Scientific Practice
Block 2, 2018

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GH700
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Week 1: intro and innate immunity
Week 2: adaptive immunity
Week 3: vaccines

PRAC: ELISA
VENUE: FNB2A 53 PCs and FNB2B 69 PCs
Date: Monday 7 May
Time: 14:00-17:00

TEST
Monday 14 May 2pm.
Flower Hall
1 hour (50 marks)

Textbooks

Janeway et al. 2001. Immunobiology, 5th edition.
Garland Science: NY
NB: this textbook is available free online at:
http://www.ncbi.nlm.nih.gov/books/NBK10757/

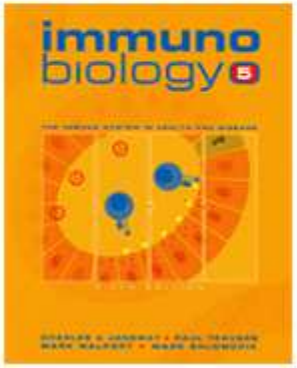
(or any other introductory Immunology textbook).
or
http://missinglink.ucsf.edu/lm/immunology_module/prologue/prologuehome.html

NCBI Resources How To

Bookshelf Books

Browse Titles Limits Advanced

By agreement with the publisher, this book is accessible by the search feature, but cannot be browsed.



Immunobiology, 5th edition

The Immune System in Health and Disease

Charles A Janeway, Jr, Paul Travers, Mark Walport, and Mark J Shlomchik.

Author Information

New York: Garland Science; 2001.

ISBN-10: 0-8153-3642-X

cell types Search this book

Contents

Immunology course outline and learning objectives

A. General introduction

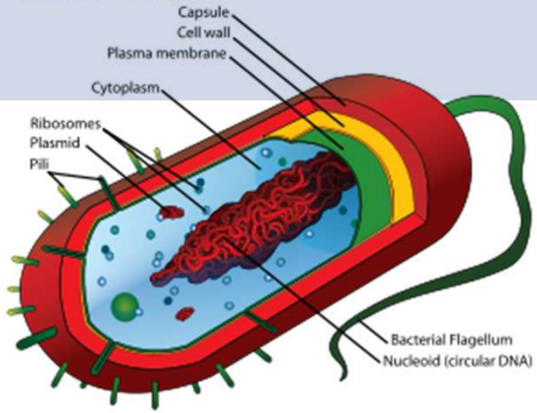
- Define basic words such as host, pathogen, micro-organism, immunology, microbiology and immune system
- Explain Koch's postulates
- Explain where in the body the immune system is found
- Describe and recognise the different types of immune cells found in human blood; identify cell subsets using CD surface markers
- Describe / explain the basic functions of immune cells, including phagocytosis, antigen presentation, and release of various effector substances .
- Compare and contrast characteristics of innate vs. adaptive immunity

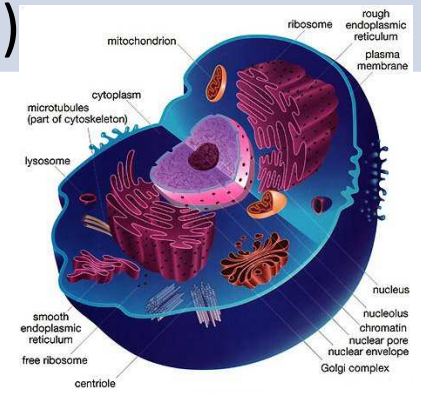
host-pathogen interactions (immunology)

What are micro-organisms?

- microscopic organism (view with a microscope)
- viewed since 1670s (Anton van Leeuwenhoek)
- Can be single-celled or multicellular
- prokaryotes or eukaryotes (without vs. with a cell nucleus)
- Most often refers to bacteria, archaea, virus*, fungus
- The study of microorganisms is called microbiology
- Robert Koch 1891 confirmed microorganisms as the cause of infectious disease (Nobel prize)

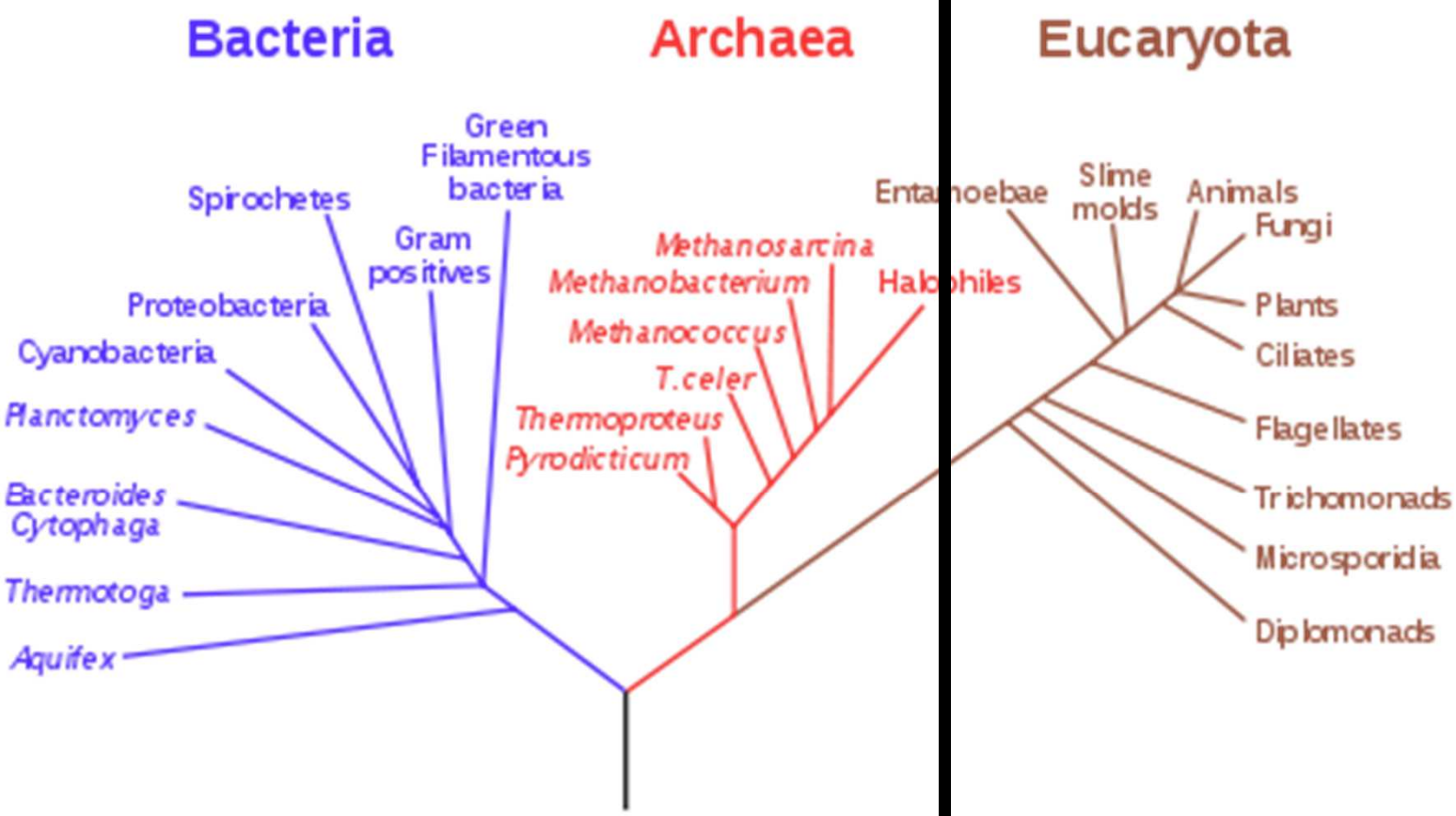
	Prokaryotes (Bacteria and Archaea)	eukaryotes:
Number of cells and cell size	Unicellular cells <5um	Unicellular or multicelluar ; cells >10um
Nucleus	no	Yes
Internal membranes and organelles	no	Yes
Cytoskeleton	no	Yes
Cell wall	yes	Sometimes (plants, fungi)





prokaryotes

eukaryotes

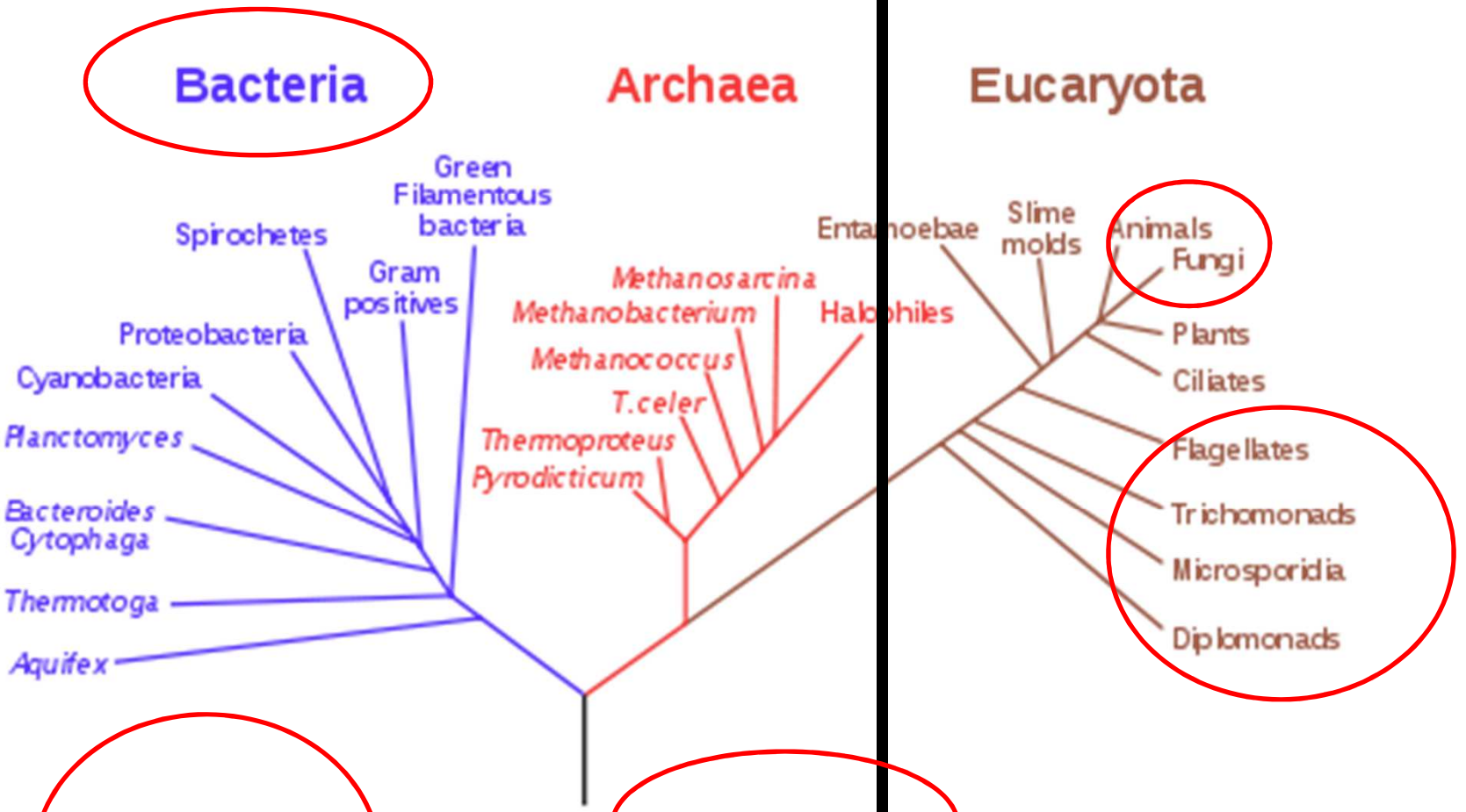


Viruses?

Woese's three domains of life (1990)

prokaryotes

eukaryotes



Common pathogens in humans

Viruses

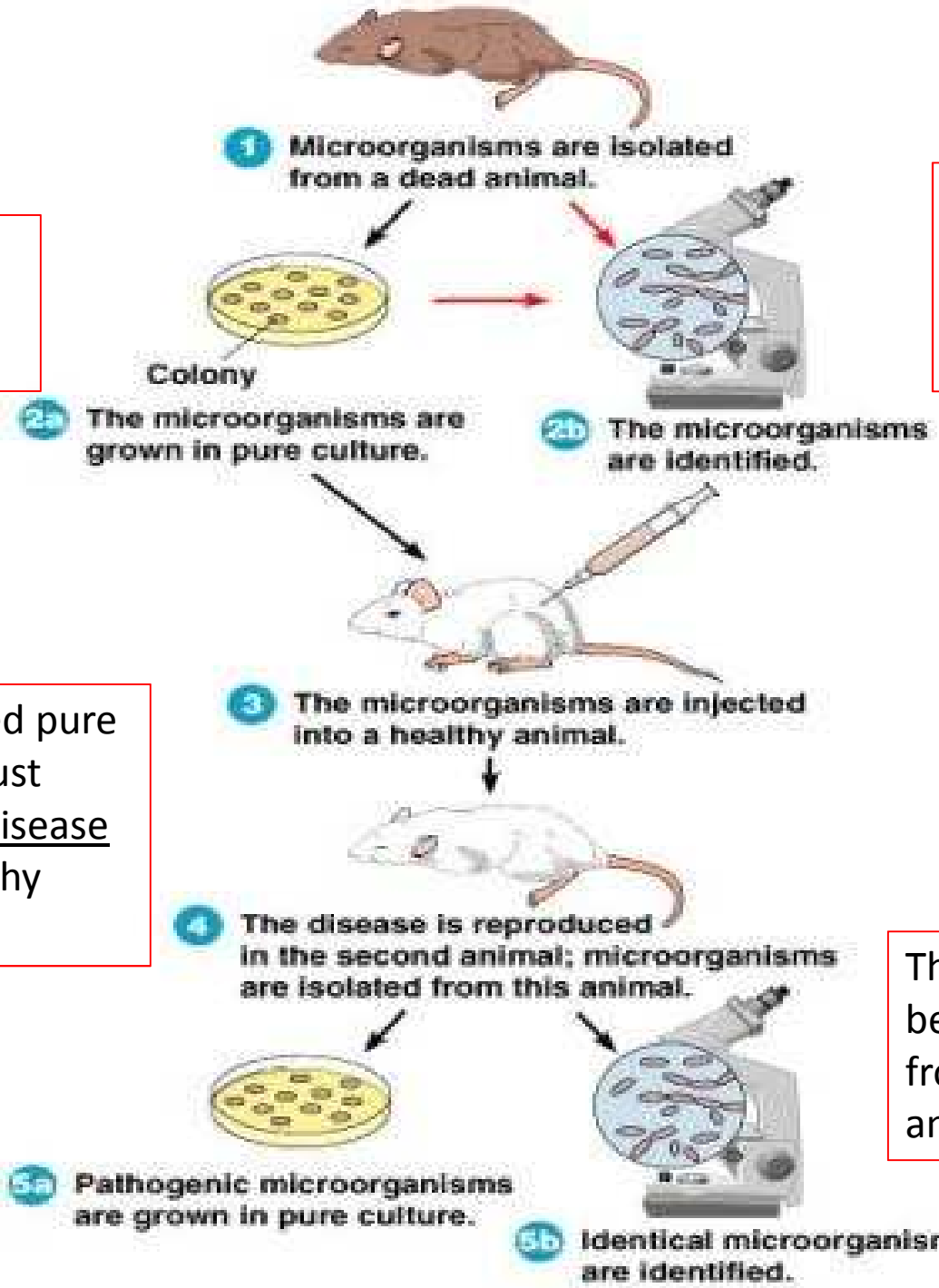
How do you prove that a specific microbe causes a specific disease? Koch's postulates

- Robert Koch, Germany, 1876
- Proved that the bacteria called *Bacillus anthracis* caused anthrax in cattle

Suggested that:

1. The same microbe must be present in every case of the disease
2. The microbe must be isolated, identified and grown in culture, then injected into a healthy animal
3. The injected pure microbe must cause the disease in the healthy animal
4. The same microbe must be present in / isolated from the newly infected animal

The microbe must be <u>isolated and</u> identified



The same microbe must be present in <u>every case</u> of the disease

The injected pure microbe must <u>cause the disease</u> in the healthy animal

The same microbe must be <u>present in</u> / isolated from the newly infected animal

host-pathogen interactions (immunology)

What is a pathogen?

- an infectious micro-organism that causes disease

- Not all micro-organisms are pathogens
- microbiome: the community of microorganisms that live on and in you (~ 1-2 kg of your body weight); are not usually pathogenic

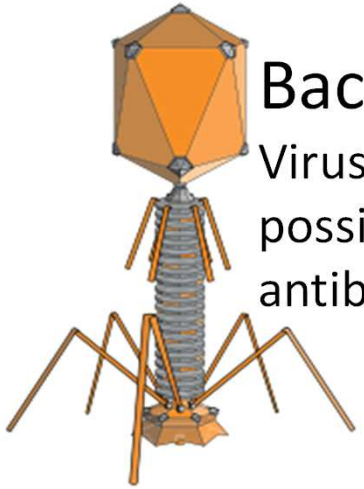
What is the host?

- It is the species / individual that gets infected and gets sick from the pathogen / mounts an immune response
- Pathogens are species-specific e.g. HIV vs SIV
- Any form of life can be a host

Some examples of common pathogens not affecting humans

plant fungus infection

Potato blight caused a famine in Ireland in the 1800s

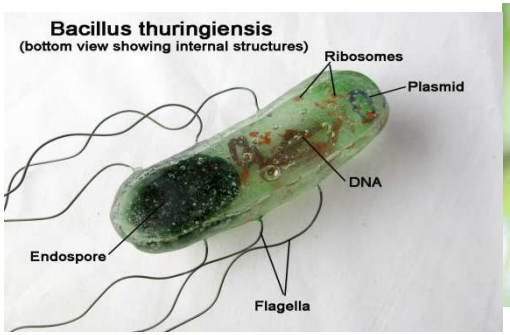


Bacteriophage

Viruses that infect bacteria; possible alternative to some antibiotics

Cattle virus

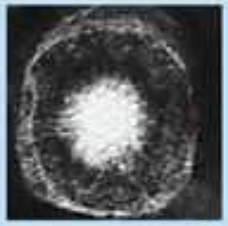
Rinderpest killed 90% of cows in SA in the 1890s (virus now eradicated)



Bacillus thuringiensis (Bt)

This bacteria kills particular insects that eat it ; has been used in commercial insecticides

Some examples of common human pathogens

Type of pathogen	Description	Human diseases caused by pathogens of that type
Bacteria *Escherichia coli* 	Single-celled organisms without a nucleus	Strep throat, staph infections, tuberculosis, food poisoning, tetanus, pneumonia, syphilis
Viruses *Herpes simplex* 	Thread-like particles that reproduce by taking over living cells	Common cold, flu, genital herpes, cold sores, measles, AIDS, genital warts, chiken pox, small pox
Fungi *Death cap mushroom* 	Simple organisms, including mushrooms and yeasts, that grow as single cells or thread like filaments	Ringworm, athlete's foot, tinea, candidiasis, histoplasmosis, mushroom poisoning
Protozoa *Giardia lamblia* 	Single-celled organism with a nucleus	Malaria, "traveler's diarrhea" giardiasis, trypanosomiasis ("sleeping sickness")

Immunology

- Immunology is the study of our protection from foreign macromolecules or invading organisms and our responses to them.
- Host – e.g. me!!!!
- Foreign macromolecule, antigen – e.g. virus protein, worm, parasite (Everything that should not be in my body)

Immunology = Study of the immune system

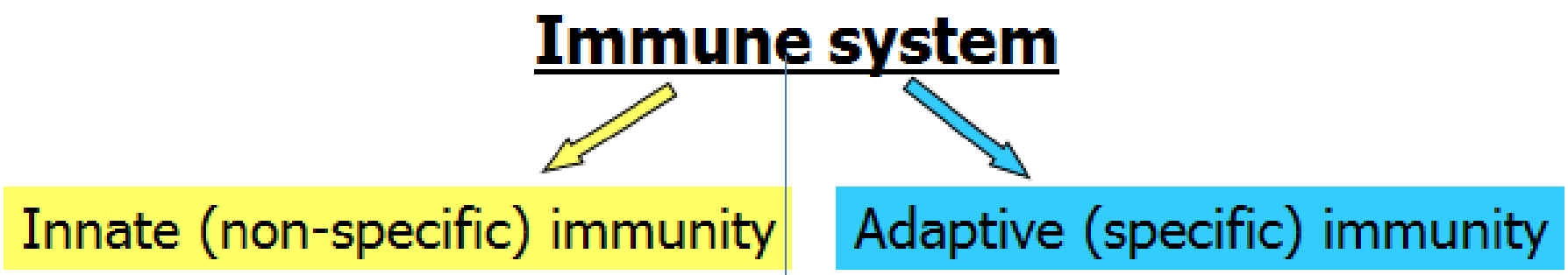
The immune system is:

- A collection of specialised tissues and cells that can
  - Recognise pathogens
  - distinguish self vs non-self
  - React to eliminate pathogens

- Who/what has an immune system?
- Unicellular prokaryotes like bacteria have very simple defense systems: enzymes, CRISPR
- Eukaryotes: invertebrates, plants, vertebrates all have innate immunity (cell-based)
- jawed vertebrates have particularly advanced immune systems (adaptive immunity)

Innate vs adaptive immunity

Human immunology:
how human immune systems respond to pathogens



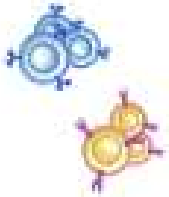
- Components naturally exist already or respond very fast to patterns, not very specific
- does not have to be taught or does not have to develop over time
- No memory

- Components respond to what you get infected with, responds to specific infections
- Has memory: remembers previous infections / responds better to them on subsequent infection

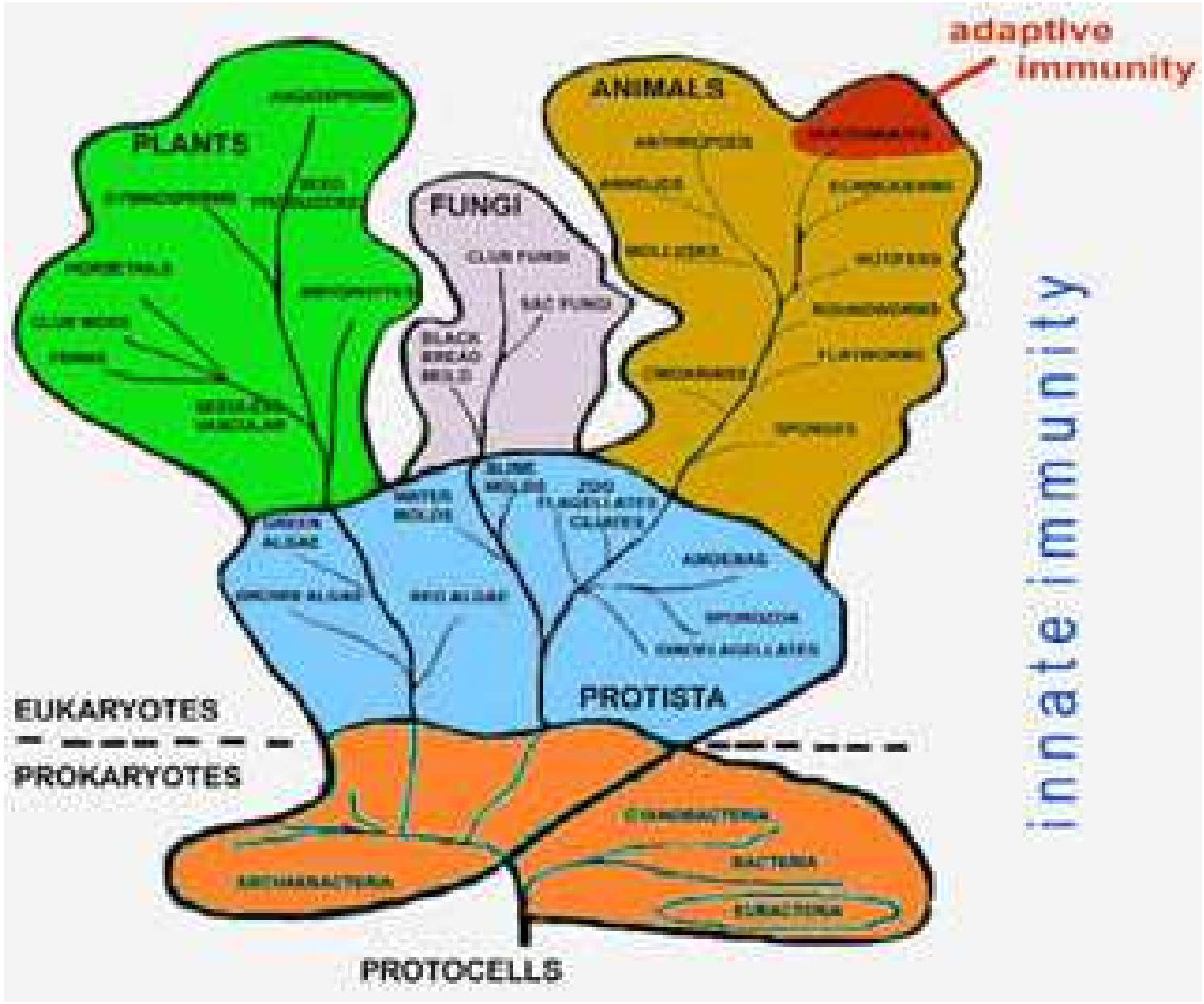
Types of Immunity

- **Innate Immunity**
  - Host defense mechanisms that act from the start of an infection but do not adapt to a particular pathogen
  - Recognize “patterns’ of a.a., saccharides, etc..
- **Adaptive Immunity**
  - Response of an antigen specific B and T lymphocytes to an antigen
  - Immunological memory

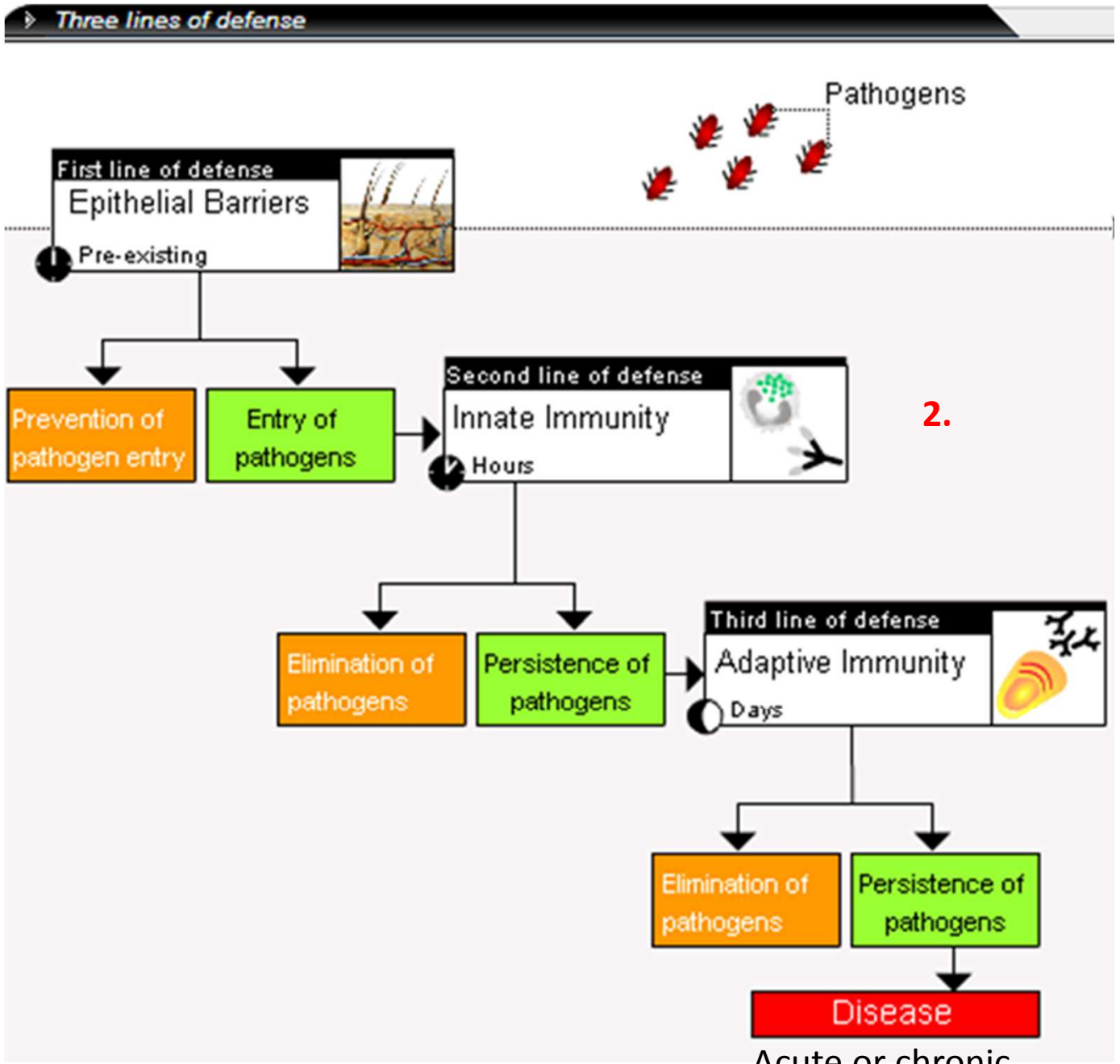
	INNATE	**ADAPTIVE**
What it recognises	Not specific – recognises pathogen patterns	Very specific - recognises particular pieces of particular pathogens
Receptors	Small set / limited variety	Very large variety
How fast it reacts	Immediate responses (hours)	Takes some time to develop (days) (unless memory response)
Memory?	no	yes - increased response on next exposure
In which species it is found	All vertebrates	Only jawed vertebrates
Cells and tissues	Barriers: Anatomical and physiological, inflammation Cells: phagocytes, Nk cells Humoral: complement, antimicrobial peptides	— Cells: B and T cells Humoral: antibodies

	Innate immunity	Adaptive Immunity
Components	 1. Physical and chemical barriers 2. Phagocytic leukocytes 3. Dendritic cells 4. Natural Killer cells 5. Plasma proteins (complement)	 1. Humoral immunity (B cells, which mature into antibody secreting plasma cells) 2. Cell-mediated immunity (T cells, which mature into effector helper and cytotoxic T cells)
Activity	Always present	Normally silent
Response and potency	Immediate response, but has a limited and lower potency	Slower response (over 1-2 weeks, but is much more potent
Specificity	General: can recognize general classes of pathogens (i.e. bacteria, viruses, fungi, parasites) but cannot make fine distinctions	Recognizes highly specific antigens
Course	Attempts to immediately destroy the pathogen, and if it can't, it contains the infection until the more powerful adaptive immune system acts.	Slower to respond; effector cells are generally produced in 1 week and the entire response occurs over 1-2 weeks. However, this course can vary somewhat during different responses in an individual.
Memory?	No--reacts with equal potency upon repeated exposure to the same pathogen.	 Yes--memory cells "remember" specific pathogens; upon re-exposure to a pathogen, these cells mount a much faster and more potent second response

Reset Show all


adaptive
immunity
PLANTS
FUNGI
ANIMALS
innate immunity
EUKARYOTES
PROKARYOTES
PROTISTA
PROTOCELLS

1.

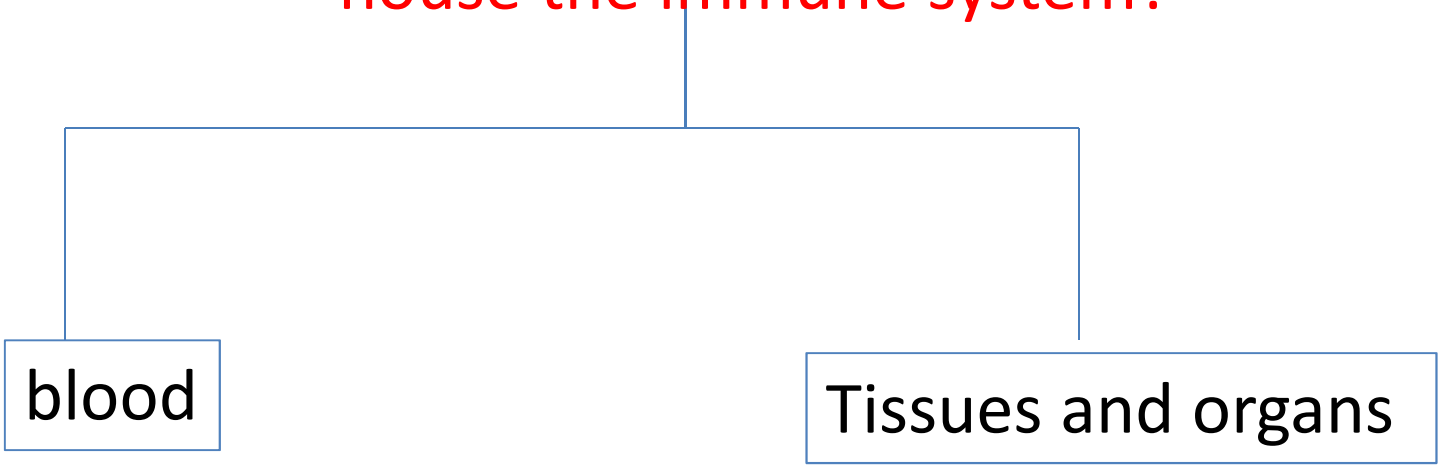


2.

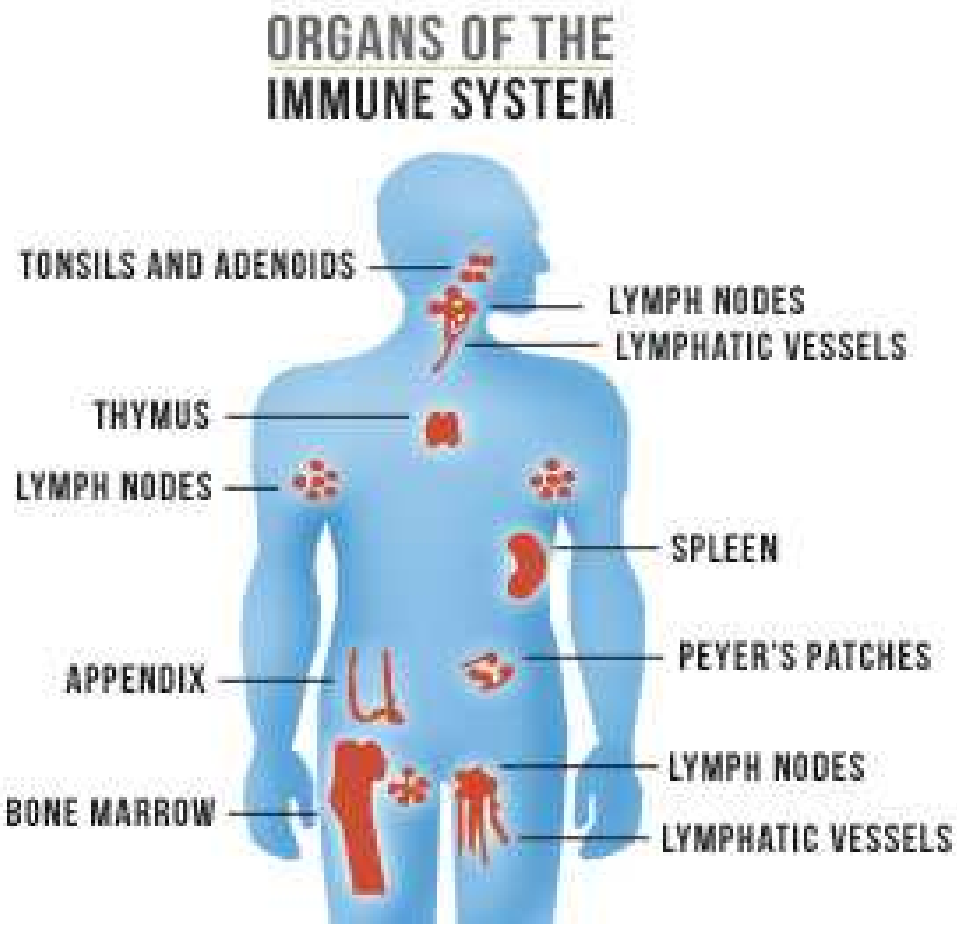
Acute or chronic

This course: focus on human immunology
Innate and adaptive responses

Which parts of the body house the immune system?

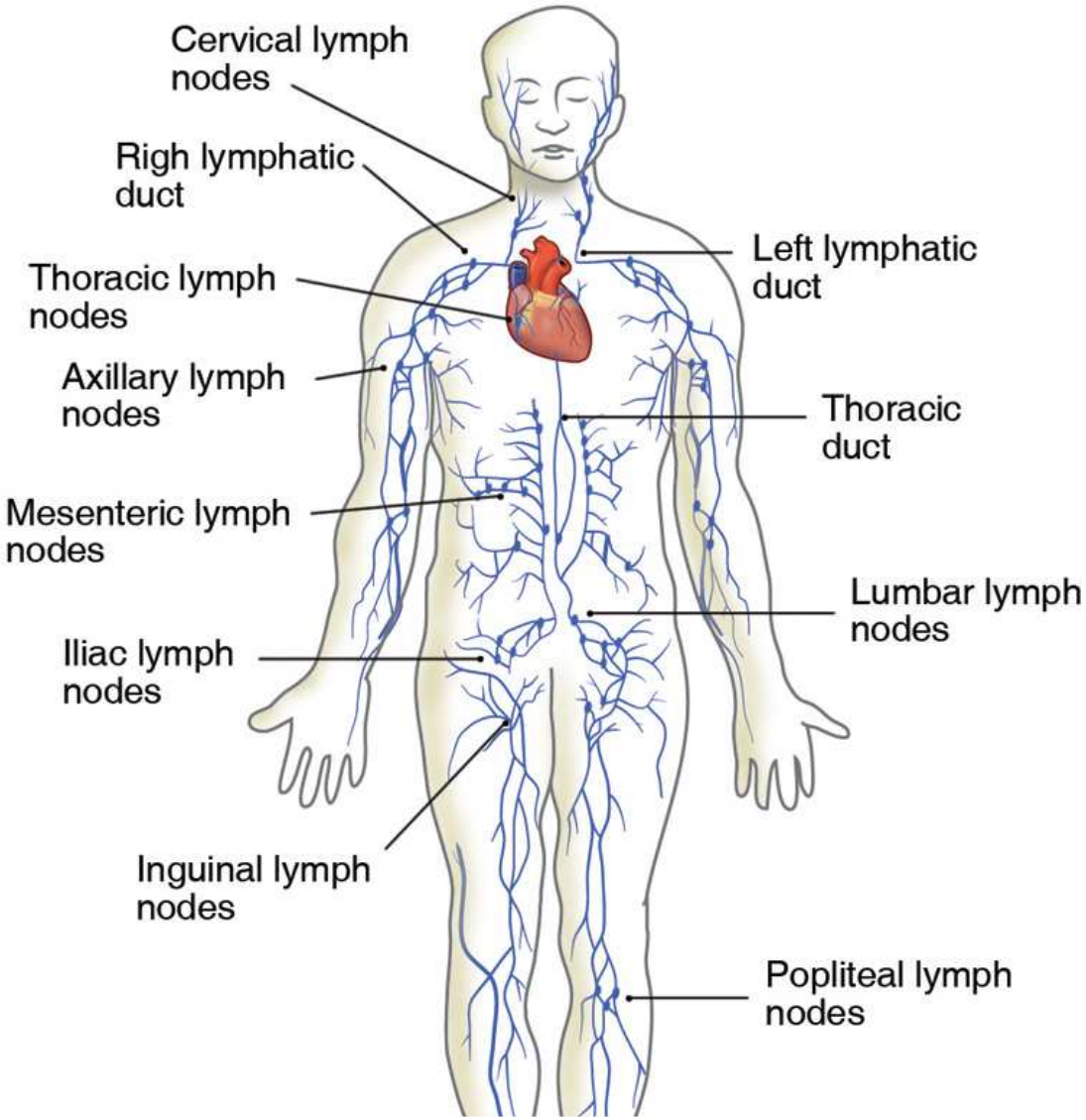


(tissue fluid; lymph)



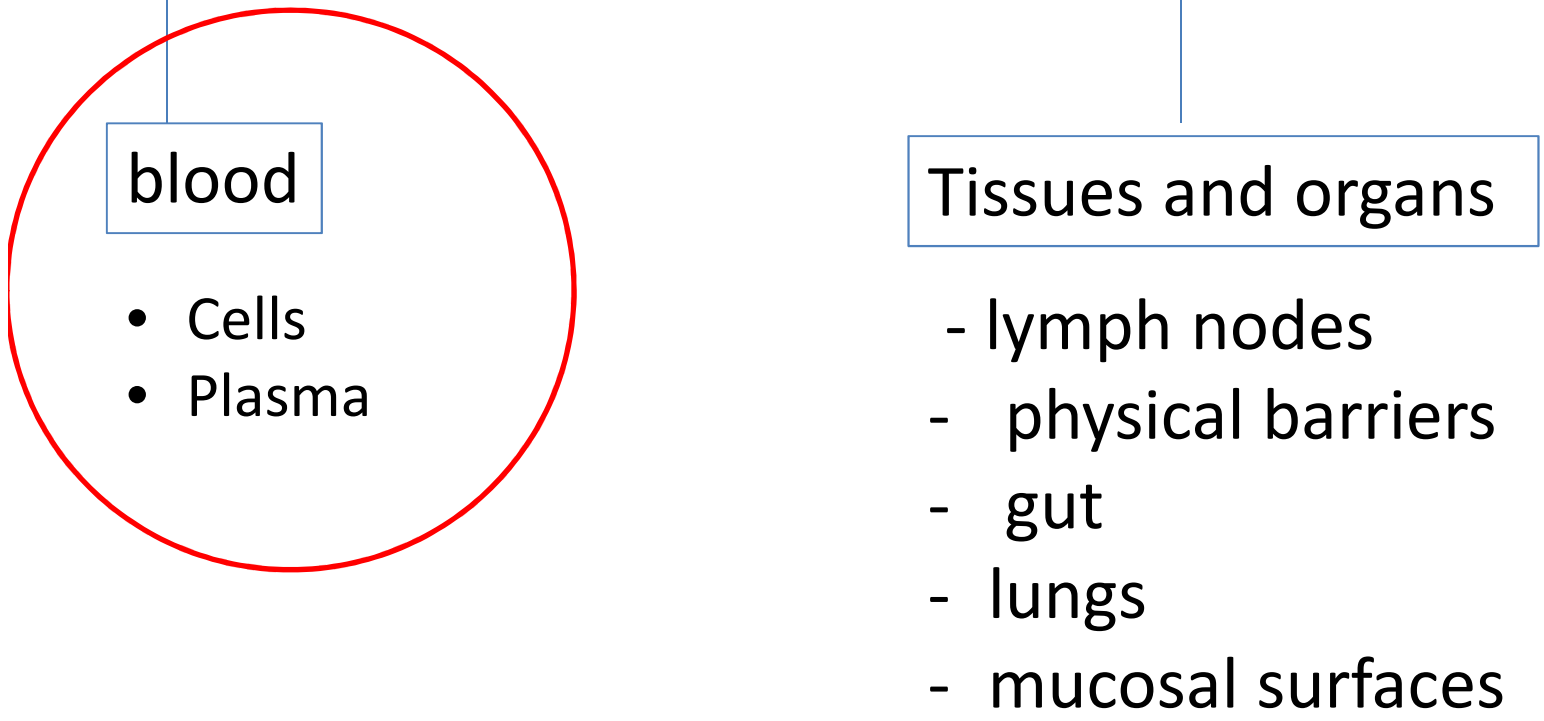
- lymph nodes and vessels
- physical barriers
- Specialised organs like appendix, tonsils
- gut and lungs
- mucosal surfaces
- Bone marrow and thymus

The Lymphatic System



- Lymph is very similar to blood plasma.
- it contains lymphocytes and other white blood cells as well as tissue fluid
- It also contains waste products and cellular debris together with bacteria and proteins.
- The lymph circulator system is not closed like blood vessels.
- Tissue fluid enters lymph system and is returned to blood via the ducts into the subclavian veins
- Lymphocytes are concentrated in the lymph nodes.

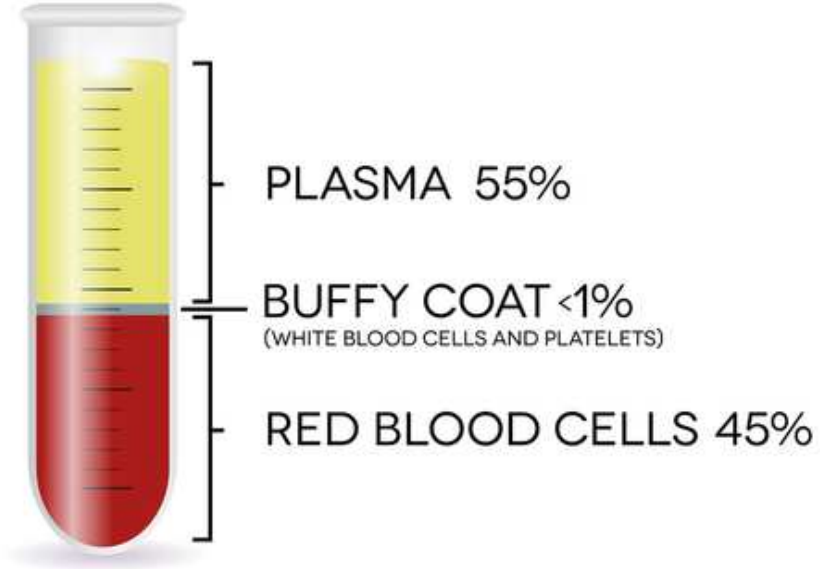
Which parts of the body house the immune system?



Cell types in human blood

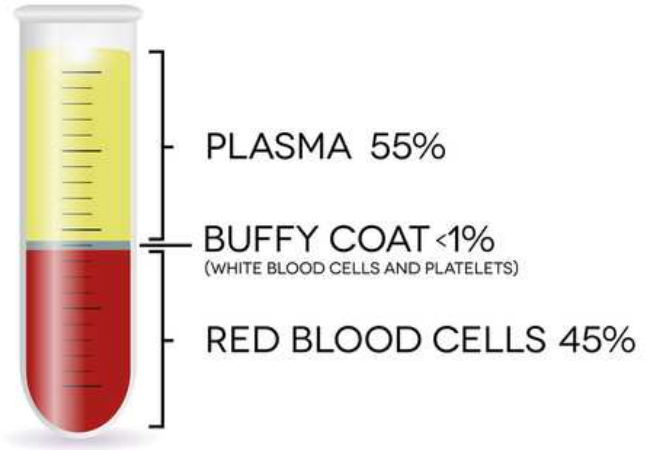
If you draw a tube of blood and either let it stand for an hour or centrifuge it gently, it will separate into 3 layers:

- 1. liquid layer = plasma
- 2. White layer or buffy coat, includes
  - white cells = leukocytes
  - platelets = thrombocytes
- 3.Red cells = erythrocytes



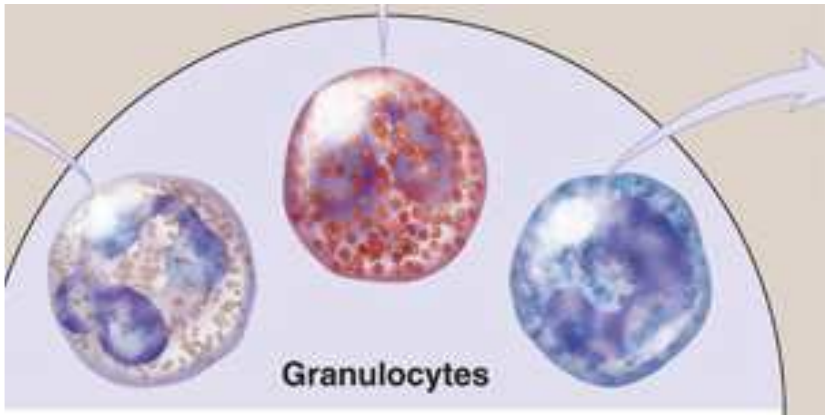
Cells in human blood – the immune system

- liquid layer = plasma (contains immune system non-cellular components such as antibodies, complement etc)
- white cells = leukocytes (your immune cells)
- platelets =thrombocytes (for clotting)
- Red cells = erythrocytes (carry oxygen)

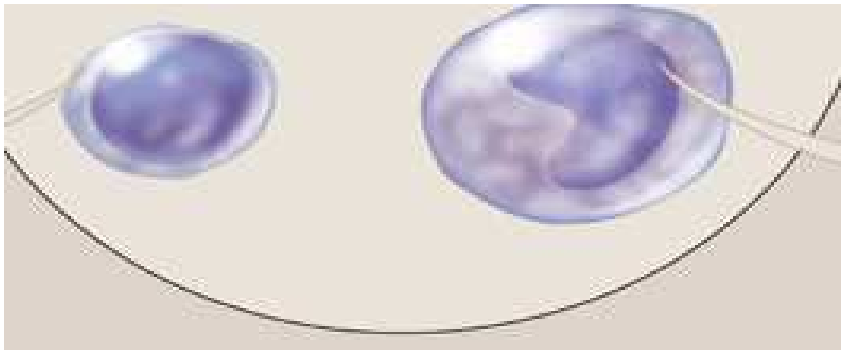


Leukocytes
(white blood cells)

- Granulocytes
- lymphocytes
- monocytes

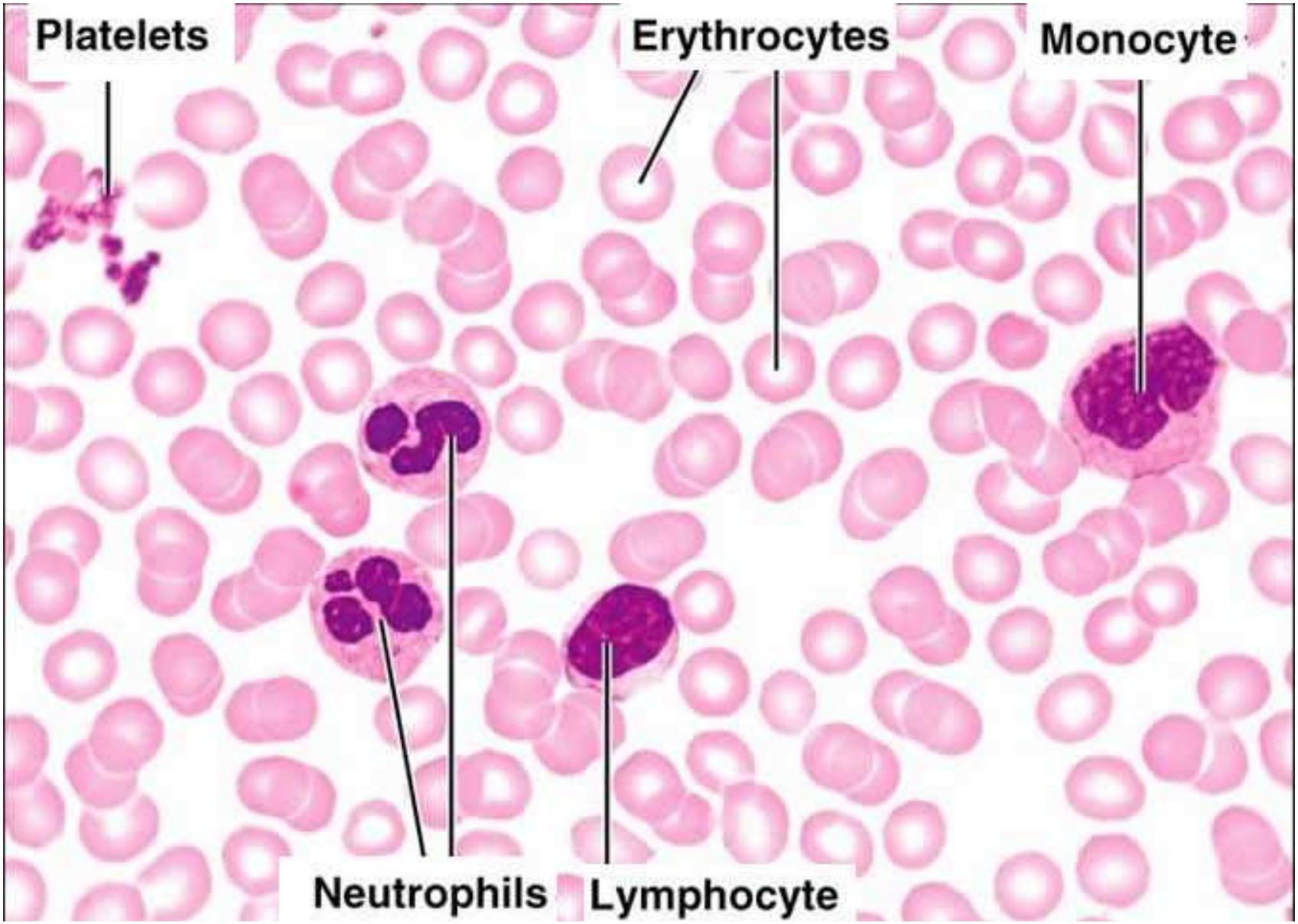


Under microscope, these cells contain lots of small granules, and the nucleus is divided into sections. Also called polymorphonuclear (PMN) cells.



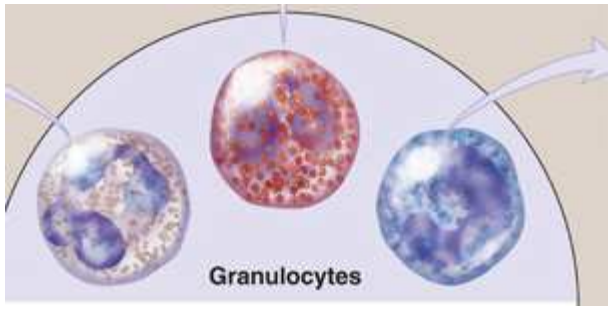
Under microscope, these cells have no granules, and the nucleus is one smooth shape .
Monocytes are a bit larger and have rougher edges than lymphocytes

Blood under microscope / types of blood cells



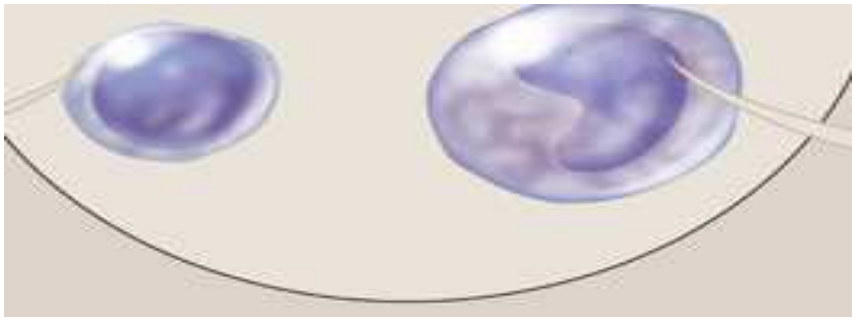
Leukocytes (white blood cells)

Granulocytes



- Neutrophils
- basophils
- eosinophils

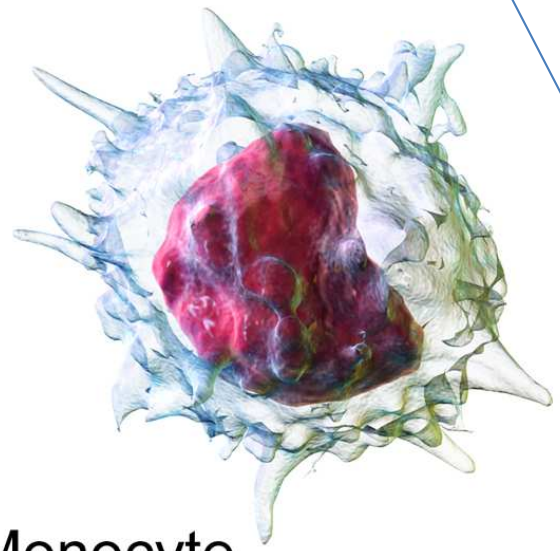
lymphocytes



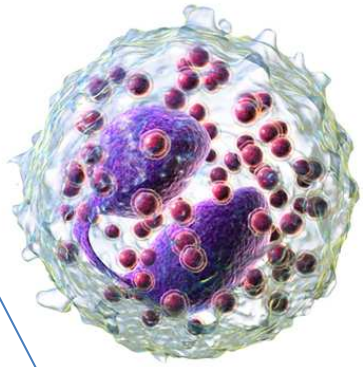
- T cells
- B cells
- NK cells

monocytes

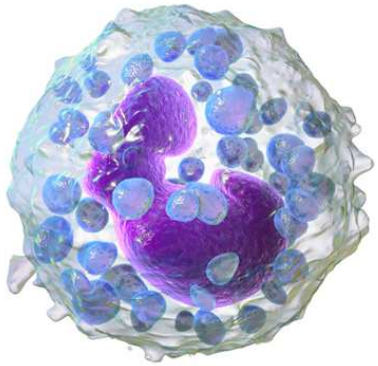
- Monocytes
- (develop into macrophages and dendritic cells in tissue)



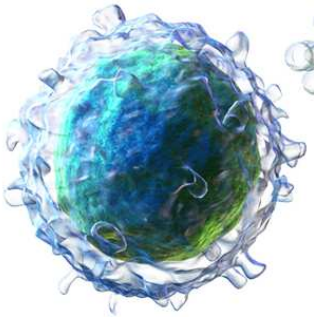
Monocyte

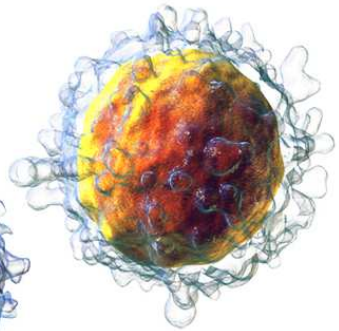


Eosinophil

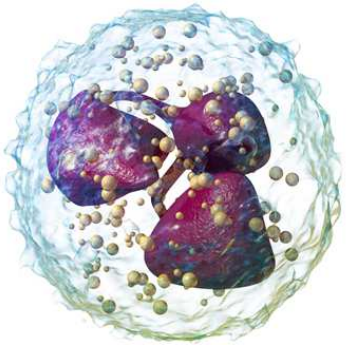


Basophil





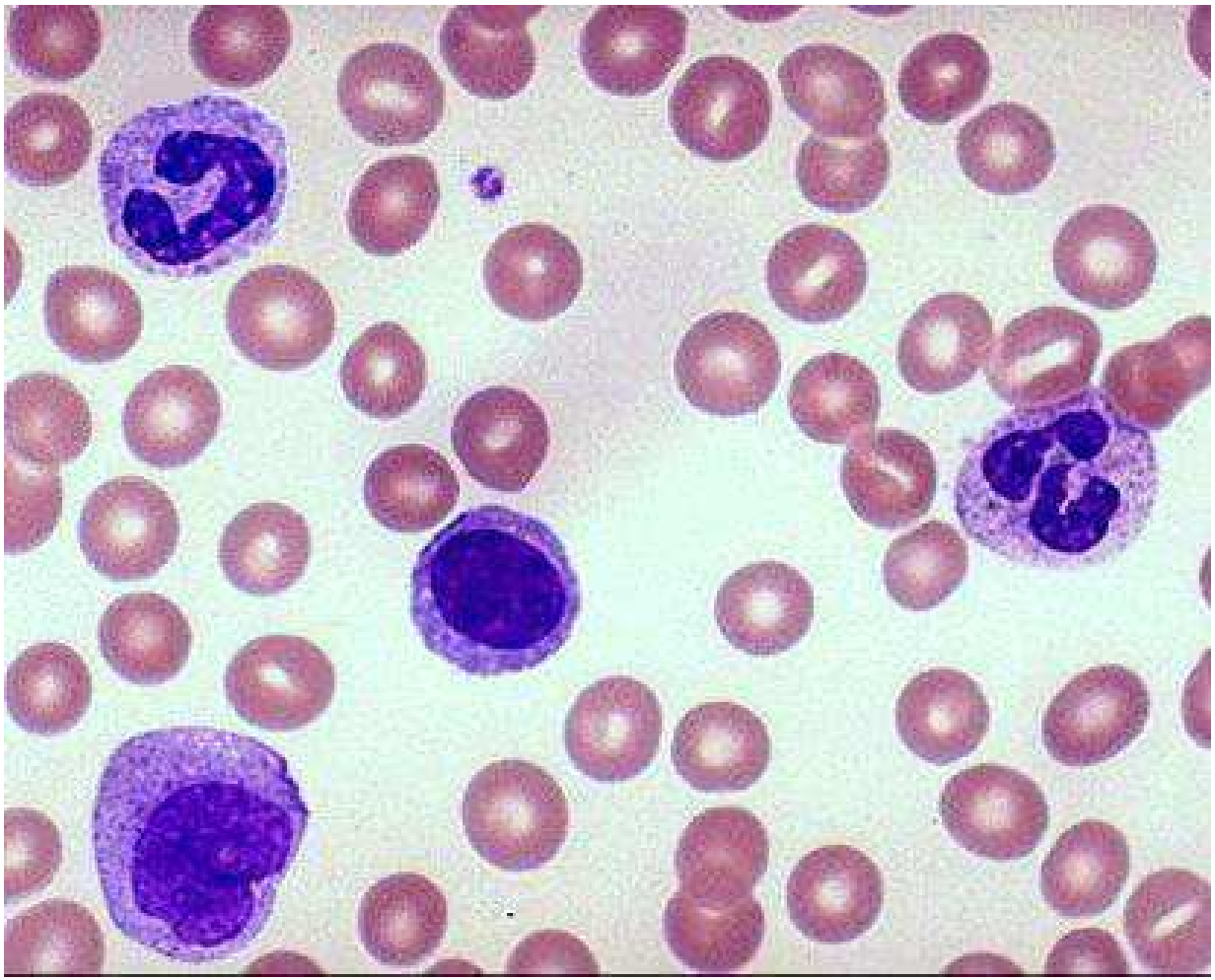
Lymphocytes



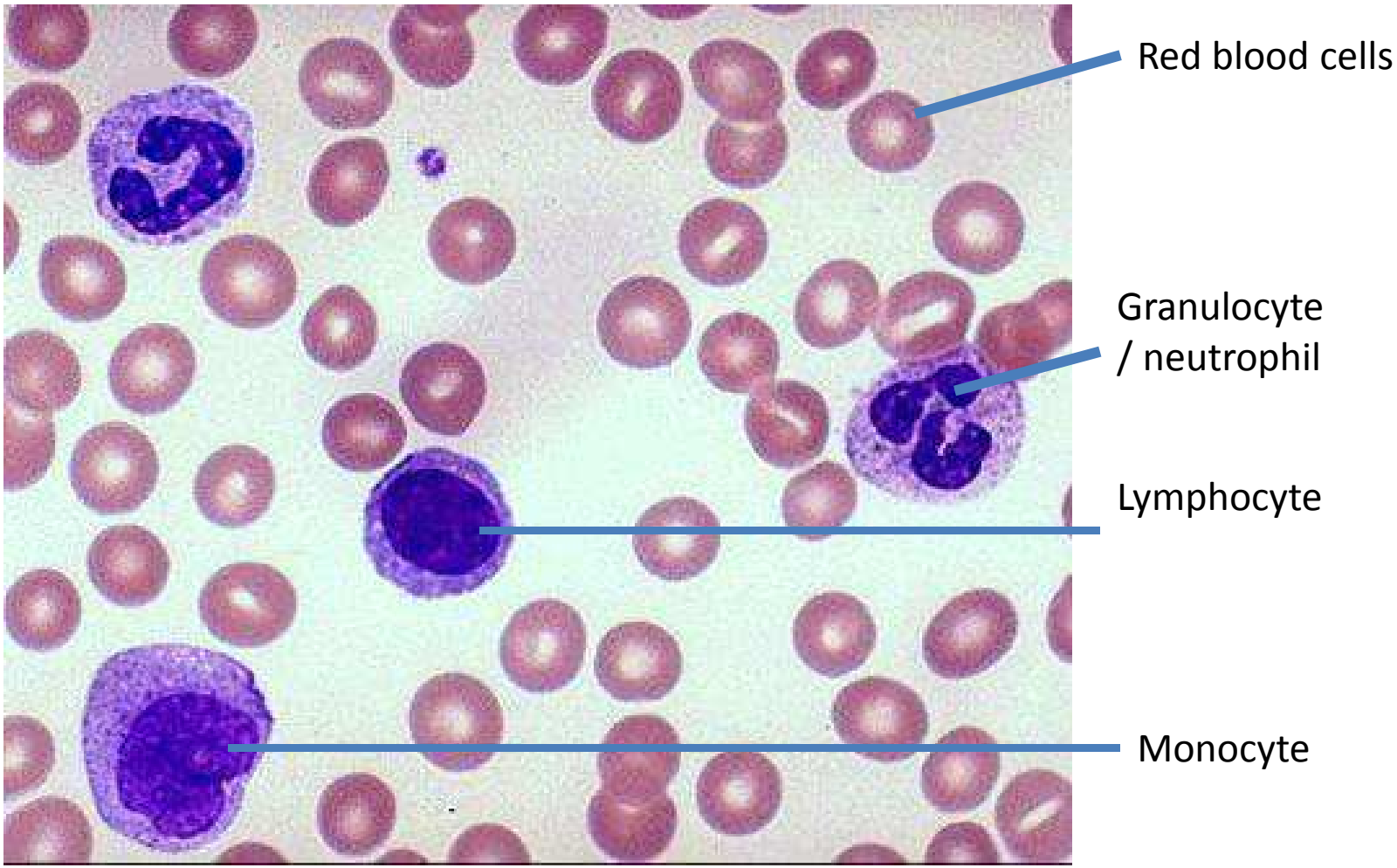
Neutrophil

White Blood Cells

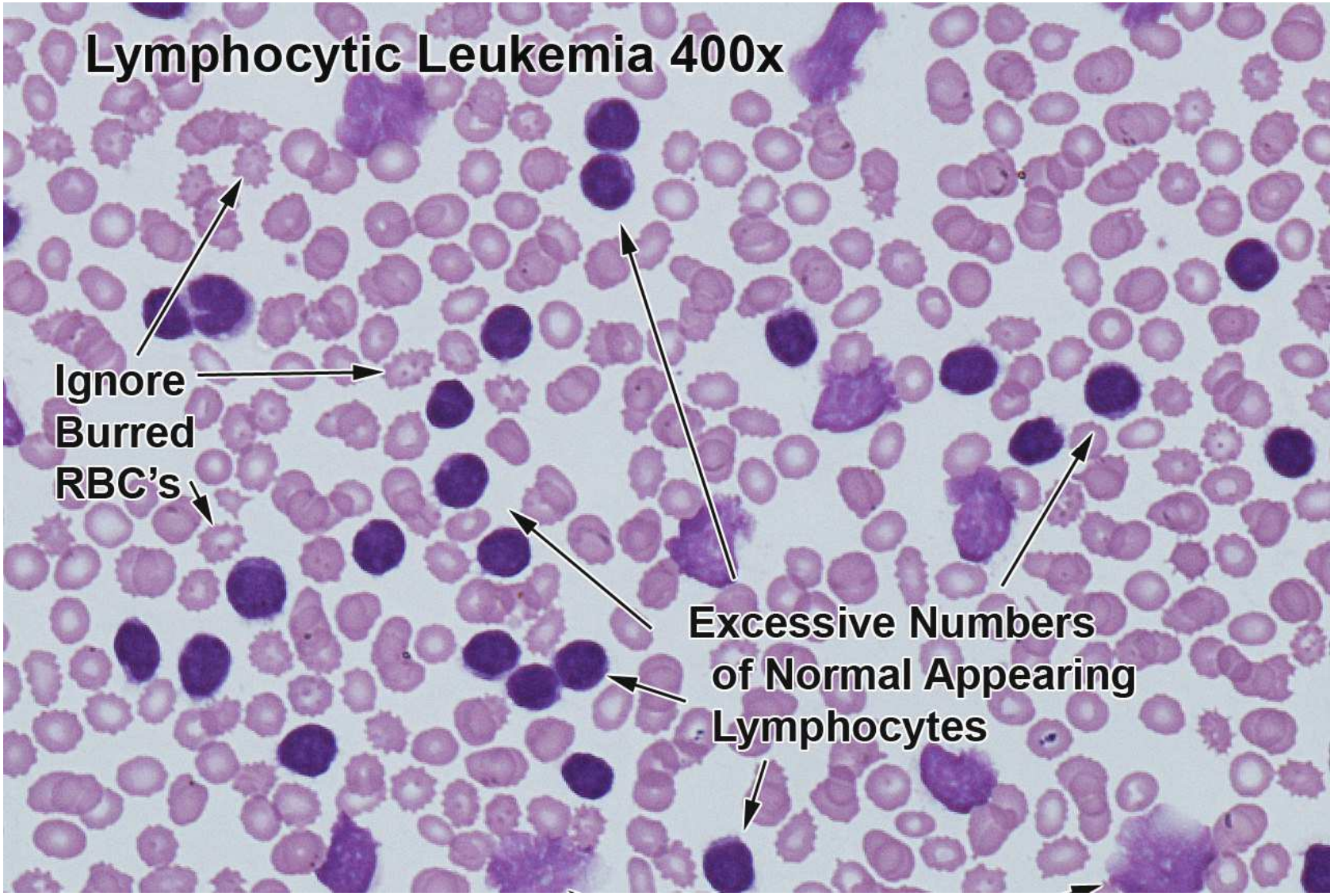
Blood under microscope –
what cell types can you see?



Blood under microscope – what cell types can you see?



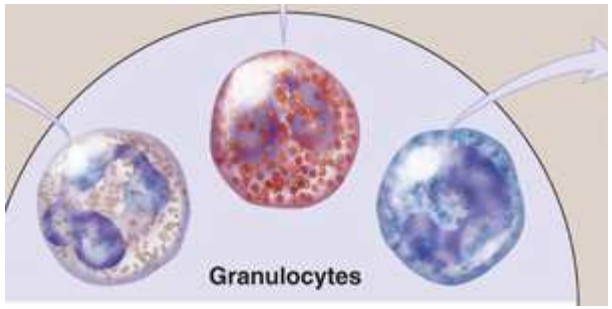
Some immune disorders can be diagnosed by looking at blood cells under a microscope



Leukocytes (white blood cells)

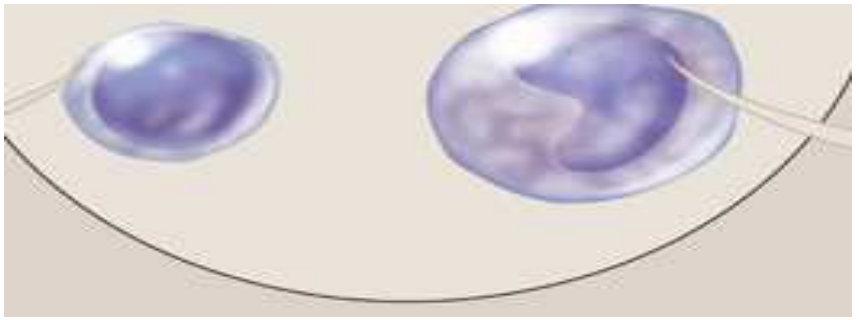
INNATE
ADAPTIVE

Granulocytes



- Neutrophils
- basophils
- eosinophils

lymphocytes



- T cells
- B cells
- NK cells

monocytes

- Monocytes
- (develop into macrophages and dendritic cells in tissue)

Q: how and where do blood cells /immune cells develop?

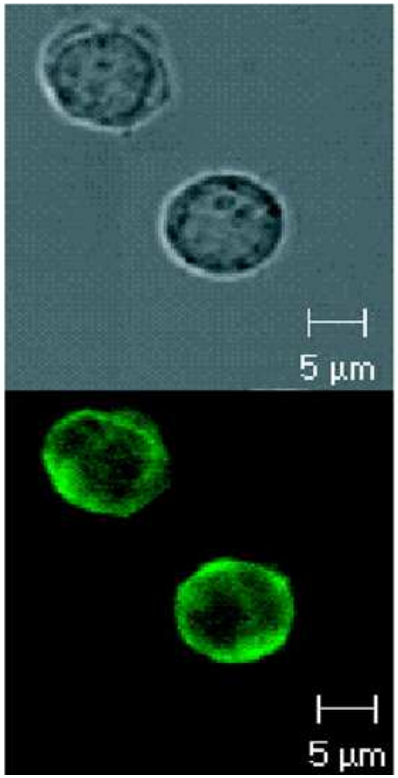
- **Haematopoiesis** is the development of blood cellular components
- Occurs from **haematopoietic stem cells (HSC)** found in the **bone marrow**
- Stem cells are undifferentiated biological cells that can differentiate into other / specialized cells
- HSC give rise to the all the blood cell types (platelets, red blood cells, white blood cells including monocyte, lymphocytes and granulocytes)
- In a healthy adult person, ~ a trillion (10^{12})new blood cells are produced daily

Identifying immune cells

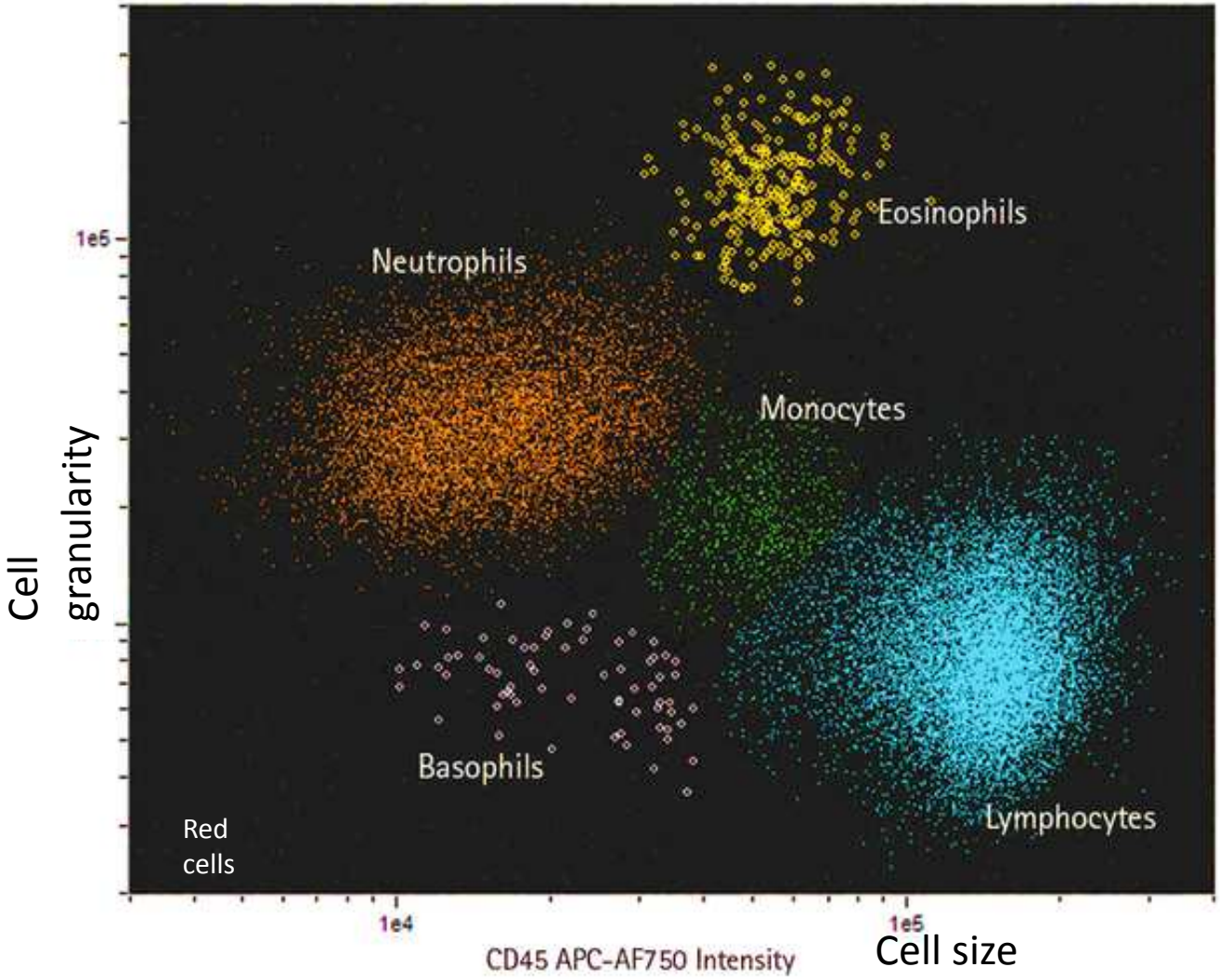
- looking at cells under the microscope: size, complexity
- Immune cells can also be identified by surface markers
- Each cell type displays particular types of proteins on its cell surface
- Known as CD markers (cluster of differentiation)

- Example: a particular subset of T lymphocytes have the CD4 marker on their surface = CD4+ T cells
- You could identify and count CD4+ T cells using a fluorescent (coloured) antibody that binds only to the CD4 protein (cell staining)
- This is typically performed using a technique called flow cytometry (sorts cells on size, granularity and fluorescent colour)
- Can also use fluorescent microscopy

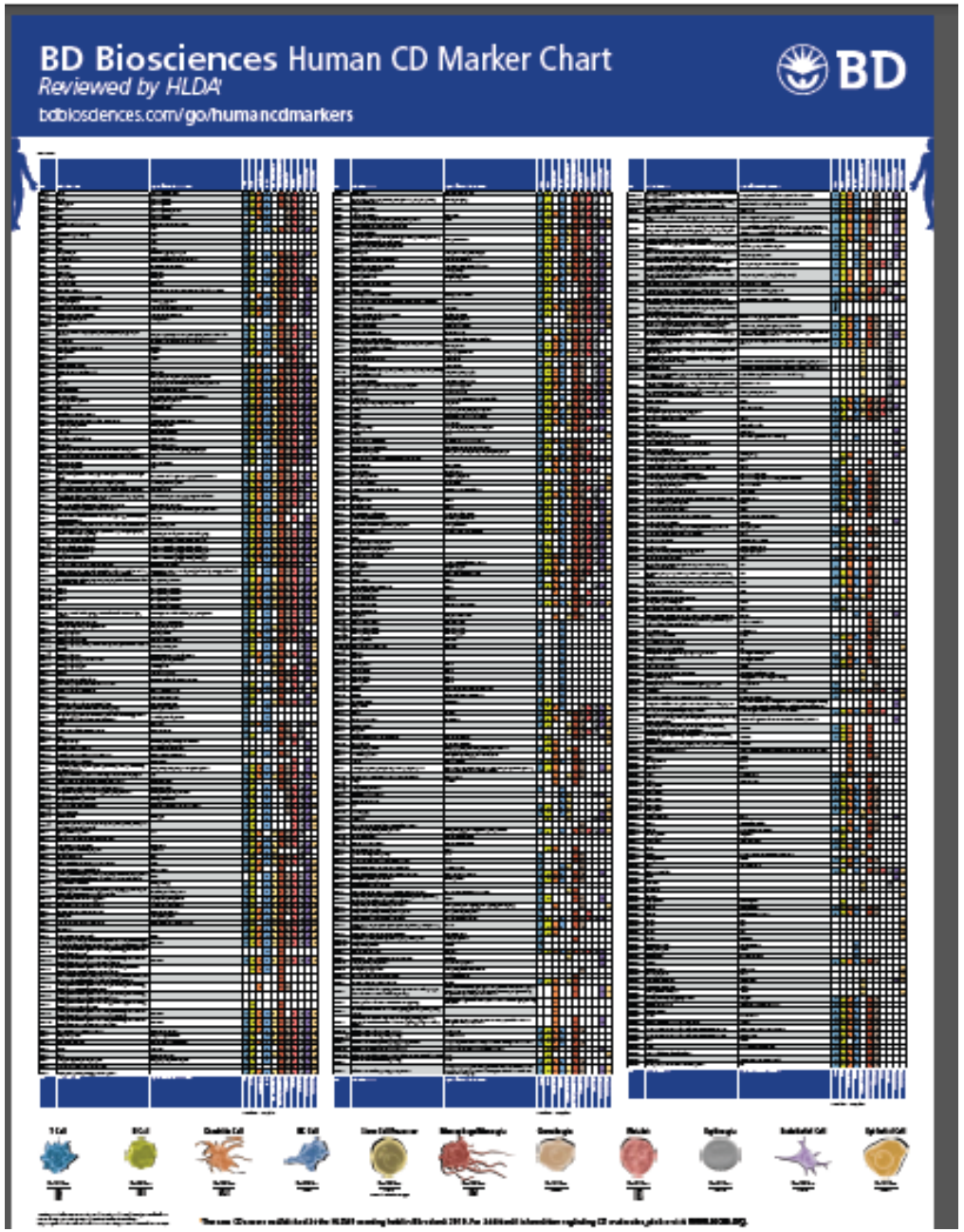
Fluorescent Microscopy



Flow cytometry

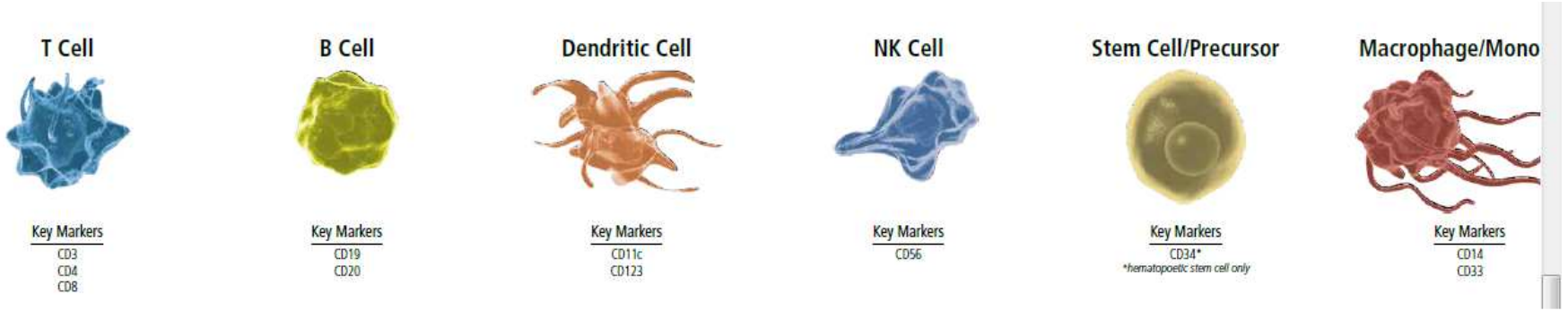


Approximately 350 CD antigens have been described in human white blood cells



Key CD markers on immune cells

Cell type	CD surface marker
HSC (stem cells)	CD34
Leukocytes (all white cells)	CD45
Monocytes	CD14
DC	CD11
Granulocytes	CD66
Lymphocytes	
NK cell	CD56
B cells	CD19
T cells	CD3
Cytotoxic T cells	CD8
Helper T cells	CD4



Check your understanding

1. Name 10 different cell types you can find in human blood.
2. What is the microbiome and does it consist of pathogens?
3. A new disease called Zika virus is causing an epidemic in Brazil. Explain how you would use Koch's postulates to prove that this disease is caused by an infectious pathogen.
4. Leukemia is a disease where too many white blood cells are abnormally produced. Explain why treatment may involve destroying the bone marrow. Do you know what a common second step of this treatment is?

Q: What do blood immune cells do?

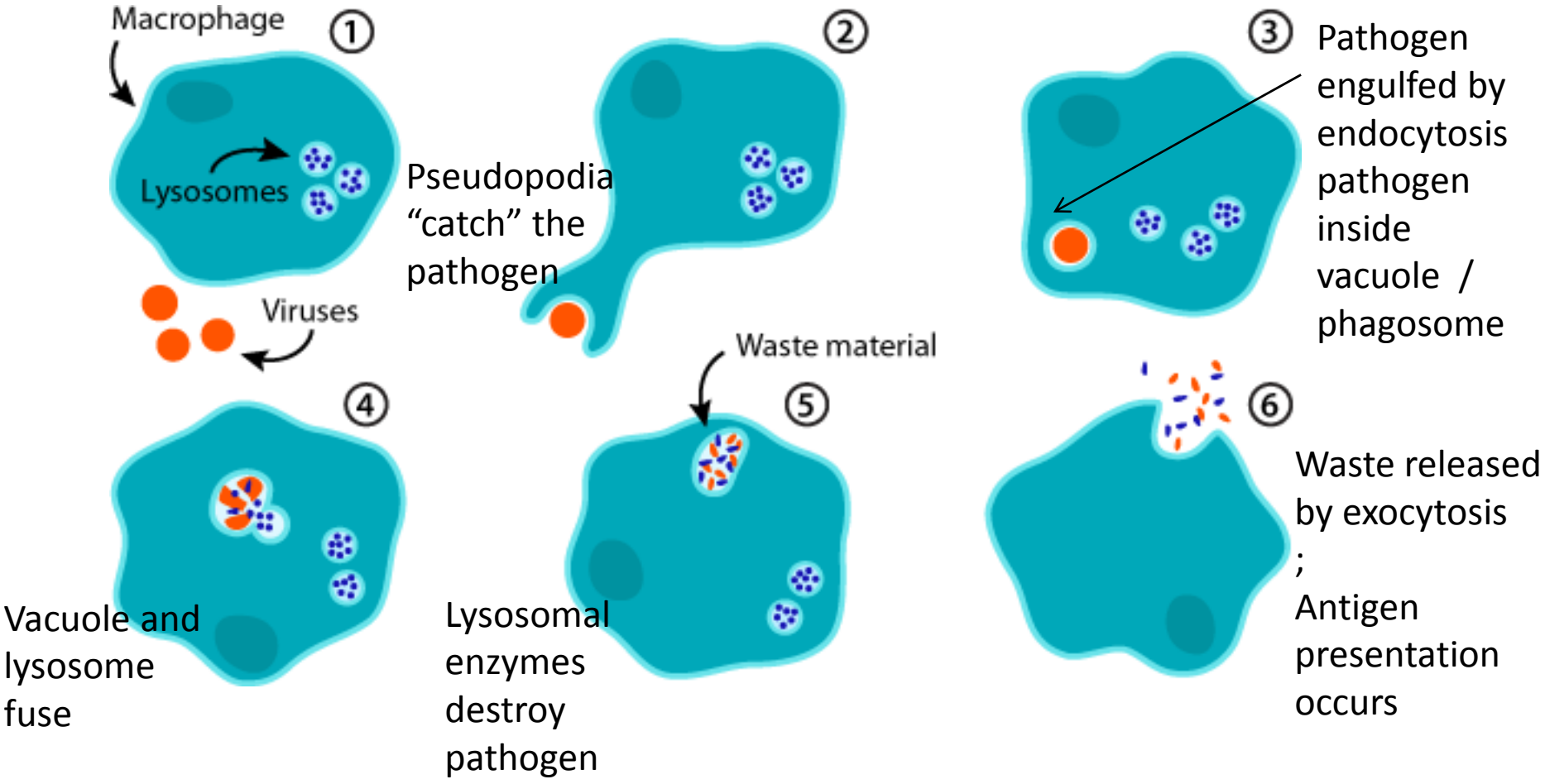
(this is a complex answer …each cell type does only some of these functions).

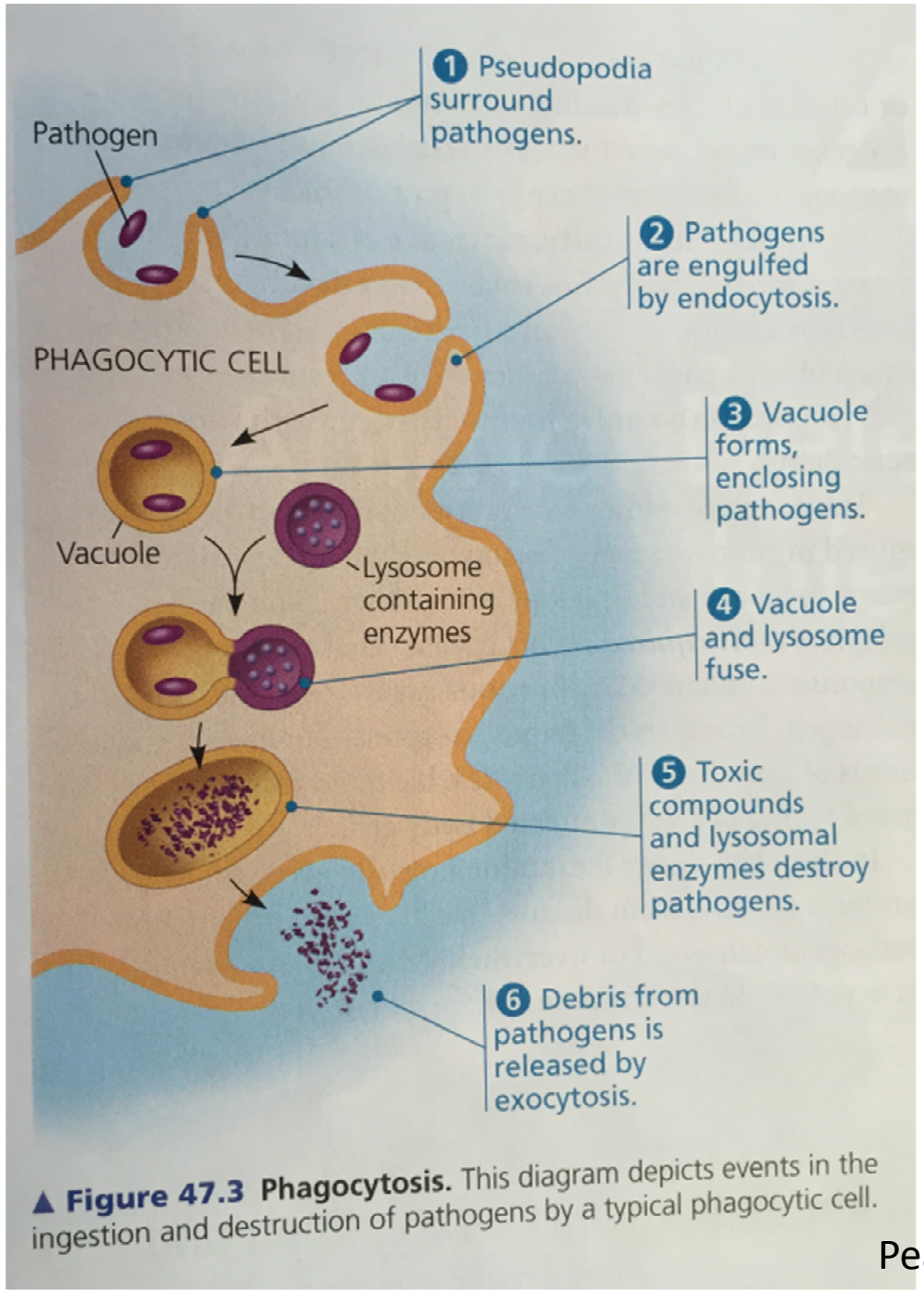
Basic functions include:

1. phagocytosis
2. Antigen presentation
3. Produce cytokines
4. Produce cytotoxic substances that will kill infected cells
5. Produce antibodies
6. Produce antimicrobial peptides
7. Activate complement
8. Interact with other immune cells to help them perform their function better
9. Cause inflammation

Phagocytosis

- phagocytic cells: macrophage , DCor neutrophil
- "eat" a pathogen
- i.e. ingests it into a membrane-bound vacuole (phagosome) where it can be destroyed with enzymes from the lysosomes





▲ **Figure 47.3 Phagocytosis.** This diagram depicts events in the ingestion and destruction of pathogens by a typical phagocytic cell.

Pearson 10th ed, Chptr 47 pg 1106, fig 47.3

Phagocytosis

=a form of endocytosis (particles enveloped by the cell membrane and internalized to form a phagosome, or "food vacuole “).

step	detail
1. chemotaxis	Chemicals from the pathogen attract phagocytes (or active chase)
2. Binding	phagocyte sticks to pathogen (catch)
3. ingestion	• Take pathogen into immune cell , by pseudopodia / projections of plasma membrane • pathogen contained inside an endosome or vacuole
4. digestion	• vacuole merges with a lysosome. • Lysosome enzymes include reactive oxygen species and proteases. • Pathogen is digested / degraded into small pieces
5. exocytosis	Waste material is released via the external cell membrane
6. Antigen presentation	Some bits of the pathogen are retained and presented at the surface cell membrane of the phagocyte

End results of phagocytosis :

1. Destruction of micro-organism
2. Antigen presentation
3. Cytokine release

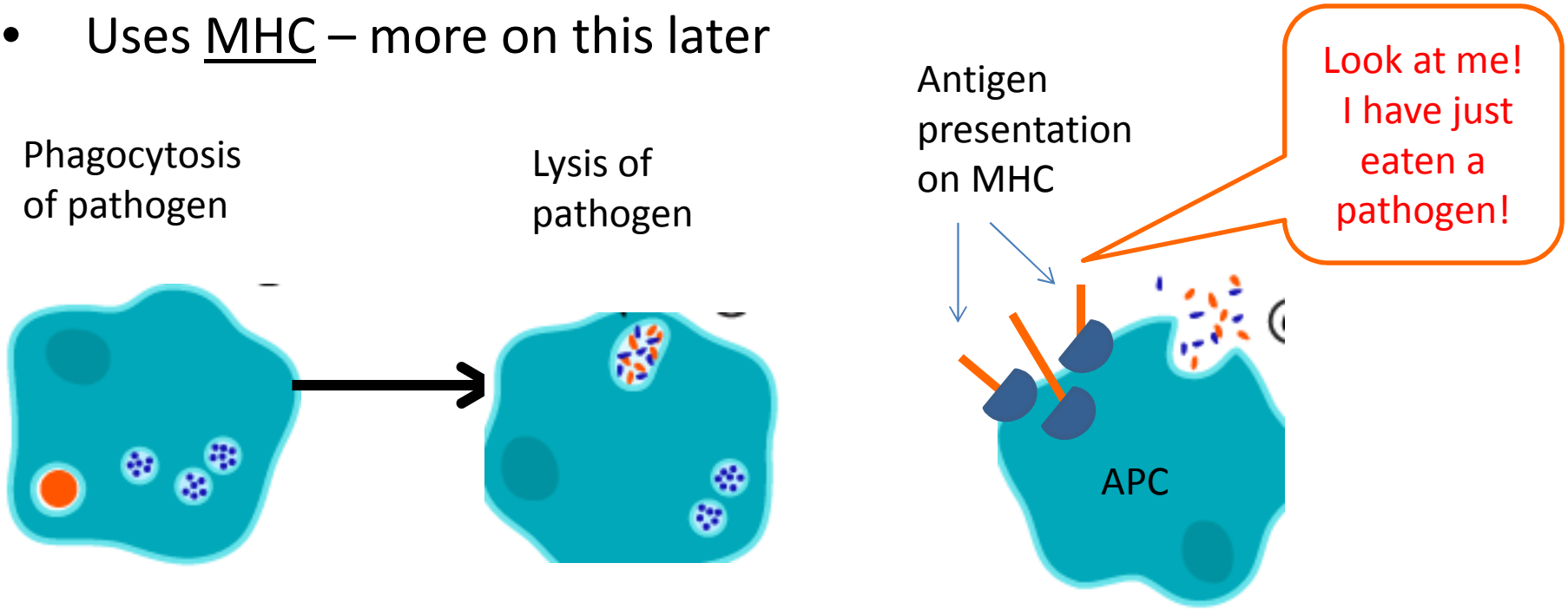
http://scientificpsychic.com/mind/whitecells.html

https://youtu.be/JnlULOjUhSQ

neutrophil chasing and phagocytosing bacteria

Antigen presentation

- After phagocytosis, the phagocyte keeps some small pieces of the pathogen known as antigens
- The antigens are displayed on the cell surface of the phagocyte
- This is a signal to show that pathogens have been detected
- This only occurs in certain phagocyte cell types known as antigen presenting cells, such as macrophages or dendritic cells
- Uses MHC – more on this later



How to defend yourself against attack from a person?

How?

EAT / DIGEST

BLOCK

STAB / PUNCTURE

TOXINS

CALL FOR HELP

How to defend host from against attack by a pathogen?

How?

EAT / DIGEST=phagocytosis

Bind and BLOCK

Cytolysis

STAB / PUNCTURE

Cytotoxic

TOXINS

CALL FOR HELP

Cytokines

More immune cell functions: stop / block / kill pathogens

How?

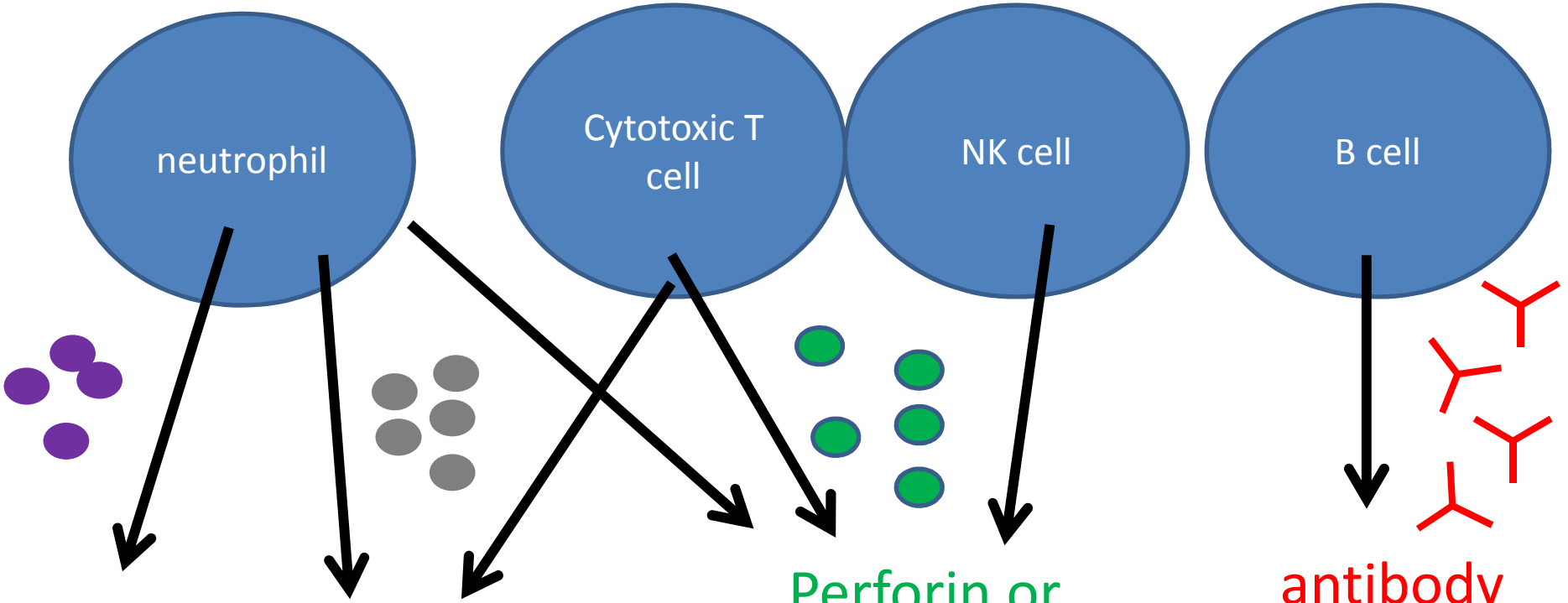
- Punch holes in the cell wall or cell membrane (this often causes the cell to burst or lyse)
- Bind to pathogen / block it from binding to other things / block its activity
- Chop it into little pieces.
- Send a message to tell other cell types to do the above

Effector functions: stop / block / kill pathogens

How?

- Punch holes in the cell wall or cell membrane (this often causes the cell to burst or lyse) e.g. complement, cytotoxic granules, antimicrobial peptides
- Bind to pathogen / block it from binding to other things e.g. antibodies
- Chop it into little pieces. e.g phagocytosis
- Send a message to tell other cell types to do the job e.g. antibodies, cytokines, antigen presentation, inflammation

Secreted substances with functional effects



Anti-Microbial peptide

Cytokines (chemical messengers)

Perforin or cytotoxic granules which lyse target cell (cytolysis)

antibody (binds to pathogen)

Major functions of Leukocytes (white blood cells)

INNATE
ADAPTIVE

Granulocytes

- Neutrophils
- eosinophils

Phagocytes
Cytotoxic
Cytokines

monocytes

- Monocytes
- macrophages and dendritic cells

Phagocytes
Antigen presentation
Cytokines

lymphocytes

- NK cells

Cytotoxic
Cytokines

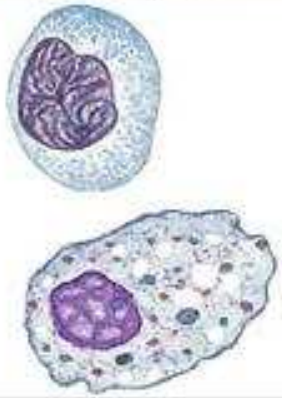
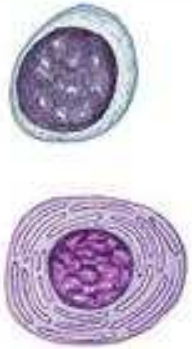
- **T cells**
- **B cells**

CD4 T cells: cytokines
CD8 T cells: **Cytotoxic**
B cells: **antibodies**
B cells: **antigen presentation**
Cytokines

Leukocyte functions

cells	types	arm	function	CD
lymphocytes	T (CD8 and CD4+)	adaptive	• Cytolysis • Cytokines	CD3 CD4 CD8
	B / plasma	adaptive	• Antibodies • cytokines • Antigen presentation	CD19 CD20
	NK	Innate	• Cytolysis • cytokines	CD56 CD16
granulocytes	Neutrophils	innate	• Phagocytosis • cytolysis	CD66
	basophils	innate	• inflammation	
	eosinophils	innate	• Cytolysis, phagocytosis • Allergy	
Monocytes	macrophages	innate	• Phagocytosis • Antigen presentation • cytokines	CD14
	Dendritic cells	innate	• Phagocytosis • Antigen presentation • Cytokines	CD11

Functions of leukocytes
(revise this at the end of the course)

	Basophils and mast cells	*Neutrophils*	*Eosinophils*	*Monocytes and macrophages*	*Lymphocytes and plasma cells*	*Dendritic cells*
						
Primary function(s)	Release chemicals that mediate inflammation and allergic responses	Ingest and destroy invaders phagocytosis	Destroy invaders, particularly antibody-coated parasites	Ingest and destroy invaders Antigen presentation phagocytosis	Specific responses to invaders, including antibody production	Recognize pathogens and activate other immune cells by antigen presentation

Innate	Adaptive	innate

Check your understanding

1. During flow cytometry I stained some blood cells and identified them as being CD14+. Which cellular subset is this and what major functions do they perform?

2. Which cell types are particularly good at antigen presentation and why?

3. What is cytolysis and which cell types can cause this?

4. What are some major difference between the innate and the adaptive immune systems?