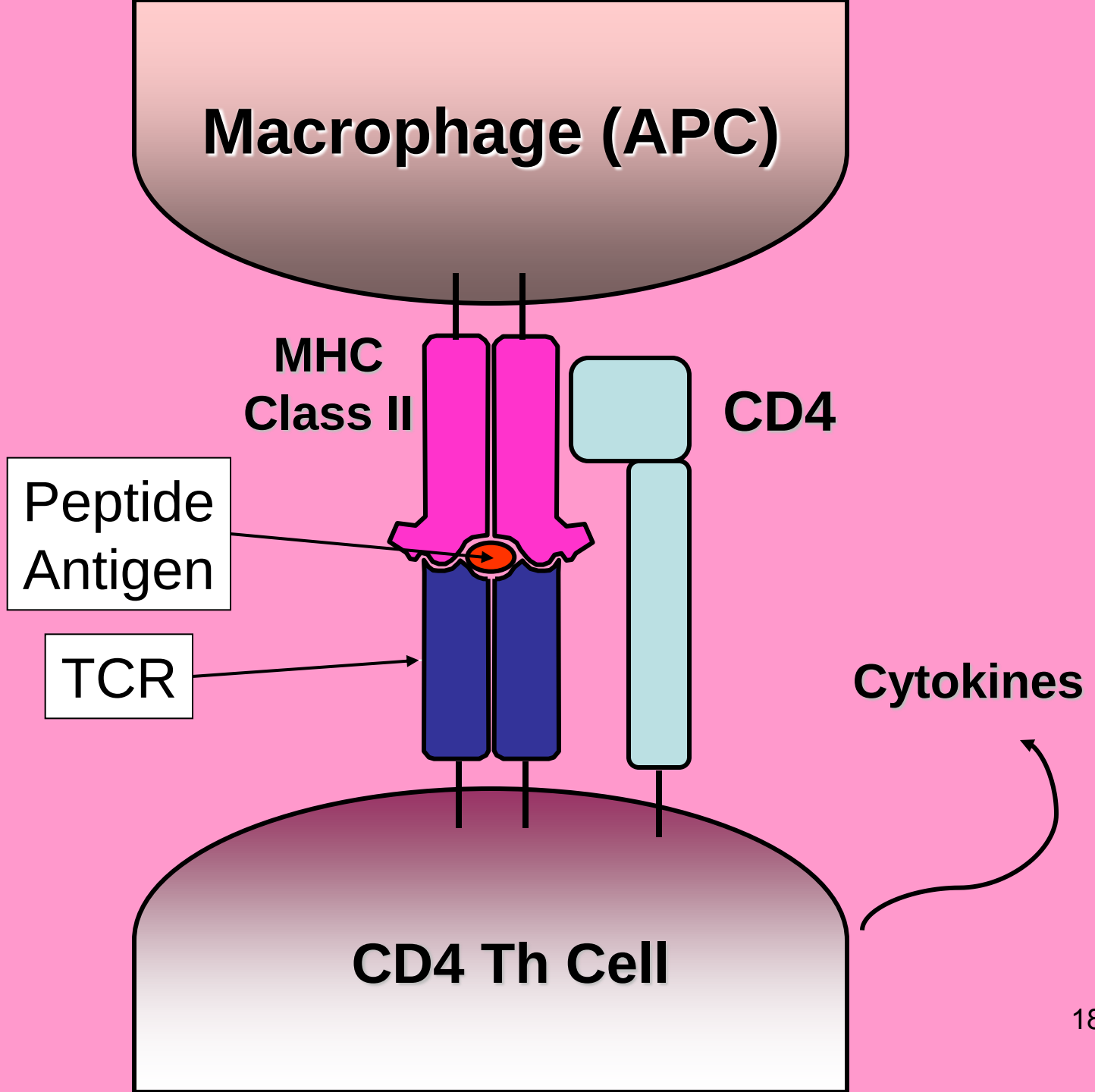
  Oxygen-independent (cationic proteins, lysozyme, TNF, porphorins

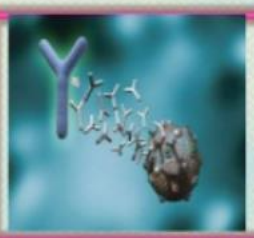
Immune Functions of Macrophages

- **Antigen processing and presentation**
- **Tumor Cytotoxicity**
- **Tumor Surveillance**

Antigen Processing and Presentation

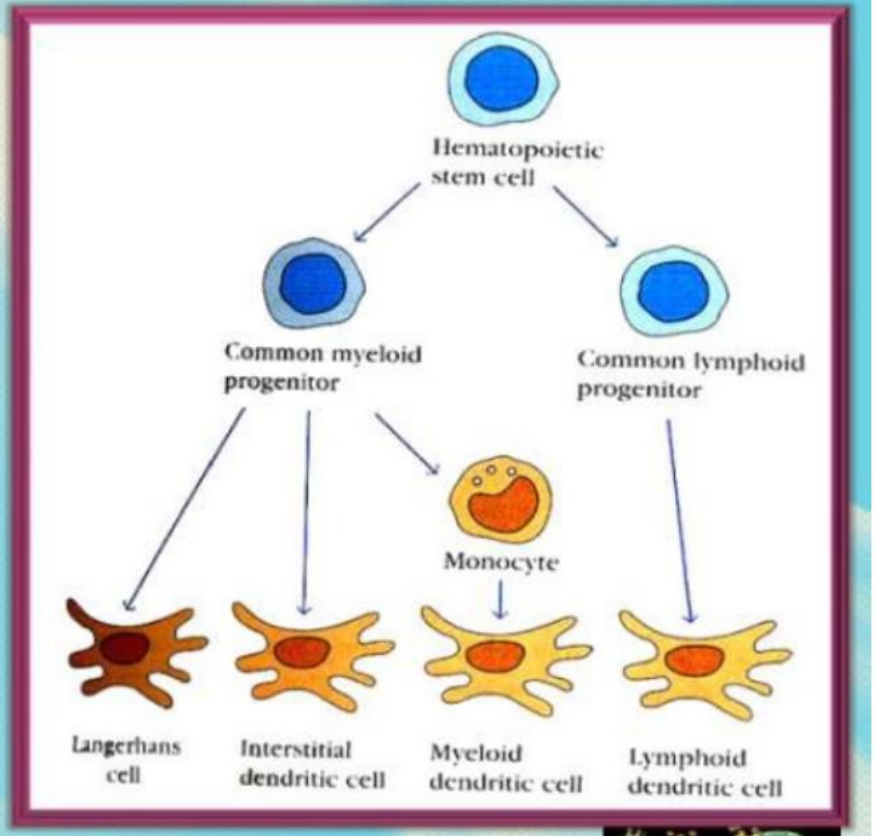


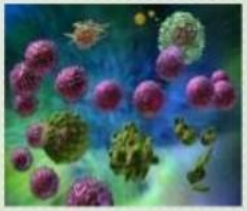

Macrophage (APC)
MHC
Class II
CD4
Peptide
Antigen
TCR
Cytokines
CD4 Th Cell

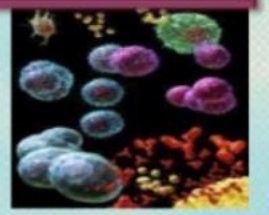


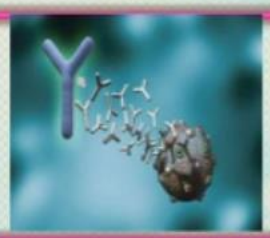
Dendritic Cells

- Antigen presenting cells
- Pleomorphic, have long needle like processes
- Present in blood & peripheral lymphoid organs
- Little or no phagocytic activity





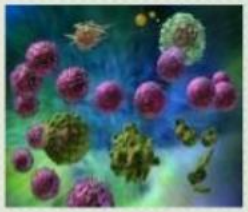


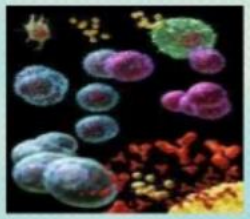


Mucosa Associated Lymphoid Tissue (MALT)

GALT- Lymphoid tissues in gut. From adenoids & tonsils to the follicles in the colon

BALT- Lymphoid tissues in respiratory tract.



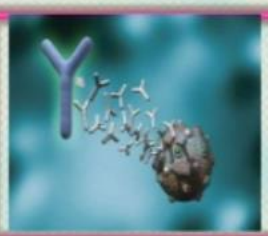


Cell Mediated Immunity

- Mediated by **T lymphocytes** which release soluble mediators (**interleukines**)

- Important in host defense against viruses, certain bacteria, fungi, transplant rejection and tumor surveillance; Type IV (delayed type) hypersensitivity (DTH) reactions

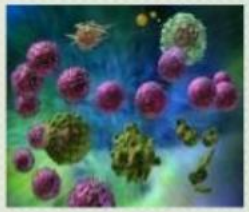
T Cells: derived from precursor cells in bone marrow; mature in thymus; become educated

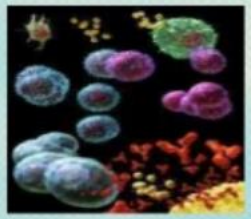


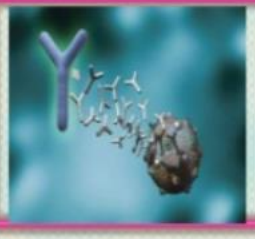
Cells of Immune System

- Central cells of the immune system-
  - LYMPHOCYTES
- They are responsible for:
  1. adaptive immunity.
  2. immunological aspects of diversity, specificity, memory & self / nonself recognition.

- Functions of other WBCs
  1. engulf and destroy micro organisms
  2. present antigens.
  3. secrete cytokines.







Immunocompetent Cells (ICC)

- Lymphocytes educated by central lymphoid organs
- Stimulated T cells produce lymphokines
- Stimulated B cells divide & transform into plasma cells which synthesize antibodies

Surface markers

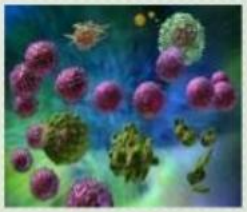
- Reflect various stages of maturation & differentiation of lymphocytes & other leucocytes
- Each surface marker given a CD (Cluster of Differentiation) number

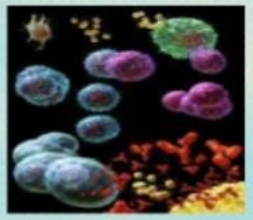
CD 4 – Helper T cells

CD 8 – Suppressor T cells or Cytotoxic T cells

CD 19 – B cells

CD16 – natural killer cells





T Lymphocyte Types

- Helper cells (CD4 cells)
- Cytotoxic cells (Killer T cells, CD8 cells)
- Suppressor cells (CD8 cells)
- Natural killer cells (NK-cells, CD16)
- Memory cells

Helper T-Cells (CD4 cells)

- Master “on-switch” of immune system
- Recognize antigens
- Secrete lymphokines that activate all other immune system cells
- Stimulate B-cells to begin antibody production

T-helper cells

Th1 cells: T helper inflammatory cells

- Involved in cell-mediated allergies,
  e.g. poison ivy
- Promote rejection of transplanted tissue
- Stimulated by cell-bound antigen, and secrete lymphokines

Th2 cells: Stimulate B cells to produce antibodies

Cytotoxic (Killer) T-Cells (CD8 cells)

- Respond to presence of antigens and lymphokines produced by CD4 cells
- Seek out, bind to, and destroy:
  - Cells infected by viruses
  - Some tumor cells
  - Cells of tissue transplants
- Can deliver lethal hits on multiple cells in sequence

Suppressor T-Cells (CD8 cells)

- Produce **interleukines** that inhibit proliferation of B and T cells
- Downregulate or dampen immune response

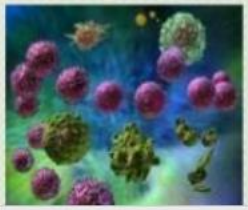
Antigen Recognition by T Cells

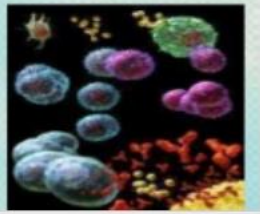
- Specific T cell receptor
- T cells are MHC restricted; only recognize antigen and MHC protein
- T-helper cells (CD4) recognize processed antigen and piece of self (MHC II)
- Cytotoxic T cells (CD8) recognize processed antigen and nonself (MHC I)

T – Cell Maturation

- CD4 cells = MHC II Restriction - TWO types – TH1 & TH2. TH1 – secrete γ interferon & IL – 2 , which activate macrophages & T cells promoting CMI. TH2 – secrete IL – 4 , IL – 5 , IL – 6 = stimulate B cells to form Abs.

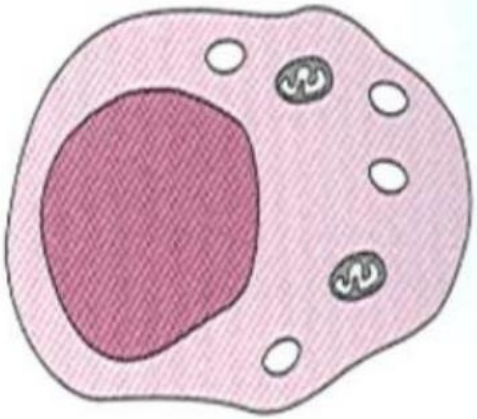
- CD8 cells = MHC I Restriction – can kill & lyse target cells carrying new or foreign Ags = down regulate immune response.





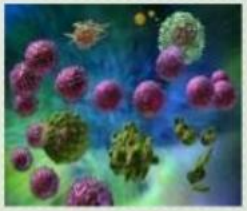
Natural Killer Cells
NK cells

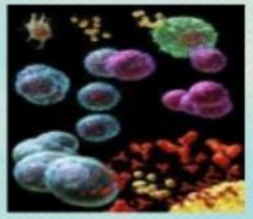
- Cytotoxic towards malignant &
  - virus infected cells
  - Natural or non immune
  - Destroy cells by apoptosis
  - Active in severe combined immunodeficiency diseases

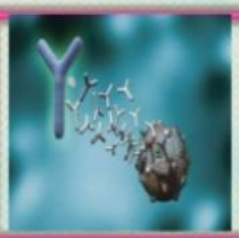


Natural killer cell

- Important cells in:
  - immune surveillance & natural defense
  - against virus infected & malignant cells.

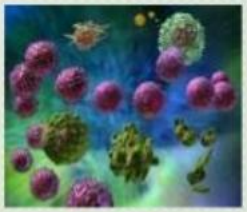


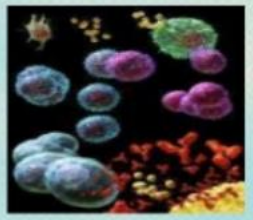




Major Histocompatibility Complex (MHC)

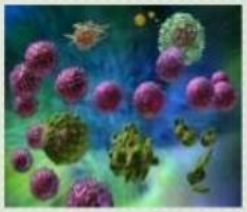
- **History**
  - Name histocompatibility complex → because of its discovery based on transplantation experiments.
  - Human MHC antigens are found on surface of leucocytes → hence synonymous with Human Leukocyte Antigens (HLA) & MHC complex of genes with the HLA complex.

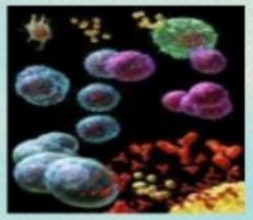


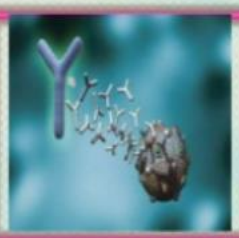


HLA Complex

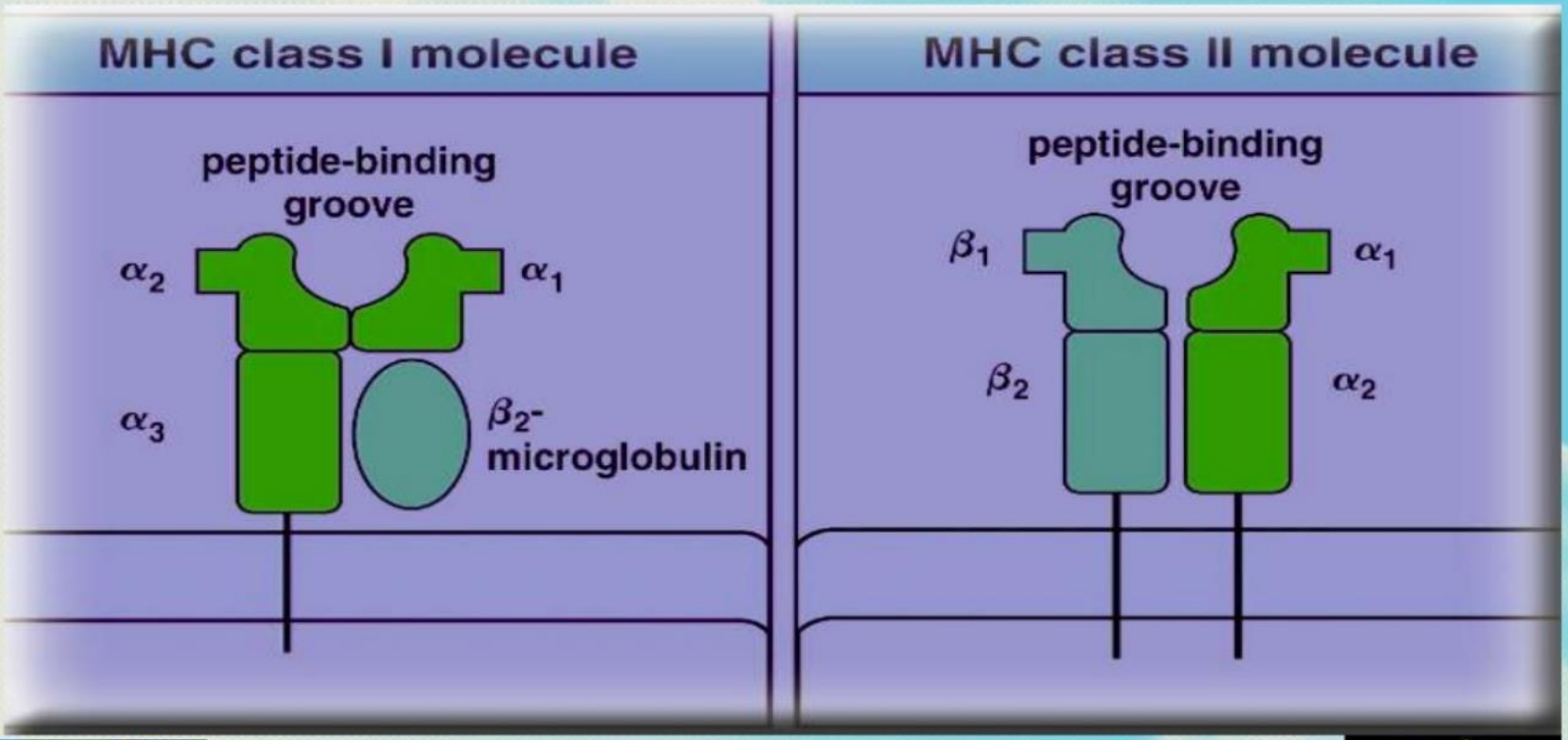
- HLA complex of genes located on short arm of chromosome 6.
- It is comprised of three separate clusters of genes:
  1. HLA class I → A,B & C loci.
  2. Class II or D region → DR DQ & DP loci.
  3. Class III or the complement region → genes for complement components C2 & C4 of the classical pathway, properdin factor B of alternative pathway, heat shock proteins, tumor necrosis factors C.

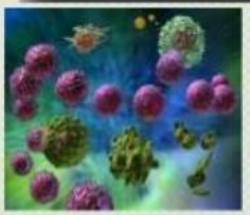


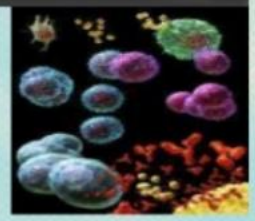




HLA Molecules







There are 2 basic types of T cell

Accessory Molecule	T cell	MHC restriction	Function
CD4	T helper	Class II	Cytokine production help for B cells and cytotoxic T cells
CD8	T cytotoxic	Class I	Cytokine production cytotoxic lysis of infected or tumour cells

Memory T-Cells

- Have previously encountered specific antigens
- Respond in enhanced fashion on subsequent exposures
- Induce secondary immune response

Humoral Immunity

Mediated by **B lymphocytes** which produce **antibodies** or **immunoglobulins (Ig)** in response to antigen challenge

Antibodies: glycoproteins; selective, highly specific; found in γ–globulin fraction of serum (humoral=blood)

Five Classes: physical, chemical and antigenic differences

Classes of Antibodies

IgM: primary immune response (7%); Type III hypersensitivity reaction; immune complexes; B cell receptor

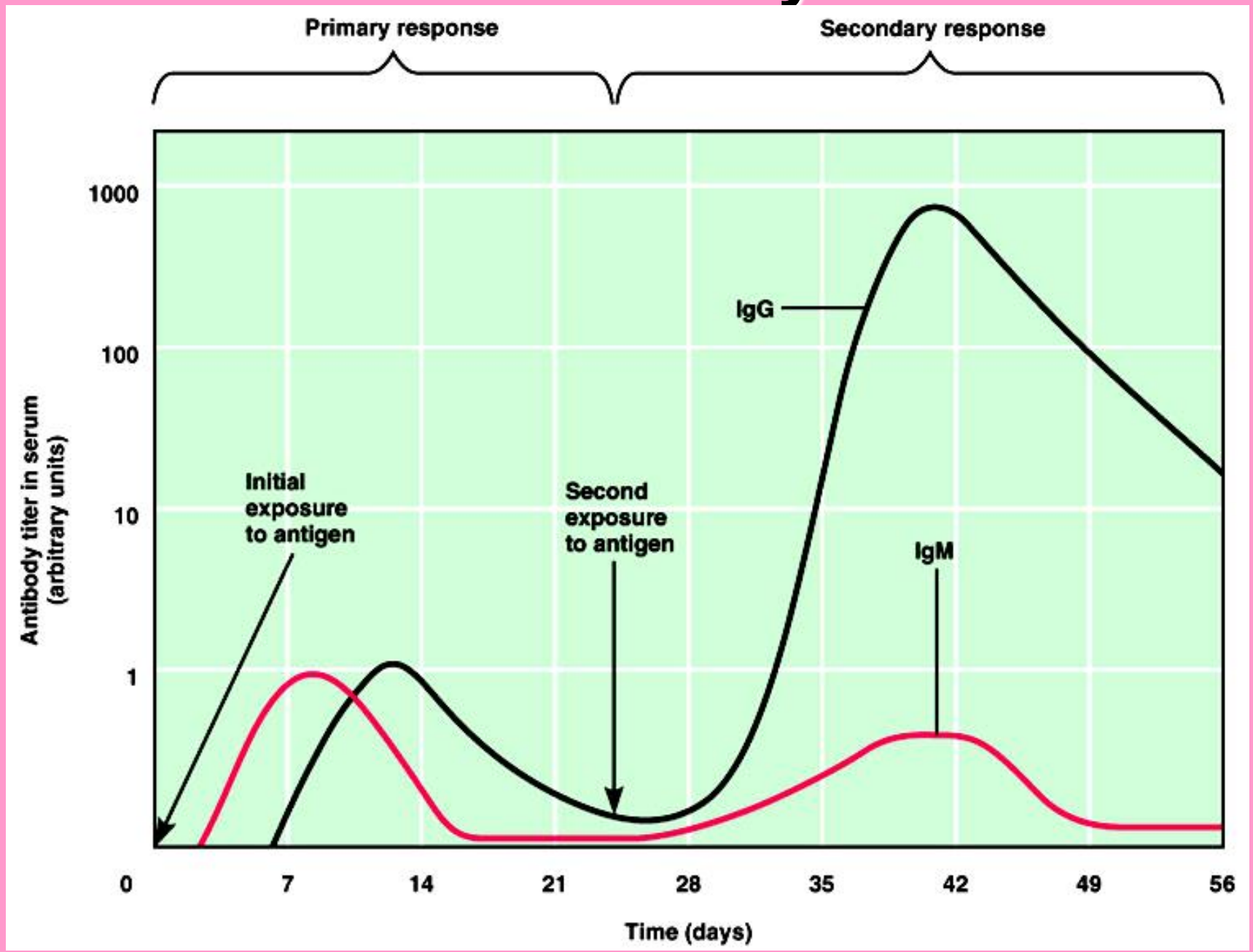
IgG: secondary immune response, B memory cells (70%)

IgA: external secretions, produced locally against bacteria and viruses (15%)

IgE: Type I hypersensitivity reactions, minute amounts

IgD: umbilical cord blood, primitive recognition or regulation; B cell receptor

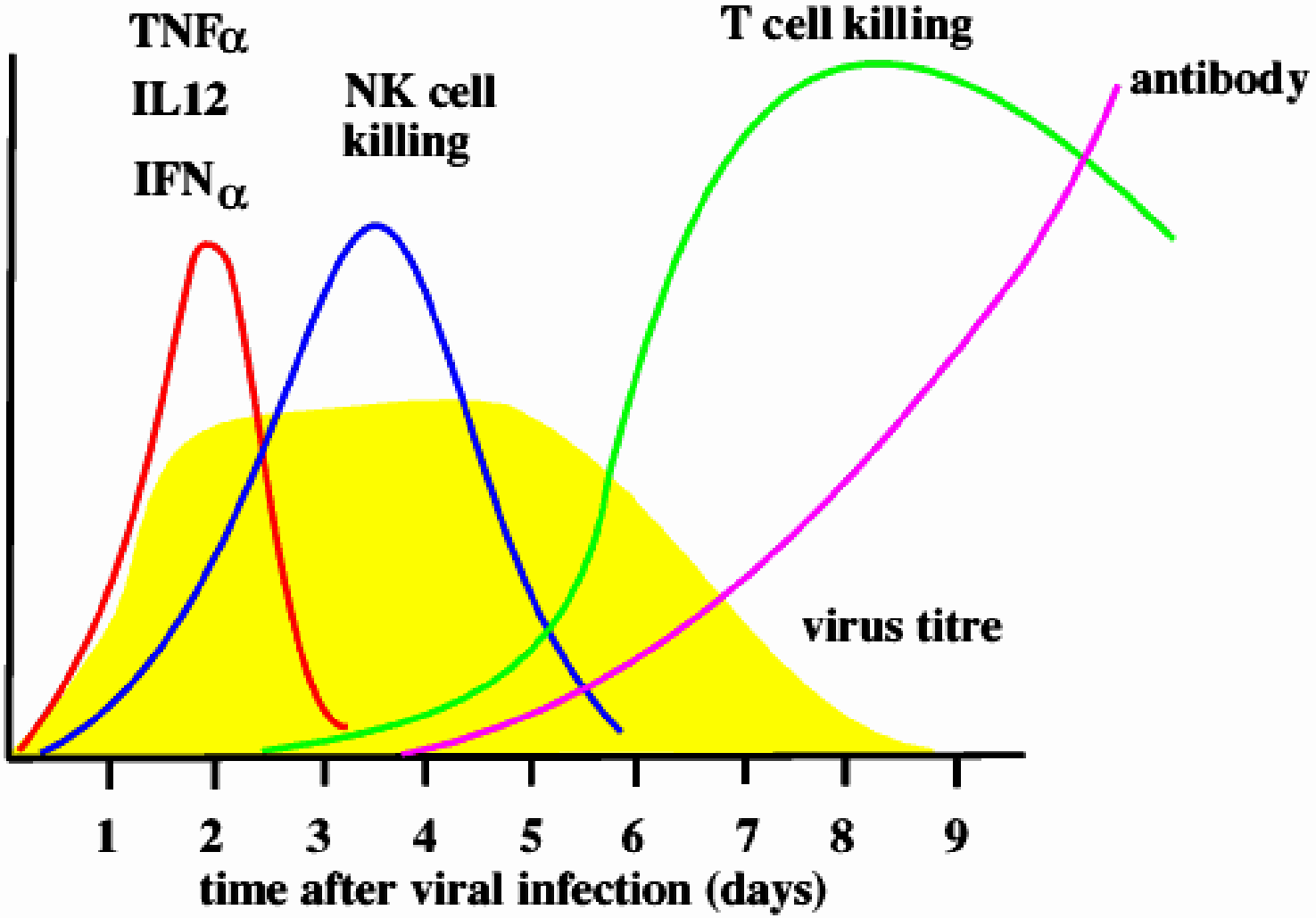
Kinetics of Antibody Production



Summary of the phases of the immune response

	Immediate	Early	Late
	0-4hrs	4-96hrs	>96hrs
Type	Innate	Innate (inducible)	Specific
Key molecules	Complement	Complement	IgM and IgG antibody
	Histamine etc	IL-1,TNFalpha,IL12	IL2,IL4,IL12,IFNgamma
		IFNalpha/beta	
		MBP, CRP	
Key cells	Macrophages	Macrophages	T cells
	Mast cells	Neutrophils	B cells
	Neutrophils	NK cells	Macrophages

Cytokines and NK cells combine to provide early defense against virus infections

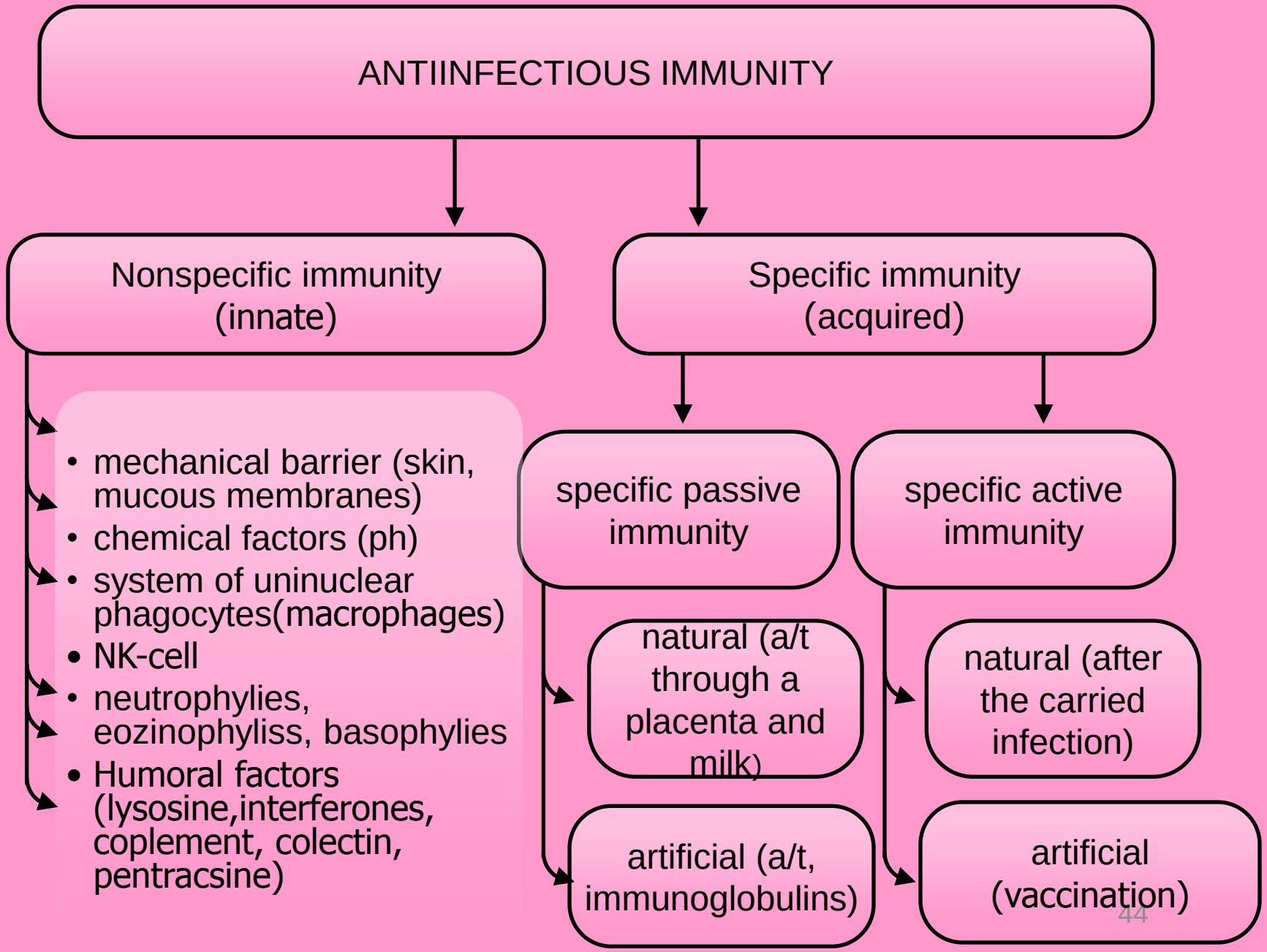


Summary of Immunity to different types of pathogens

class	subclass	key immune mechanisms	memory?	example
Extracellular				
	bacterial			
		complement (alternative pathway)	No	Staph aureus
		phagocytosis via innate receptors	No	Strep pneumoniae (capsule -ve)
		IgM/IgG antibody/ complement (CP)	Yes	Staph aureus
		IgG/IgM iC3bR/FcγR phagocytosis	Yes	Strep pneumoniae (capsule+ve)
	helminths			
		IgG/ADCC (granulocytes+eosinophils)	Yes	Schistosoma mansoni

Summary of Immunity to different types of pathogens

class	subclass	key immune mechanisms	memory?	example
Intracellular				
	bacteria + protozoa	activated macrophage (by NK cells)	No	Listeria monocytogenes
		activated macrophage (by T_H1 cells)	Yes	Leishmania major
		cytotoxic T cell	Yes	Chlamydia trachomatis
	viruses	interferon α/β	No	Influenza virus
		NK cell killing	No	Cytomegalovirus
		Cytotoxic T cell killing	Yes	Smallpox (Variola major)
		activated macrophage (by T_H1 cells)	Yes	Herpes Simplex virus
		neutralising antibody	Yes	Influenza virus

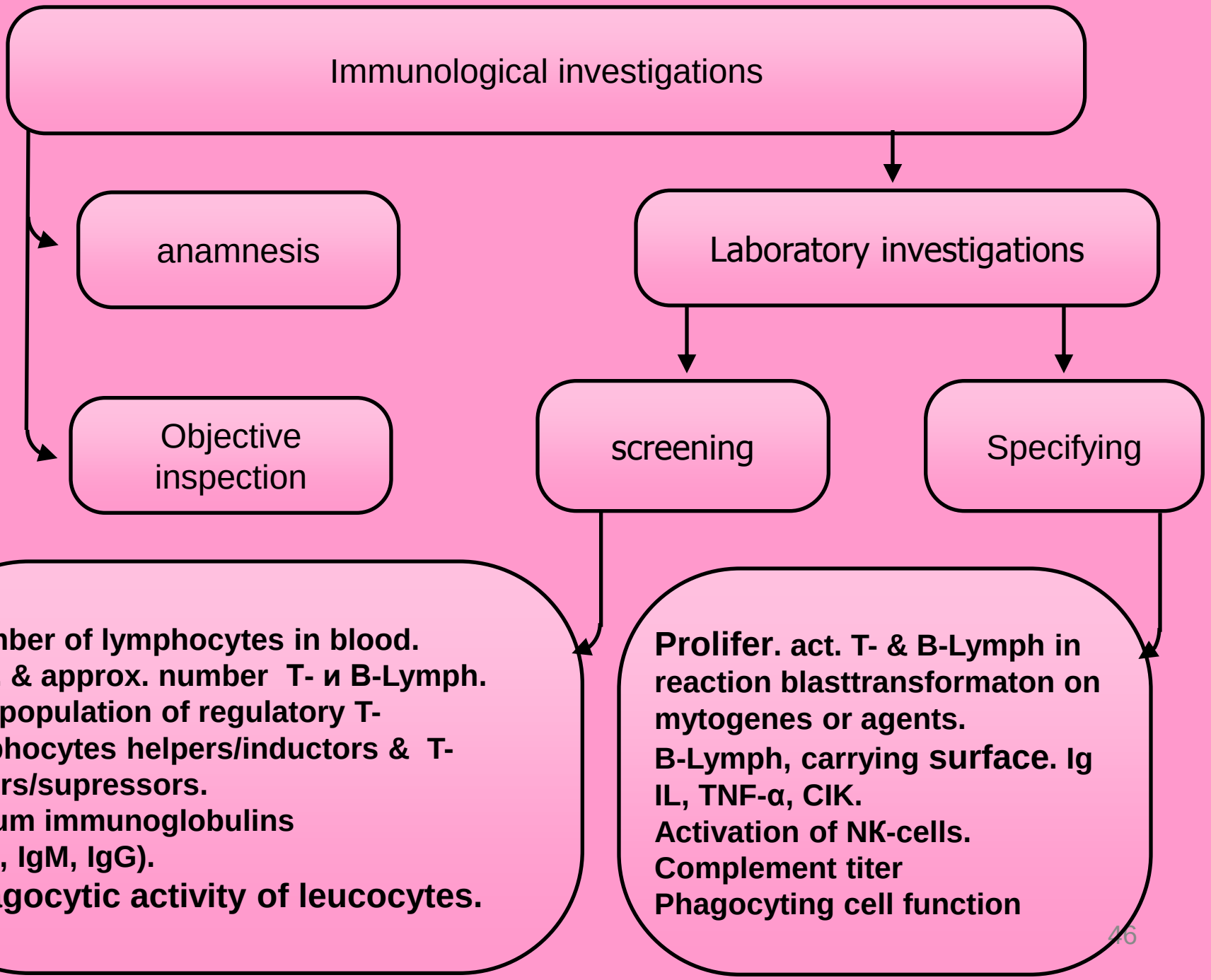

ANTIINFECTIOUS IMMUNITY
Nonspecific immunity (innate)
Specific immunity (acquired)
mechanical barrier (skin, mucous membranes)
chemical factors (ph)
system of uninuclear phagocytes(macrophages)
NK-cell
neutrophylies, eozinophyliss, basophylies
Humoral factors (lysosine,interferones, coplement, colectin, pentracsine)
specific passive immunity
specific active immunity
natural (a/t through a placenta and milk)
artificial (a/t, immunoglobulins)
natural (after the carried infection)
artificial (vaccination)

IMMUNE STATUS OF ORGANISM

- Immune status of organism is quantitative and functional descriptions of separate links of the immune system on the certain stage of development of organism or on the certain stage of development of disease

- PRINCIPLES OF DETERMINATION OF IMMUNE STATUS:

estimation;
-determining the connection of quantitative indexes with their functional activity;
-determining the connection of immunological mechanisms with the clinical features of disease;
-account of connection of individual reactivity with genetic factors;
-comparing of indexes of immune status of organism to the norm;
-looking after the state of immune status in dynamics


Immunological investigations
anamnesis
Laboratory investigations
Objective inspection
screening
Specifying
Number of lymphocytes in blood.
abs. & approx. number T- и B-Lymph.
Subpopulation of regulatory T-limphocytes helpers/inductors & T-killers/supressors.
Serum immunoglobulins (IgA, IgM, IgG).
phagocytic activity of leucocytes.
Prolifer. act. T- & B-Lymph in reaction blasttransformaton on mytogenes or agents.
B-Lymph, carrying surface. Ig
IL, TNF-α, CIK.
Activation of NK-cells.
Complement titer
Phagocyting cell function

Variants of changes of laboratory indexes of immunity

Syndrome	Indexes of immunogramm
Signs of infectious syndrome	**Decline of natural resistance, level of T-cell, IRI<1,5 due to T-helpers, decline of level of V-lymphocytes, immunoglobulins.**
Signs of allergic syndrome	**Decline of level of T-cell, IRI >3,5 due to the increase of T-helpers and decline of T-suppressor-cell, increase of V-lymphocytes and level of IGE, positive tests of hypersensitiveness of immediate or slow type.**
Signs of autoimmune syndrome	**Decline of level of T-cell, IRI>3,5 due to the considerable increase of level of T-helpers and decline of level T-suppressor-cell, increase of level of V-lymphocytes, Circulatory immune complexes, decline of indexes of phagacytosis and complements, presence of specific autoantibodies.**
Combined violations	**mixed changes with approximately equivalent defects .**

Immunogram in acute bacterial infection (pneumonia), the patient L., 22 years old

Index		Result		Rate				
Hemoglobin		102		F - 115- 145. M- 132 - 164 g/l				
Erythrocytes		3,1		F – 3,7 – 4,7. M - 4.0 - 5.1x10^{12} /l				
Platelets		160		150 - 320x10^{9}/l				
ESR		42		2-15 mm/h				
Leukocytes		10,1		4-9x10^{9}/l				
Neutrophils 43-71 % 2000-6500	Stick-nuclear 1 - 4 % 80-400	Segment-nuclear	Eosinophils 0,5 - 5% 80-370	Basophils 0-1% 20-80	Monocytes 3 - 9% 90-720	Lymphocytes 25 -37% 1600-3000	Large granular lymphocytes 1-5% 80-500	Plasmocytes 0-1% 20-80
84	9	75	0	0	7	9	0	0
8480	900	7580	0	0	710	910	0	0

Immunological indexes		Result	Rate	Immunological indexes		Result	Rate
T- lymph. CD3	%	49	50 - 80	Ig G		26,34	8,0-18,0 g\l
	The abs. number	446	1000-2200				
T-help. CD4	%	29	33-46	IgM		2,96	0,2-2,0 g\l
	The abs. number	263	309-1571				
T-suppress. CD8	%	21	17-30	Ig A		5,36	0,3-3,0 g\l
	The abs. number	191	282-999				
Immune regulatory index	CD4/CD8	1,38	1,4-2,0	CIC		217	30 - 50 IU. density.
NK-cells CD16	%	25	12-23	Power activity	Ph. Index		60 - 80%
	The abs. number	227	72-543		Ph. Value		1,5-3,5
B-lymph. CD22	%	23	17-31	NBT-test	spontaneous		to 10%
	The abs. number	209	109-532		Inductive		-

Immunogram during acute viral infection. Patient 26 years old.

Index		Result	Rate					
Hemoglobin		134	F- 115-145. M- 132 - 164 g/l					
Erythrocytes		3,9	F – 3,7-4,7. M-4,0-5,1x10^{12}/l					
Platelets		270	150 - 320x10^{9}/l					
ESR		18	2-15 mm/h					
Leukocytes		10,2	4-9x10^{9}/l					
Neutrophils 43-71 % 2000-6500	Stick-nuclear 1 -4% 80-400	Segment-nuclear	Eosinophils 0,5 - 5% 80-370	Basophils 0-1% 20-80	Monocytes 3 - 9% 90-720	Lymphocytes 25 -37% 1600-3000	Large granular lymphocytes 1-5% 80-500	Plasmocytes 0- 1% 20-80
36	5	31	0	0	11	43	10	0
3670	510	3160	0	0	1120	4390	1020	0

Immunological indexes		Result	Rate	Immunological indexes		Result	Rate
T-lymph. CD3	%	60	50 - 80	IgG		19,8	8,0-18,0 g/l
	The abs. number	2630	1000-2200				
T-help. CD4	%	24	33-46	IgM		3,25	0,2-2,0 g/l
	The abs. number	1053	309-1571				
T- uppress. CD8	%	36	17-30	IgA		2,07	0,3-3,0 g/l
	The abs. number	1580	282-999				
Immune regulatory index	CD4/CD8	0,67	1,4-2,0	CIC		65	30 – 50 IU Density
NK-cells CD16	%	29	12-23	Power activity	Ph. Index	83	60 - 80%
	The abs. number	1270	72-543		Ph. value	4,2	1,5-3,5
B-lymph. CD22	%	16	17-31	NBT -test	spontaneous	15	to 10%
	The abs. number	480	109-532		Inductive	32	-