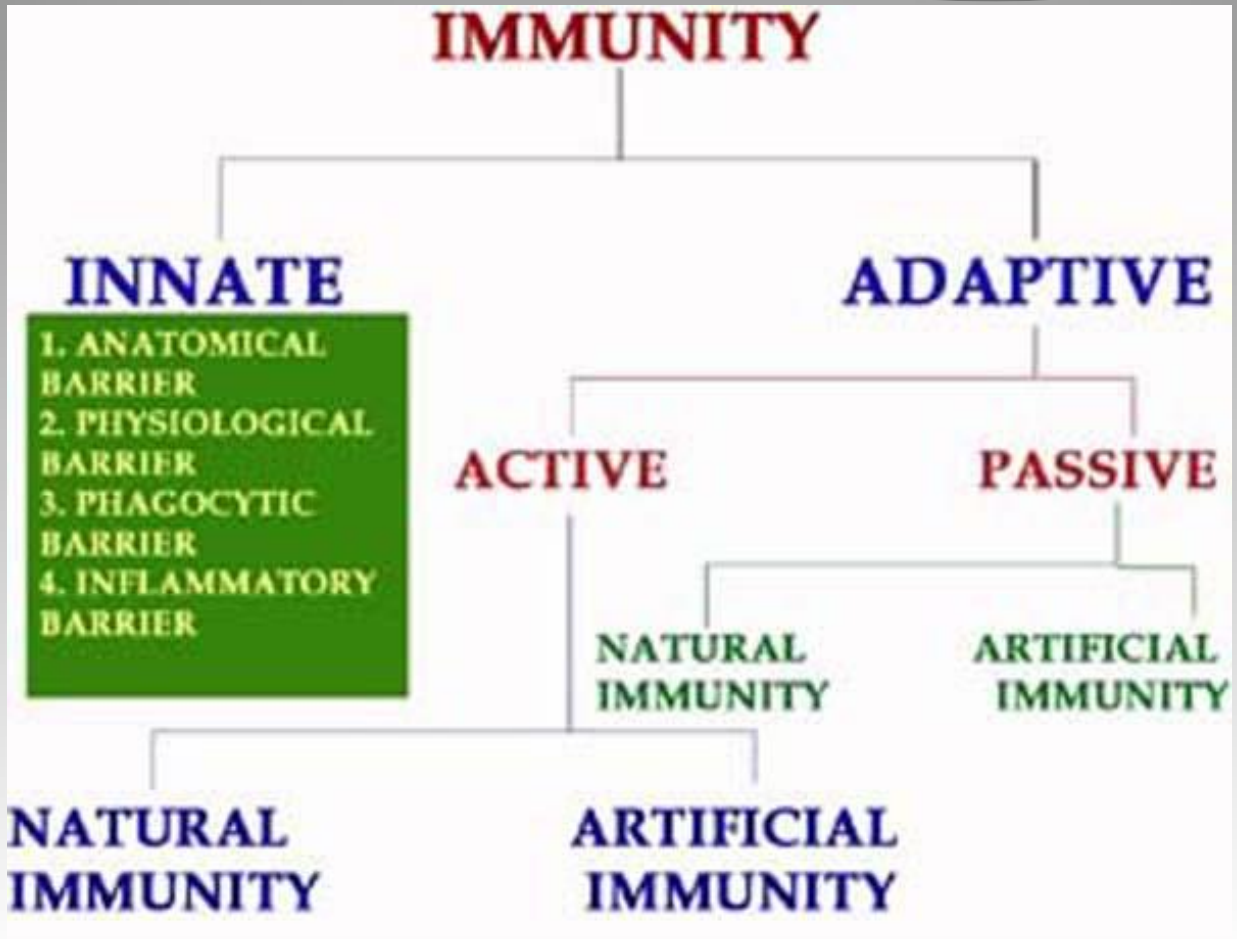
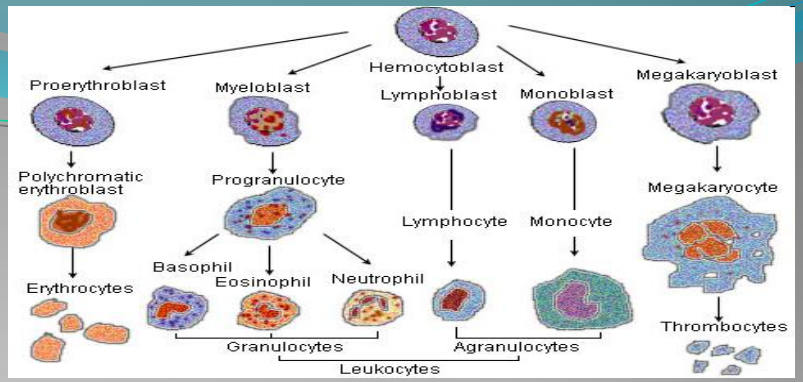
Immunology – practical 1

cells of the immune system


IMMUNITY
INNATE
ADAPTIVE
1. ANATOMICAL BARRIER
2. PHYSIOLOGICAL BARRIER
3. PHAGOCYTIC BARRIER
4. INFLAMMATORY BARRIER
ACTIVE
PASSIVE
NATURAL IMMUNITY
ARTIFICIAL IMMUNITY
NATURAL IMMUNITY
ARTIFICIAL IMMUNITY

hematopoiesis



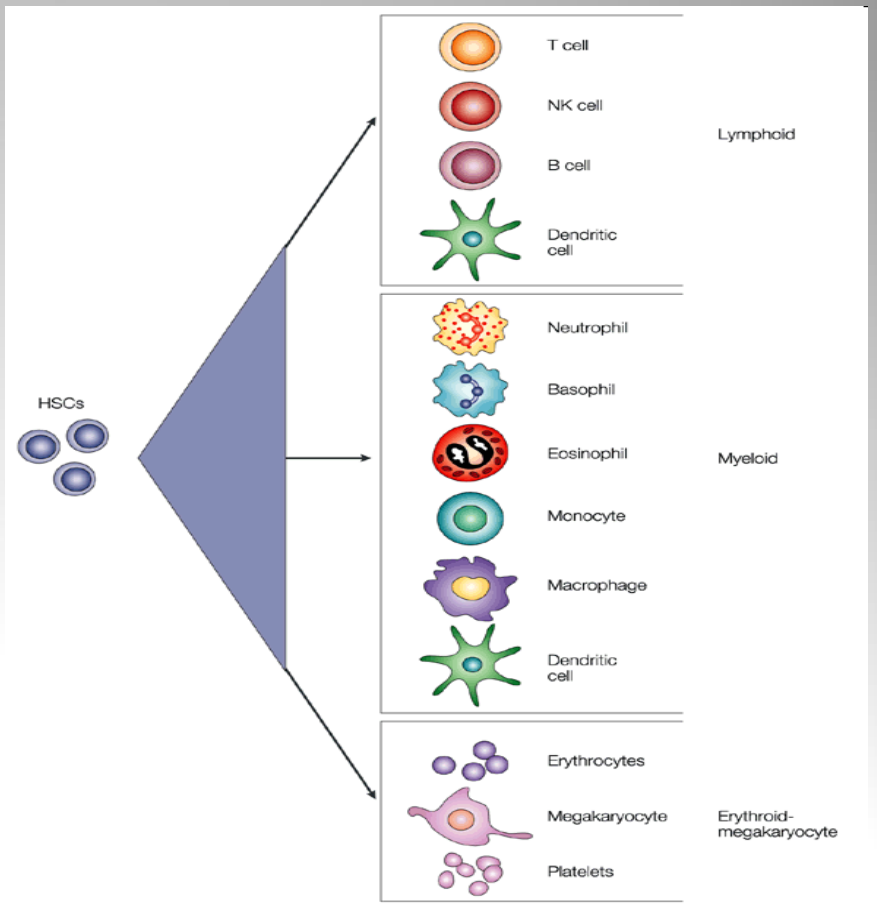
- Three main classifications of blood cells derive from haematopoetic stem cells (HSCs)
- ***Myeloid cells*** - this includes
  - macrophages (monocytes)
  - granular white blood cells (or granulocytes; neutrophils, basophils and eosinophils).
- ***Erythroid-megakaryocytes***
  - Erythrocytes (red blood cells)
  - platelets
- ***Lymphoid cells*** - this includes

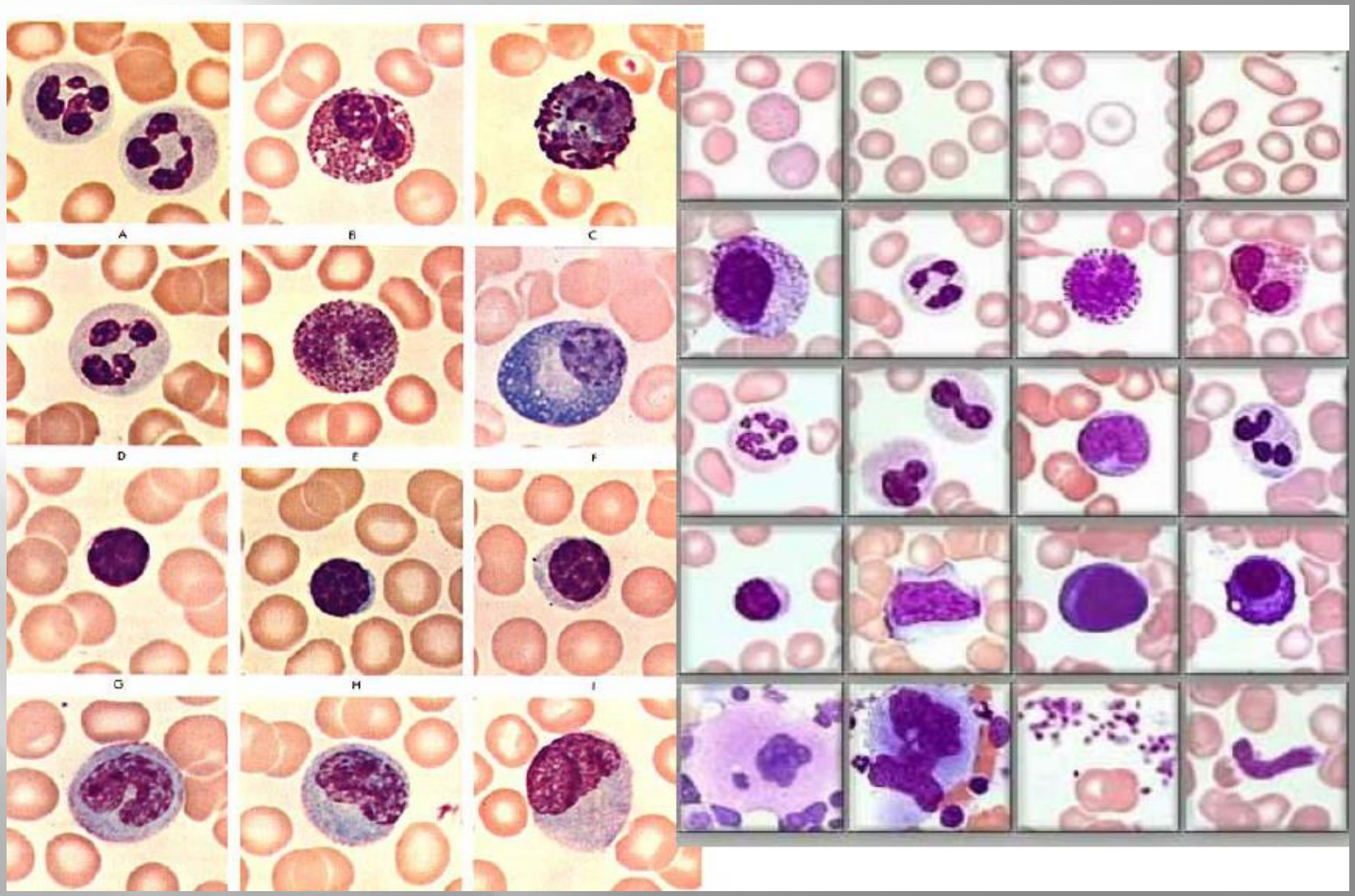
T-cells

B-cells

Natural killer (NK) cells are thought to be the prototype of T cells.

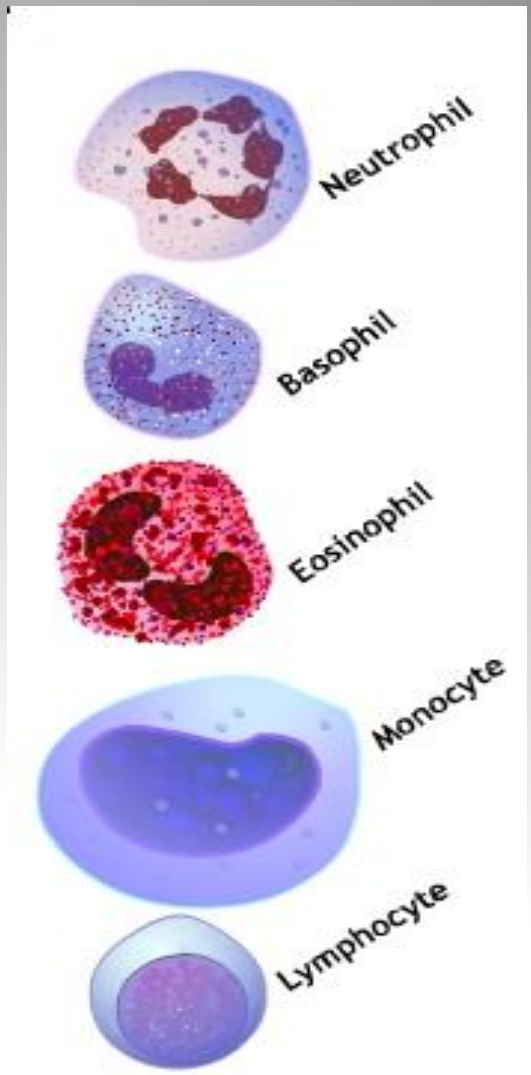
- **<u>T-cell progenitors are able to generate dendritic cells.</u>**

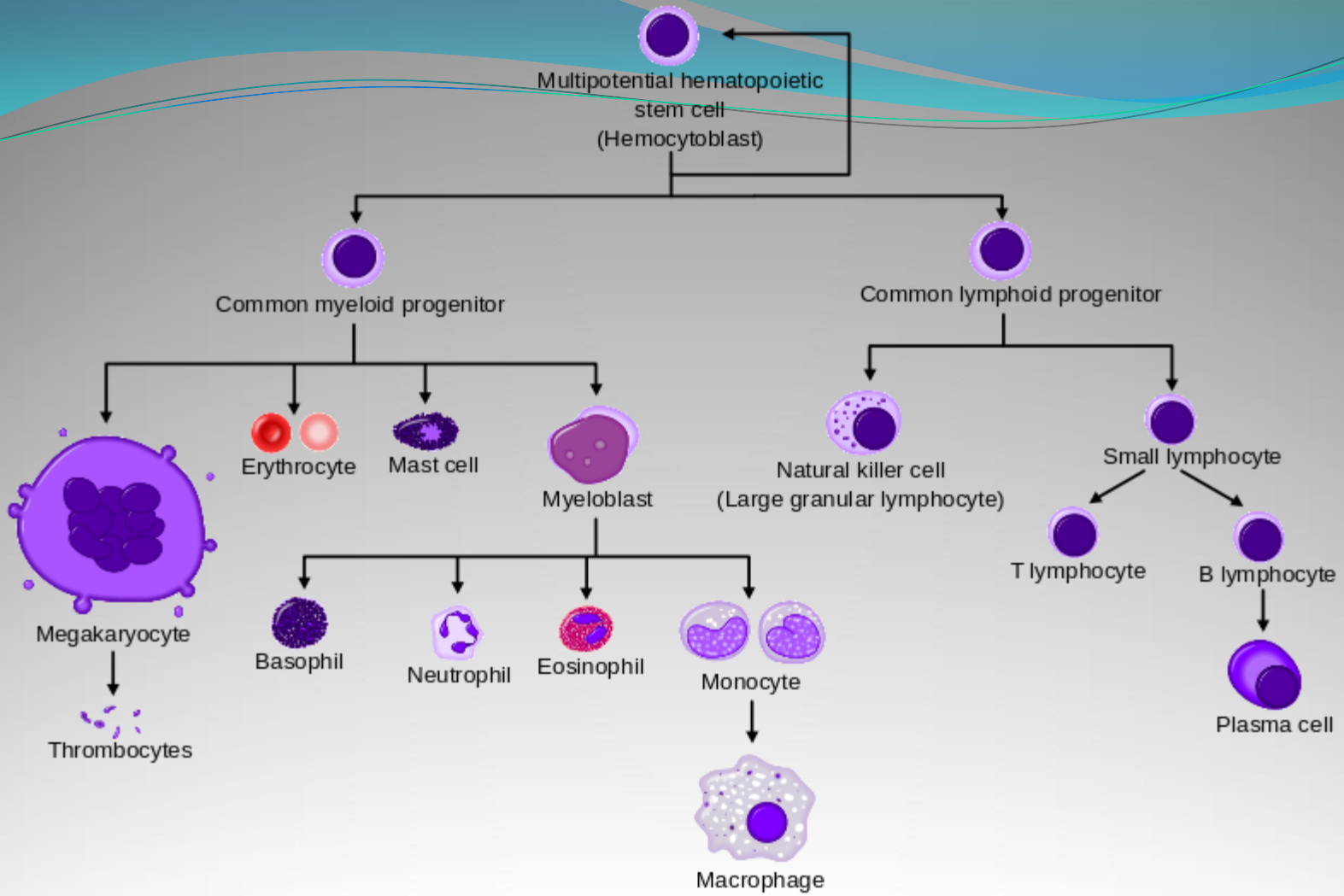



A
B
C
D
E
F
G
H
I

White blood cells (or leucocytes)

- have nuclei & do not contain hemoglobin
- typical concentration is 5,000 - 9,000 per cubic millimeter
- Types f WBCs:
  - granular white blood cells include:
    - neutrophils (50 - 70% of WBCs)
    - eosinophils (1 - 4%)
    - basophils (less than 1%)
  - agranular (or non-granular) white blood cells include:
    - lymphocytes (25 - 40%)
    - monocytes (2 - 8%)




Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Erythrocyte
Mast cell
Myeloblast
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
Megakaryocyte
Basophil
Neutrophil
Eosinophil
Monocyte
T lymphocyte
B lymphocyte
Plasma cell
Thrombocytes
Macrophage

Blood smear preparation - aim of blood smear

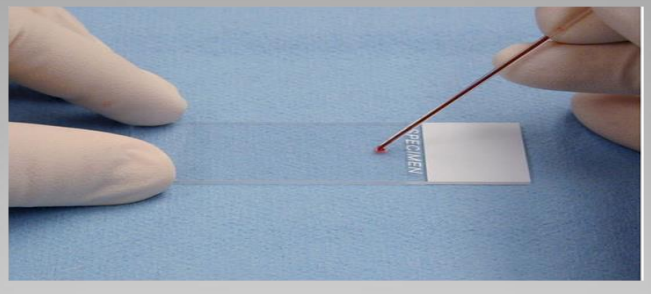
- **Value of blood films:**
  - Examination of thin blood films is important in the investigation and management of anaemia, infections, and other conditions which produce changes in the appearance of blood cells and differential white cell count.
  - A blood film report can provide rapidly and at low cost, useful information about a patient's condition.

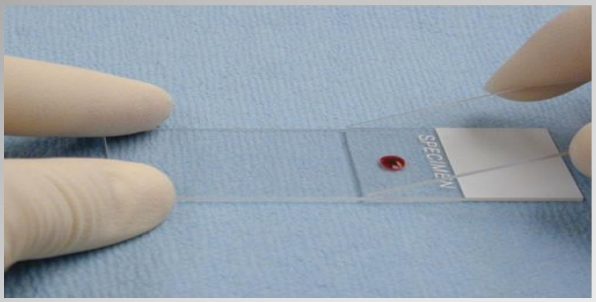
Making blood films

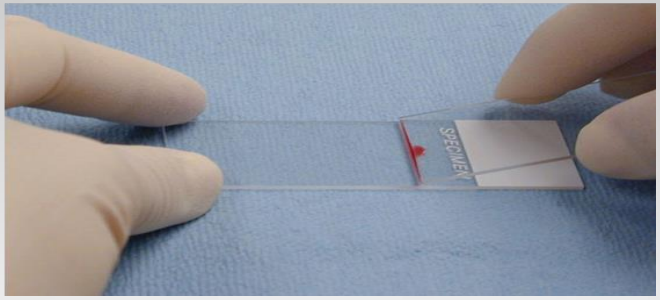
Three basic steps to make blood film:

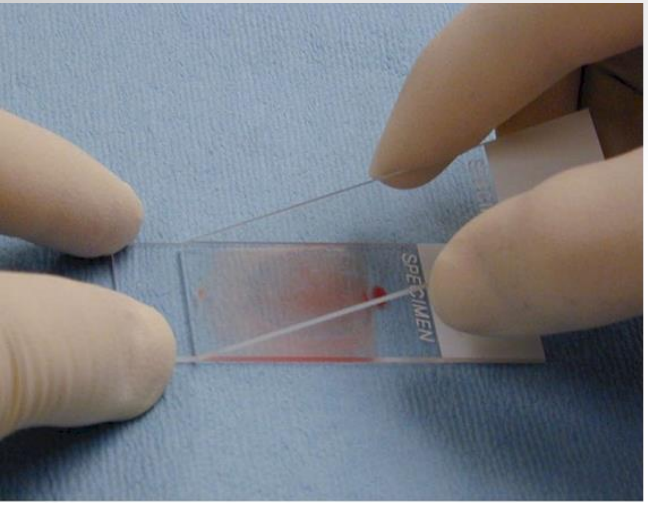
1. Preparation of blood smear.
2. Fixation of blood smear.
3. Staining of blood smear.

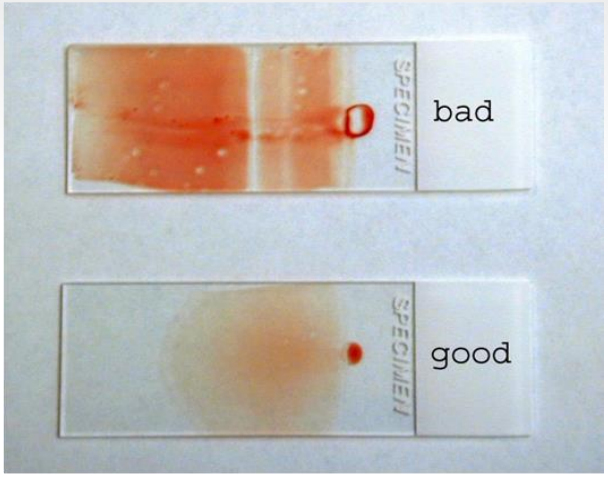
Steps for Blood Film

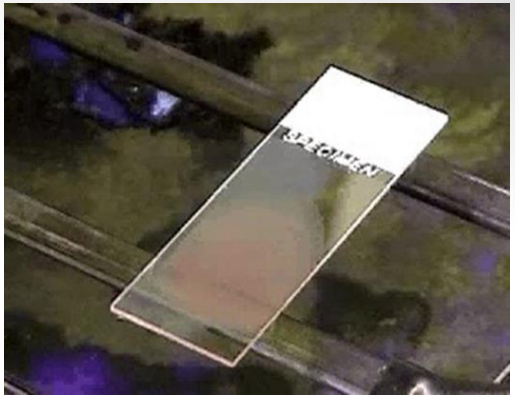




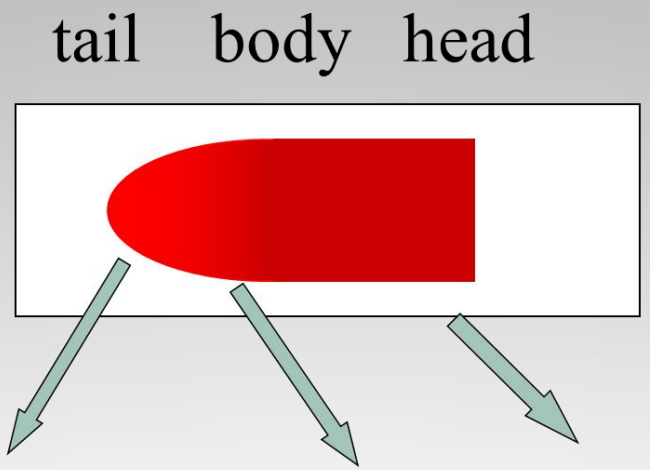


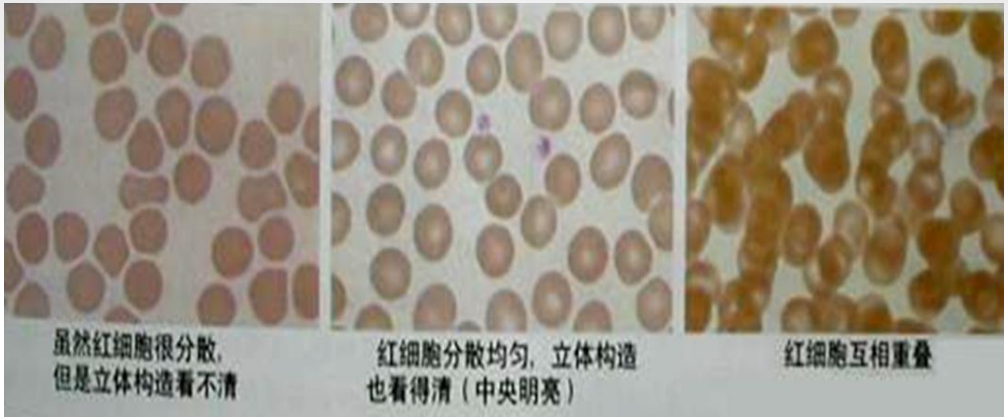






The shape of blood film





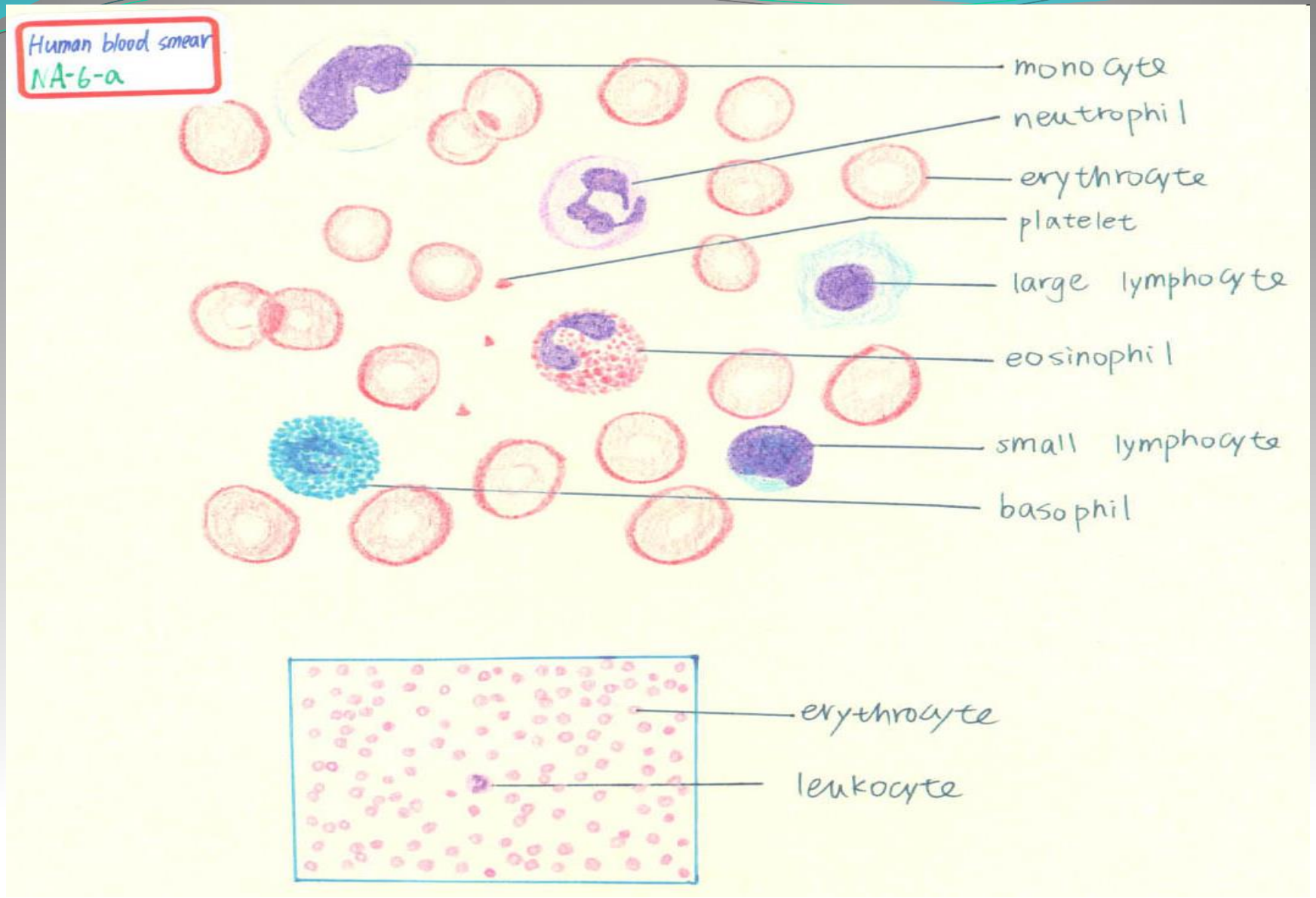
Staining

- May Grünwald solution (4 min)
- add destiled water (4 min)
- lift slide to drain the staining solution
- add Romanovski solution (18 min)
- wash
- dry

- **Staining pattern:**
- **Erythrocytes** - pale pink,
- **Thrombocytes** - small and blue,
- **Lymphocytes** - round blue nucleus almost filling the whole cell,
- **Neutrophiles** - segmented blue nucleus within pale cytoplasm,
- **Eosinophil**es - segmented blue nucleus and pink granules in cytoplasm,

Basophils - segmented blue nucleus and blue granules in cytoplasm,

Monocytes - round blue nucleus within pale cytoplasm

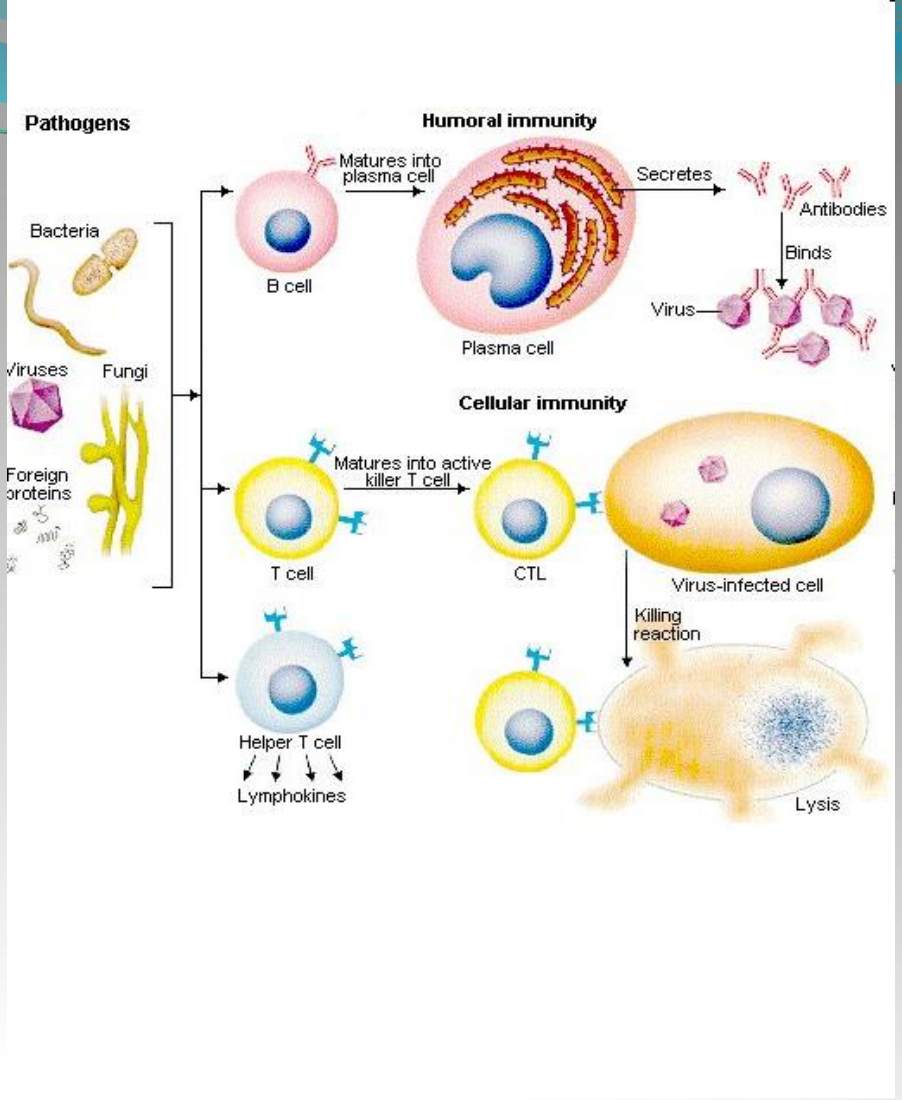

Human blood smear
NA-6-a
monocyte
neutrophil
erythrocyte
platelet
large lymphocyte
eosinophil
small lymphocyte
basophil
erythrocyte
leukocyte

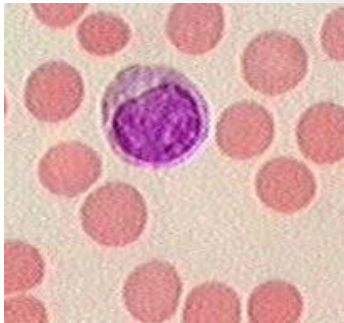

【MAKOTO】

lymphocytes

- Small white blood cells which are responsible for much of the work of the immune system.
- Lymphocytes can be divided into three classes:
- B cells,
- T cells
- null cells (NK)

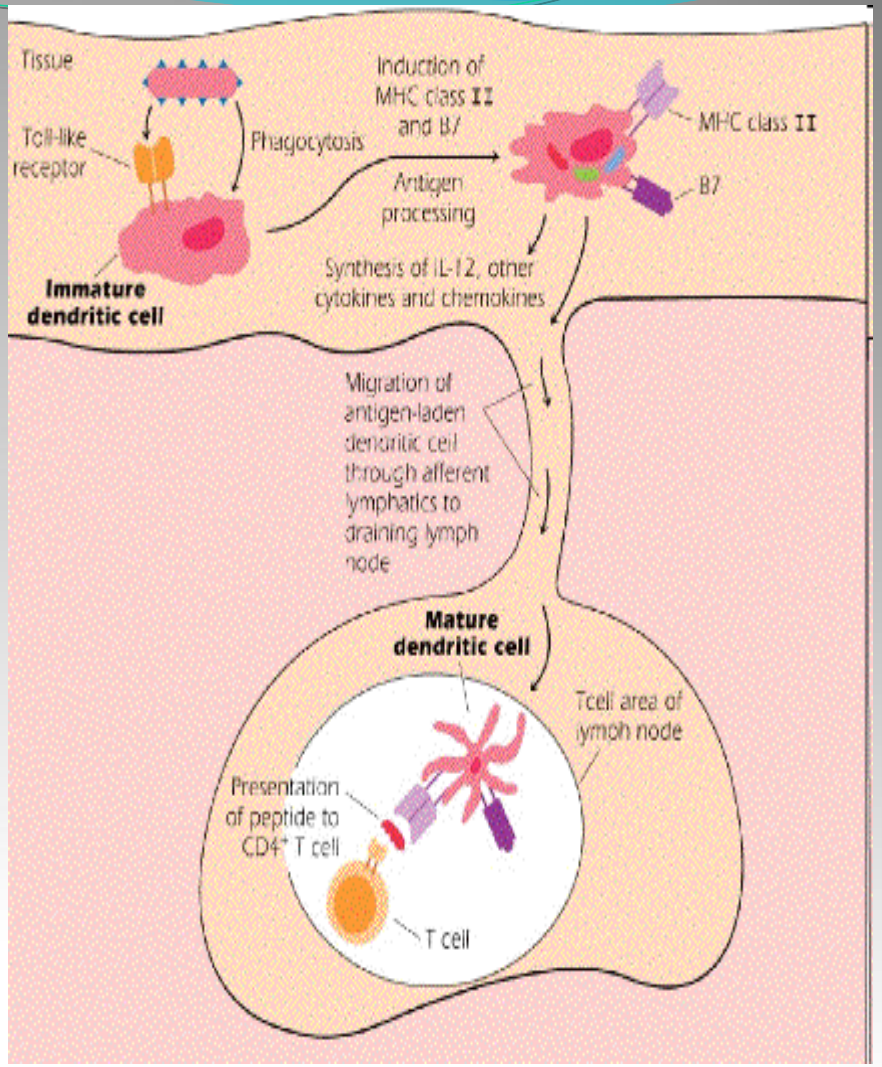
- **T–lymf. (65–75%)**
- **B–lymf.(20–30%)**





lymphocytes

- CD 4 (+) T-cells become activated by antigen presenting cells (APC's).
- Naive CD4(+) cells are activated by dendritic cells.
- Memory CD4(+) cells interact well with macrophages.



lymphocytes

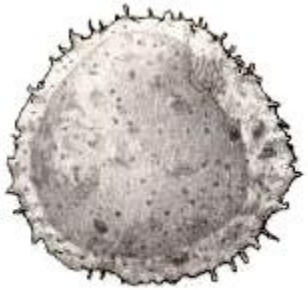
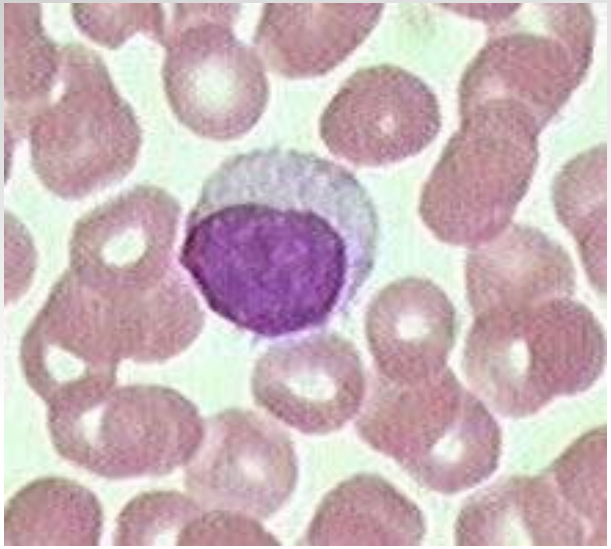


Fig. 11 - Lymphocyte



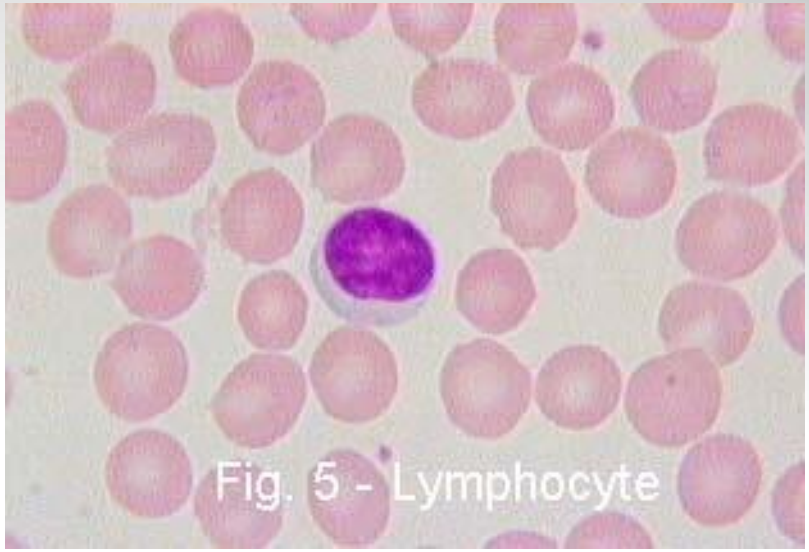
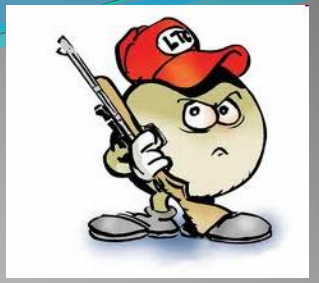


Fig. 5 - Lymphocyte

T lymfocytes



TH(helpers) CD4:

- These cells travel through the blood and lymph, looking for antigens (such as those captured by *antigen-presenting cells*). Upon locating an antigen, they notify other cells to assist in combating the invader.
- This is sometimes done through the use of cytokines (or specifically, lymphokines) which help destroy target cells and stimulate the production of healthy new tissue. Interferon is an example of such a cytokine.

TS(supresors) CD8 :

TC(cytotoxic) CD8:

Killer T cells only recognize antigen in the grasp of Class I MHC markers. Here a resting cytotoxic T cell recognizes virus fragments, which are displayed by a macrophage in combination with a Class I MHC marker.

A receptor on a circulating, resting cytotoxic T cell (and CD8 protein) recognizes the antigen-protein complex and binds to it. The binding process and an activated helper T cell activate the cytotoxic T cell.

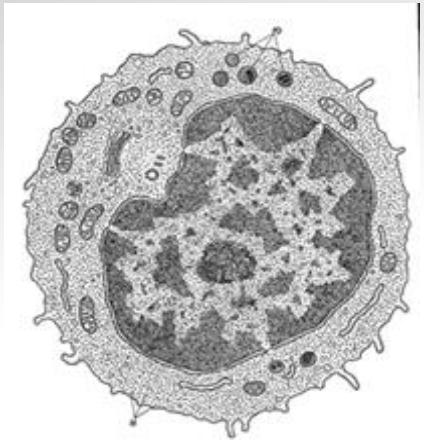
Because the surfaces of other infected cells bear the same virus fragments in combination with Class I MHC markers, the activated cytotoxic T cells can quickly recognize, attack, and destroy the diseased cell.

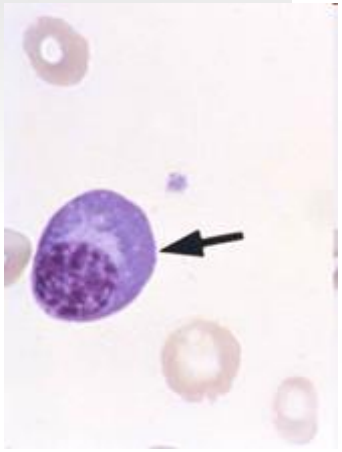
B lymfocytes

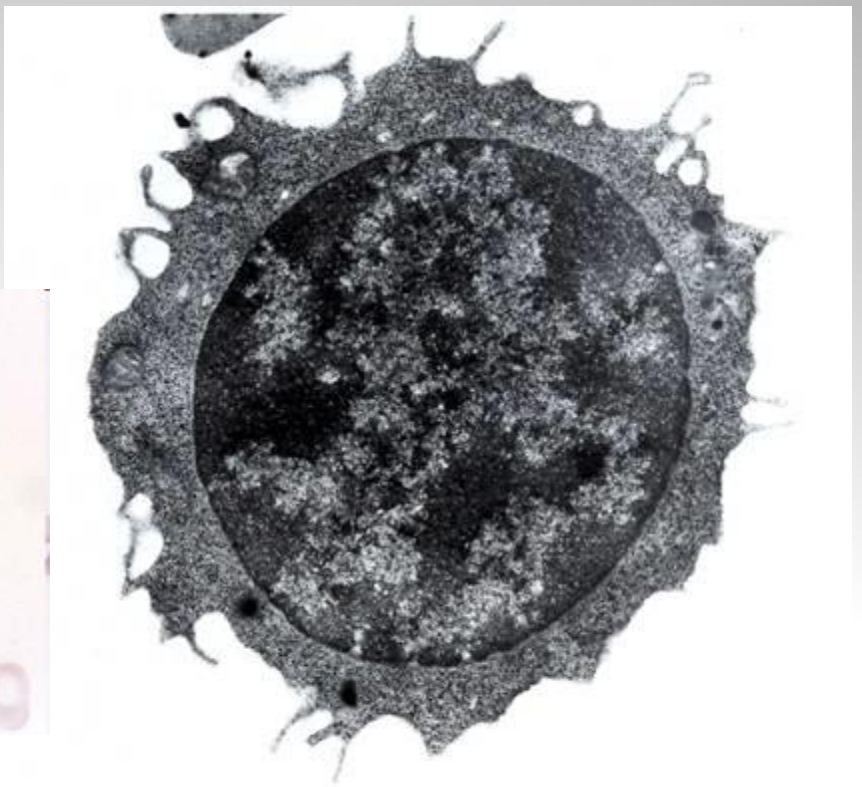
- B cells spend their entire early life in the bone marrow.
- Upon maturity, their job is to travel throughout the blood and lymph looking for antigens with which they can interlock.
- Once a B cell has identified an antigen, it starts replicating itself.
- These cloned cells mature into antibody-manufacturing *plasma cells*.
- *Memory cells* - specialized B cells which grant the body the ability to manufacture more of a particular antibody as needed, in case a particular antigen is ever encountered again.

B-lymfocytes

- *plasma cells*
- *memory cells*







Isolation of human mononuclear cells

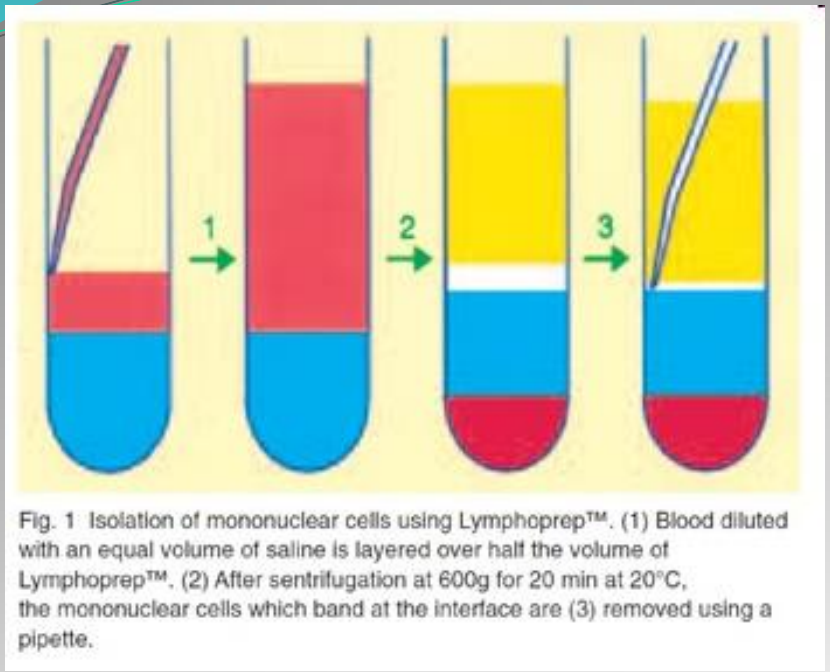
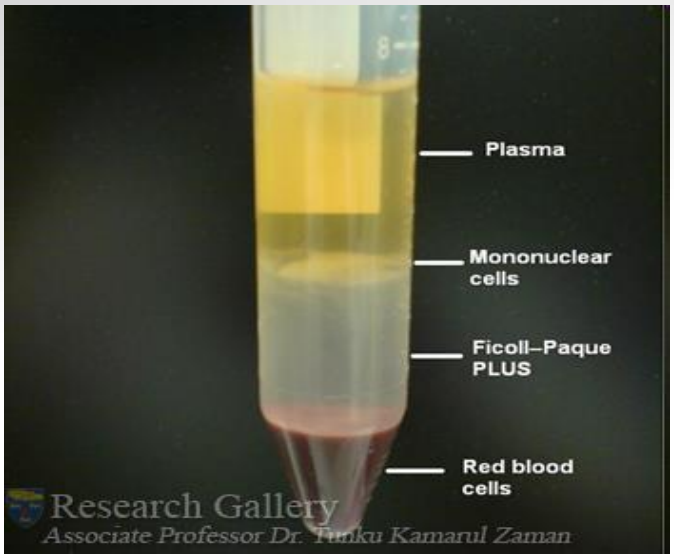


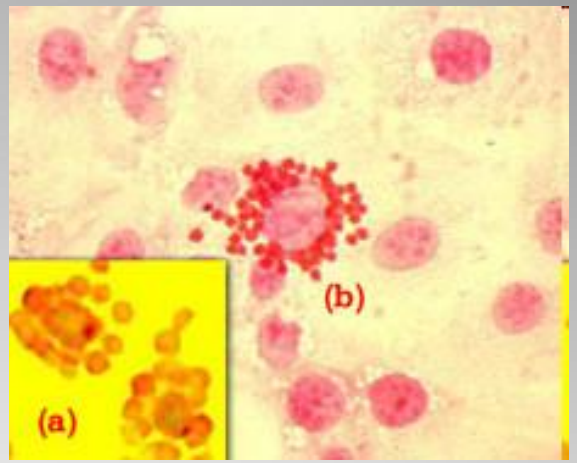
Fig. 1 Isolation of mononuclear cells using Lymphoprep™. (1) Blood diluted with an equal volume of saline is layered over half the volume of Lymphoprep™. (2) After sentrifugation at 600g for 20 min at 20°C, the mononuclear cells which band at the interface are (3) removed using a pipette.



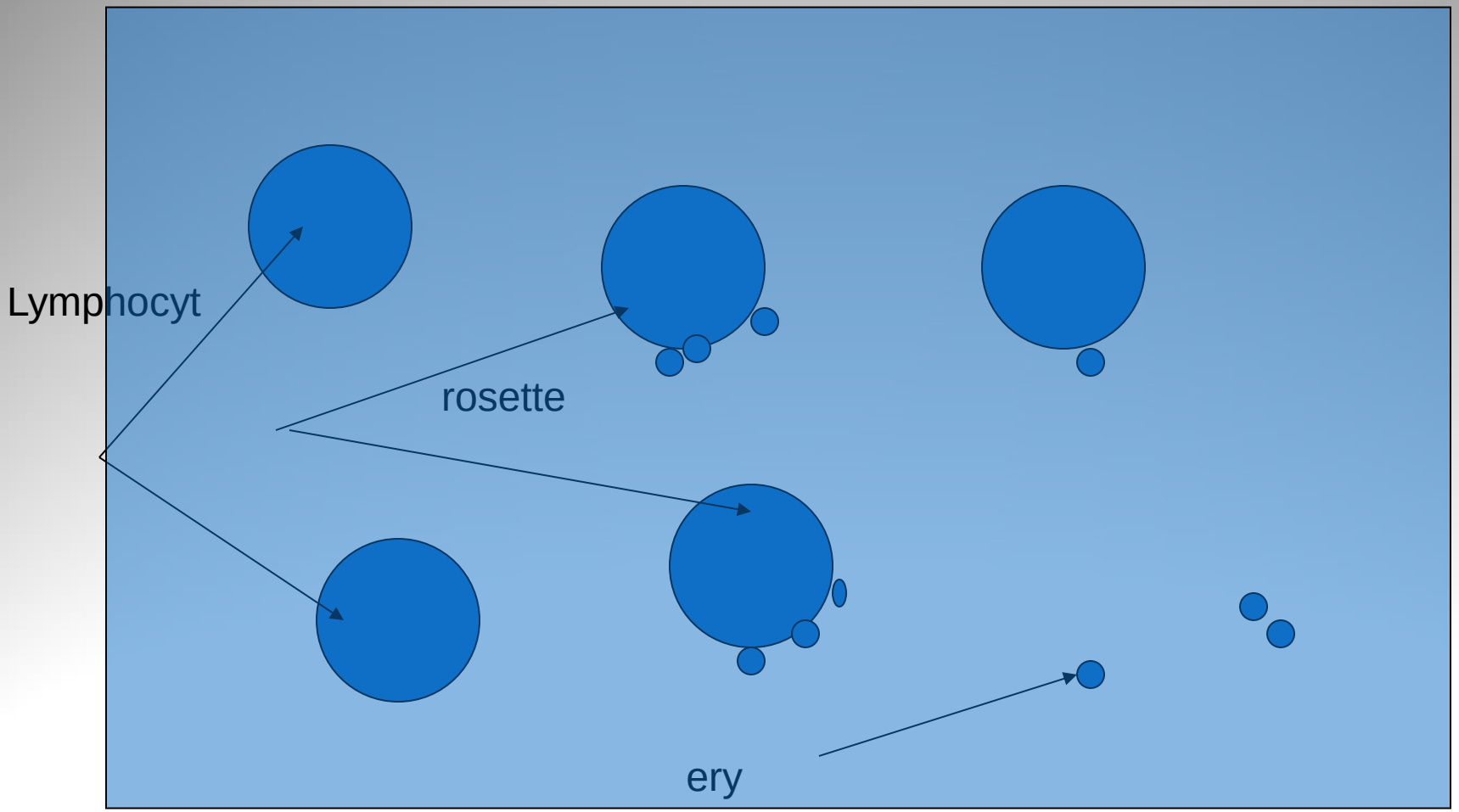
- Gradient density centrifugation
- Mononuclear cells (monocytes and lymphocytes) have a lower buoyant density than the erythrocytes and the polymorphonuclear leucocytes (granulocytes).
- The vast majority of mononuclear cells have densities below 1.077 g/ml. These cells can therefore be isolated by centrifugation on an isoosmotic medium with a density close to 1.077 g/ml, which allows the erythrocytes and the granulocytes to sediment through the medium while retaining the mononuclear cells at the sample/medium interface

Separation of T and B lymph. – Rosette test

- T a B lymf. : different receptors for animal erytrocytes
- T lymf. : receptor for sheep ery – E-rosettes
- B lymf. : receptor for mouse ery – M-rosettes
- **For T lymphocytes.**
- **Rosettes formed with erythrocytes.**



- T lymphocytes, have the characteristic of **forming E-rosettes when they bind selectively to sheep red blood cells (a).**
- Lymphocytes can be quantified by **acridine orange labeling** and afterwards observed with a fluorescence and ordinary light microscope.
- If T and B lymphocytes are labeled with acridine orange, only **T lymphocytes form E-rosettes; B lymphocytes** are actually stained but **do not form rosettes.**


Lymphocyt
rosette
ery