Haematology and blood transfusion

Andrea Knight, PhD

Definition & function

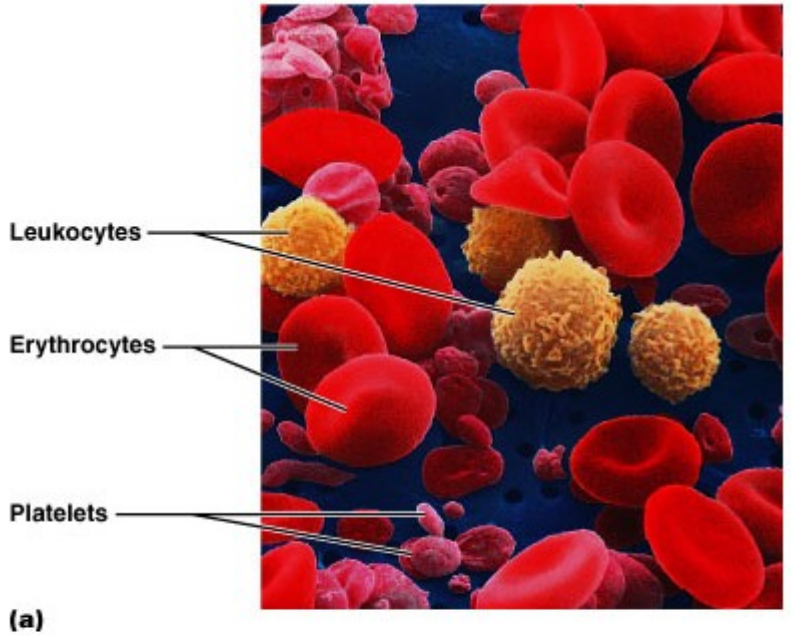
- the branch of medical science concerning blood and blood-forming tissues
- study of etiology, diagnosis, treatment, prognosis & prevention
- pathophysiology
  - Variations from normal blood element counts
  - Bleeding disorders - hemophilia
  - Malignant disorders – leukaemia, lymphoma, myeloma
  - Haemoglobinopathies
- bone marrow and stem cell transplantation
- blood transfusion & blood banking

Scanning EM

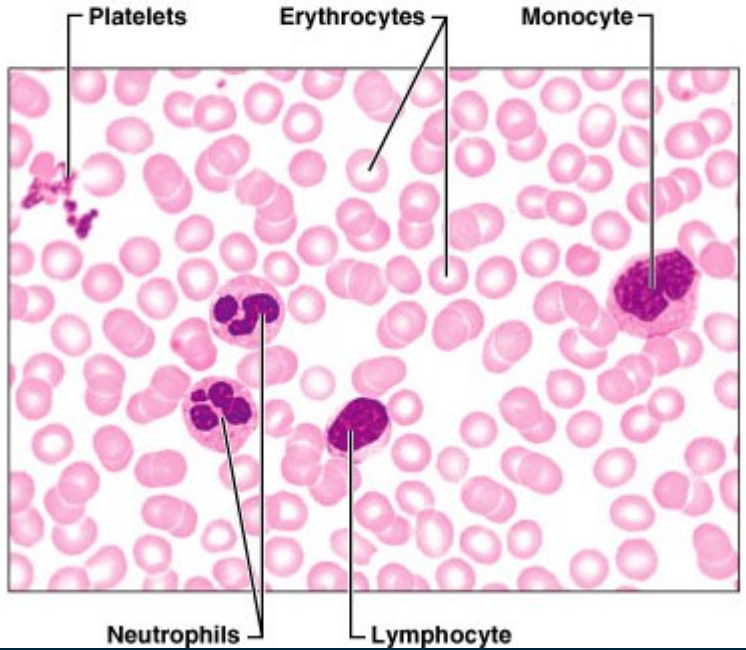
Organs & tissues

- peripheral blood
- bone marrow

- spleen
- lymph nodes
- (liver)



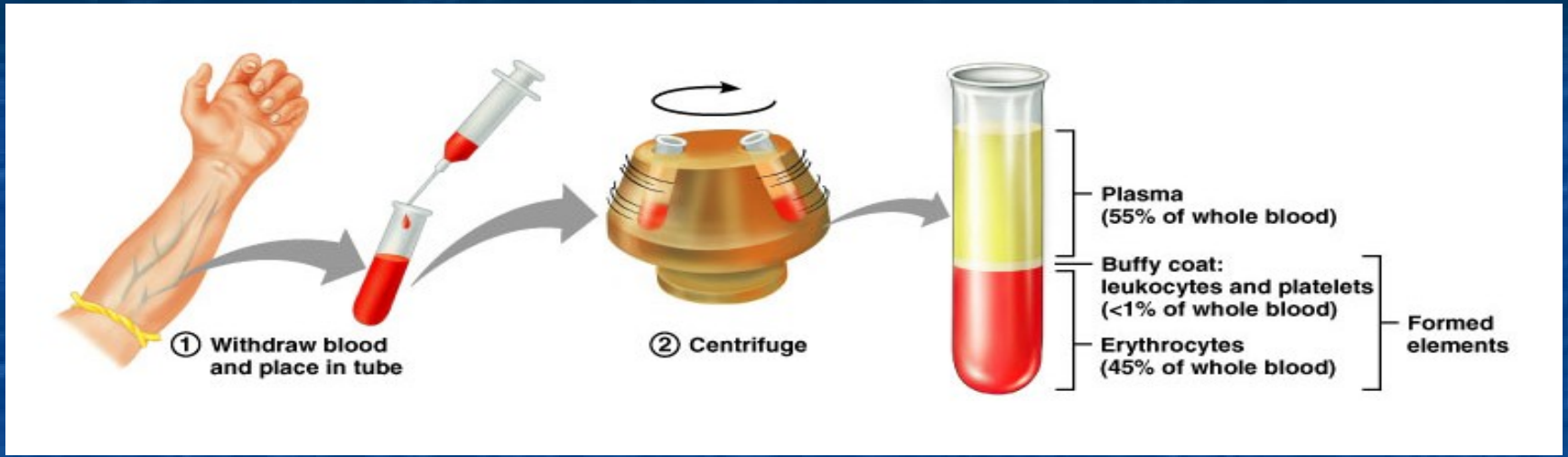
Light microscope



Composition of blood

- Specialized connective tissue
- Blood cells (elements) suspended in plasma
- Blood volume: 5-6 litres in males and 4-5 litres in females
- Clinically important hematocrit
  - % of blood volume consisting of erythrocytes (red blood cells)
  - Male average 47; female average 42
  - Plasma contains water, ions, 3x important proteins: albumin, globulins, fibrinogen

- Serum
  - Blood that is allowed to stand will clot
  - plasma without the clotting factors



- Density gradient centrifugation
- Solution: Lymphoprep, Ficoll (1.077 g/ml)
  - layered over with whole blood or bone marrow as 1;1 volumes
- Buffy coat

- Practicals: small groups 4-6 students welcome to learn in our labs; to arrange by email: knight@med.muni.cz

Anticoagulants:

-to prevent the coagulation (clotting) for:

renal dialysis

deep vein trombosis (DVT)

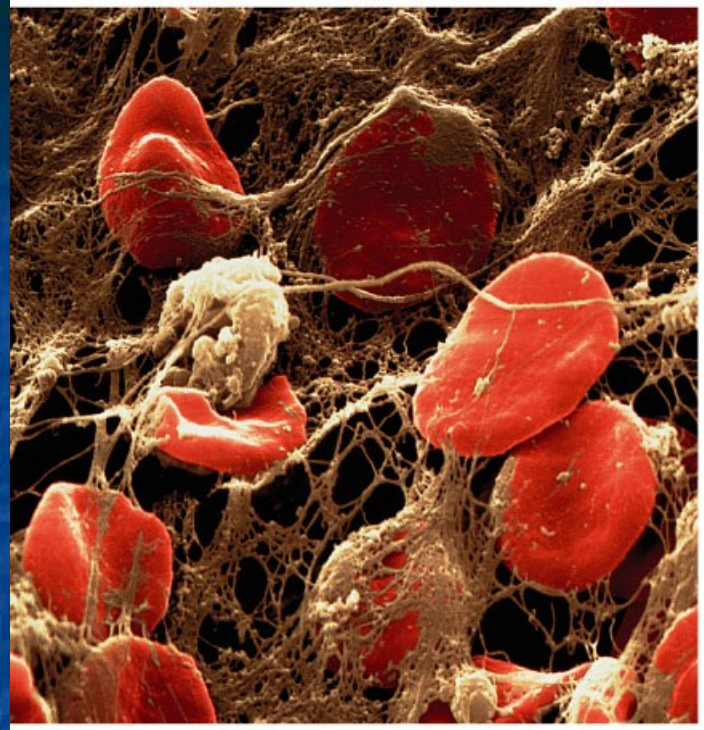
pulmonary embolism

myocardial infarction

ischemic stroke

-food supplements with blood-thinning effects: beer, papaya, cranberries

-encouraging clotting are avocado, spinach



A key event in the blood coagulation is the conversion of fibrinogen into fibrin by the serine protease enzyme thrombin.

Thrombin is produced from prothrombin, by the action of an enzyme, prothrombinase (Factor Xa along with Factor Va as a cofactor), in the final states of coagulation.

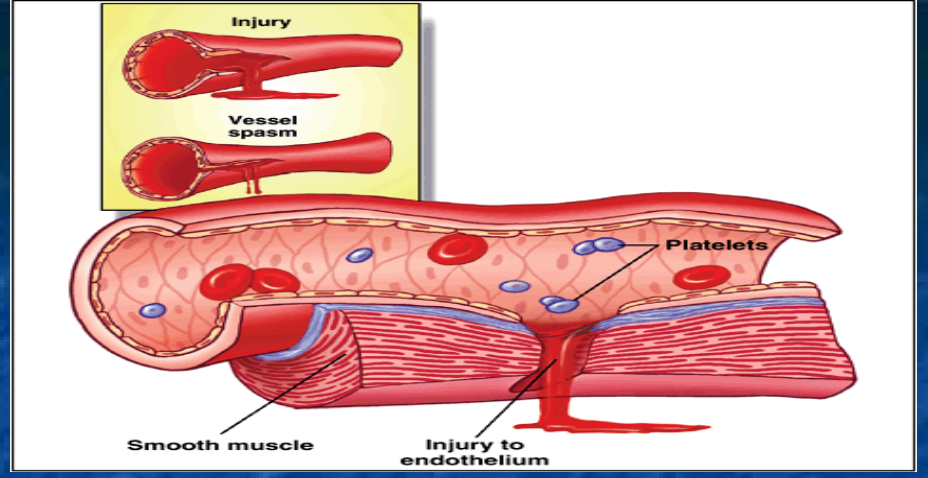
Fibrin is then cross linked by factor XIII (Fibrin Stabilizing Factor) to form a blood clot.

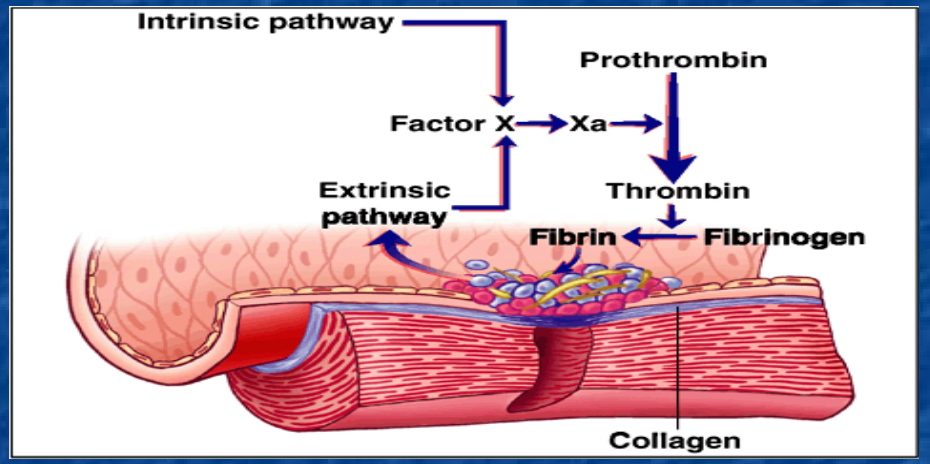
The principal inhibitor of thrombin in normal blood circulation is antithrombin.

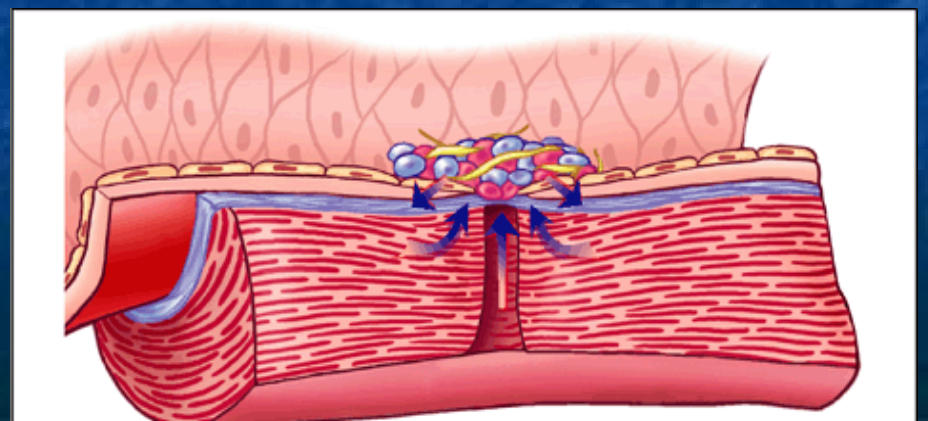
Zástava krvácení

- hemostáza má tři složky:

1. reakce cév
2. reakce trombocytů
3. koagulace: srážení krve

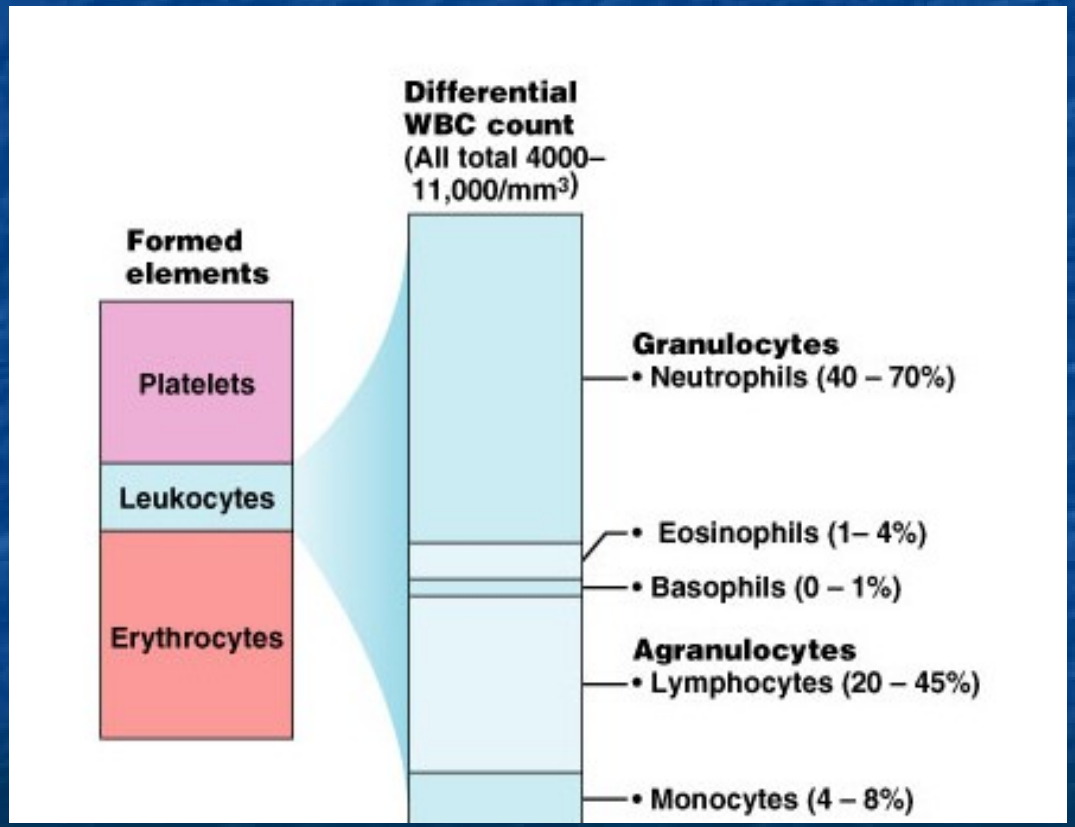






CBC test = complete blood count

- RBC count (red blood)
- WBC (white blood)
  - in thousands/cumm
  - differential WBC
- Platelet count in thousands/cumm



Adult Normal Ranges in FBC

Parameter		Male	Female
Haemoglobin	g/dL	13.5 - 18.0	11.5 - 16.0
WBC	$x10^9/L$	4.00 - 11.00	4.00 - 11.00
Platelets	$x10^9/L$	150 - 400	150 - 400
MCV	fL	78 - 100	78 - 100
PCV		0.40 - 0.52	0.37 - 0.47
RBC	$x10^{12}/L$	4.5 - 6.5	3.8 - 5.8
MCH	pg	27.0 - 32.0	27.0 - 32.0
MCHC	g/dL	31.0 - 37.0	31.0 - 37.0
RDW		11.5 - 15.0	11.5 - 15.0
Neutrophils		2.0 - 7.5	2.0 - 7.5
Lymphocytes		1.0 - 4.5	1.0 - 4.5
Monocytes		0.2 - 0.8	0.2 - 0.8
Eosinophils		0.04 - 0.40	0.04 - 0.40
Basophils		< 0.1	< 0.1

- MCV: mean cell volume
- PCV: packed cell volume (or haematocrit) – percentage of the blood volume that is made from RBCs
  - Hypoxia can lead to increased amount of RBC
- MCH: mean cell haemoglobin – average amount of haemoglobin per RBC
- MCHC: mean cell Hb concentration
- RDW: red cell distribution width

http://www.pathology.leedsth.nhs.uk/pathology/ClinicalInfo/CommonTestsampInvestigations/FullBloodCount.aspx

Variations from normal

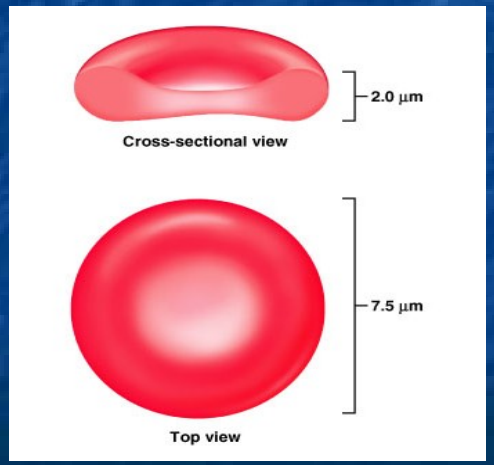
- Lymphopenia : too few lymphocytes
- Neutropenia: too few neutrophils
- Thrombocytopenia : too few platelets

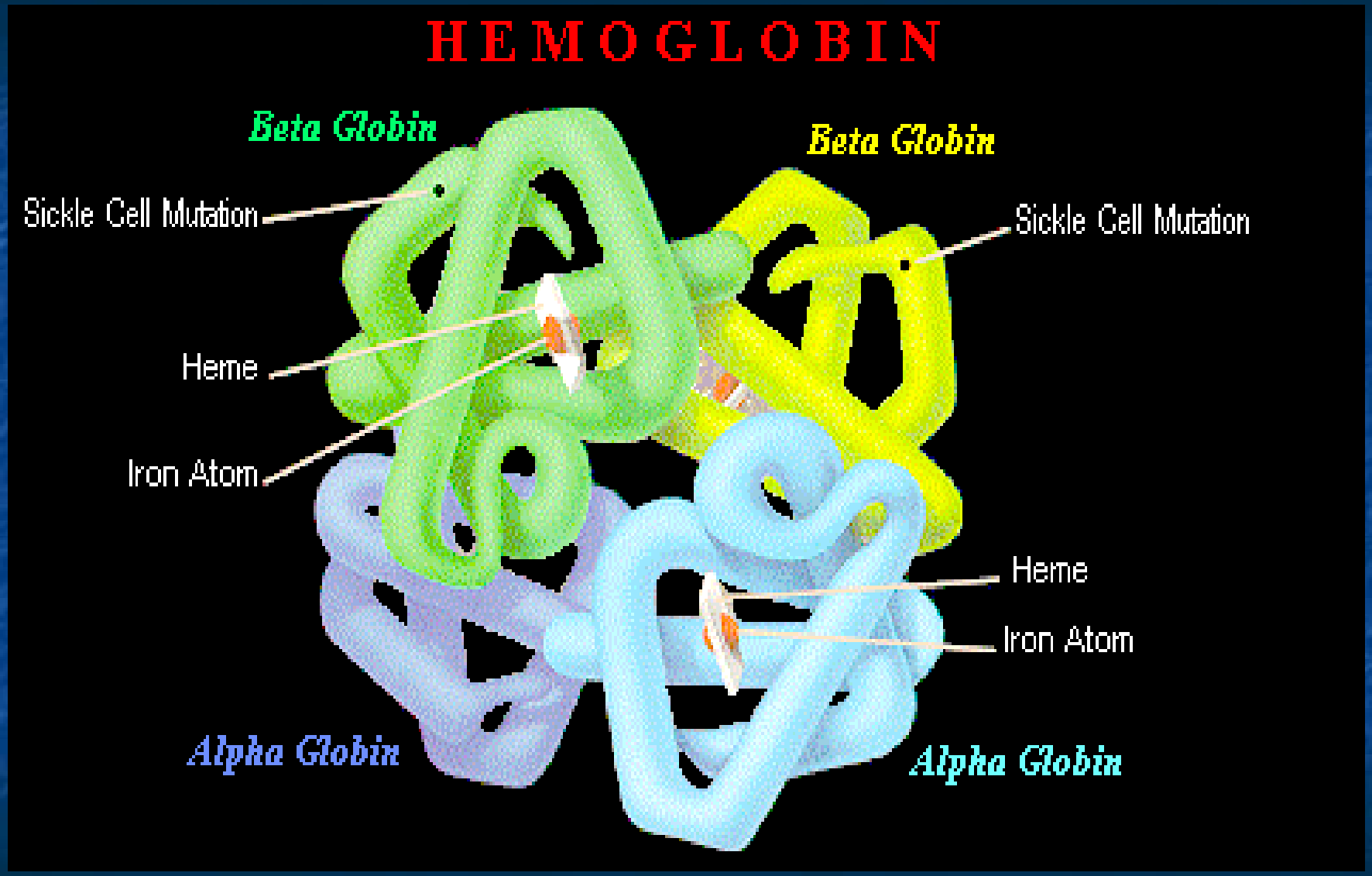
- Neutrophilia: too many neutrophils
- Thrombocytosis: too many platelets
- Leucocytosis : too many WBC

Erythrocytes

- Also called red blood cells (RBC)
- Biconcave discs and flexible
- Plasma membrane but no nuclei or organelles
- Packed with hemoglobin molecules
  - Oxygen carrying protein
  - 4 chains of amino acids, each with iron which is binding site for oxygen/CO2
  - young RBC still containing ribosomes are called ***reticulocytes***
- Lifespan 100-120 days

Parameter	Male	Female
Haemoglobin g/L	135 - 180	115 - 160
RBC x10^{12}/L	4.5 - 6.5	3.8 - 5.8



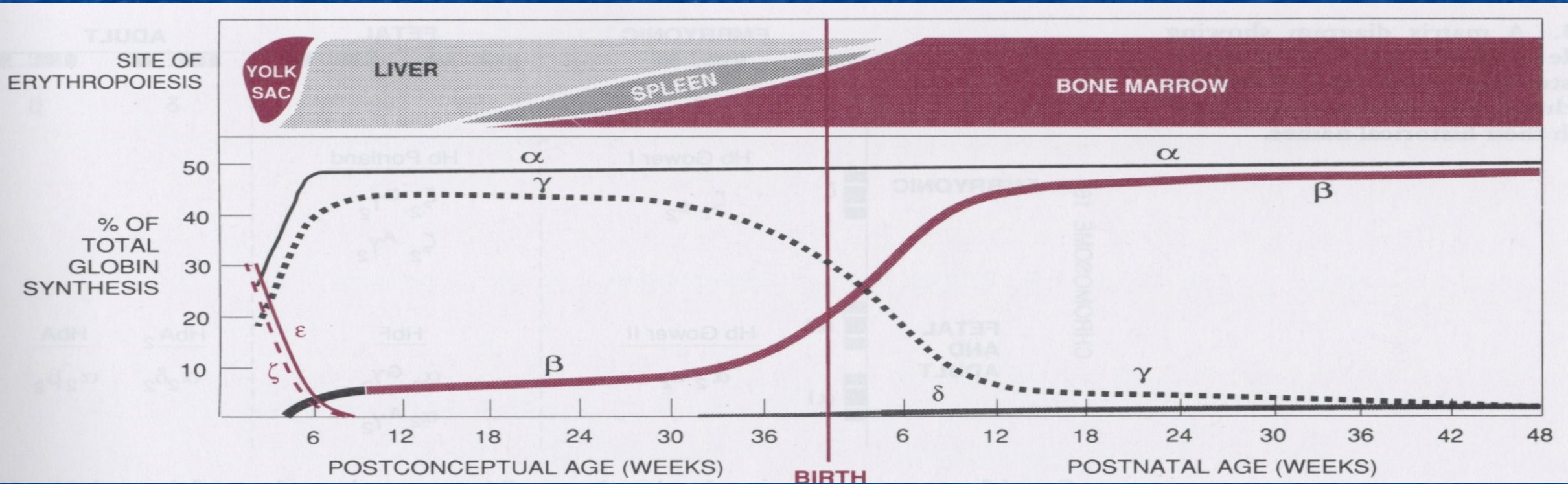

HEMOGLOBIN
Beta Globin
Beta Globin
Sickle Cell Mutation
Sickle Cell Mutation
Heme
Iron Atom
Heme
Iron Atom
Alpha Globin
Alpha Globin

Hemoglobin

- Heterotetramer
- HbA_1 $\alpha_2\beta_2$ 96-98%
- HbA_2 $\alpha_2\delta_2$ 2%
- HbF $\alpha_2\gamma_2$ this dominates until 6 weeks of age

- Afterwards, Hb A dominates through life

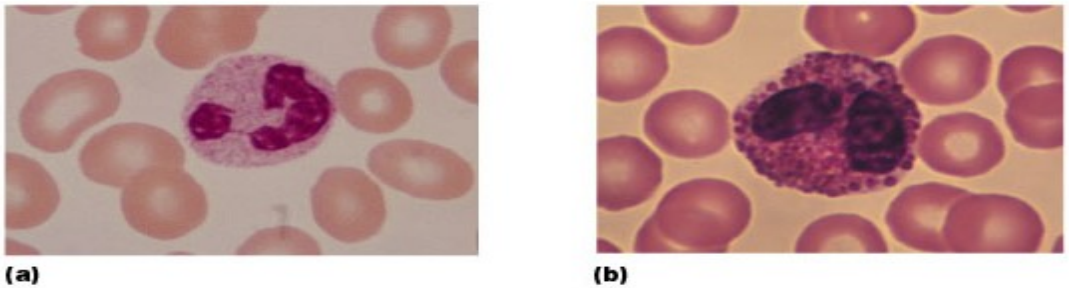
Erythropoesis

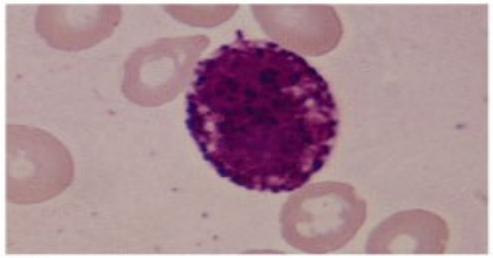


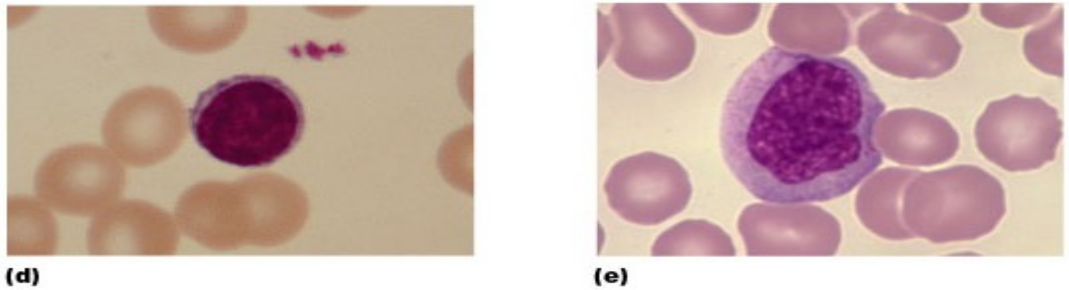
Methemoglobin

- MetHb is the derivative of Hb, in which the iron of the heme group is oxidized from Fe2+ to Fe3+
- MetHb is no longer completely capable of reversibly binding O2 (brown)
- MetHb forms continuously (present in RBC 1-2% c HB)
  - must be reduced actively by normal red cell metabolism or by ascorbic acid
  - cyanosis & fatigue 10%, coma & fatal 50-70%
  - nitrates in food and water, medication-local anesthetics, G6PD deficiency

Leukocytes



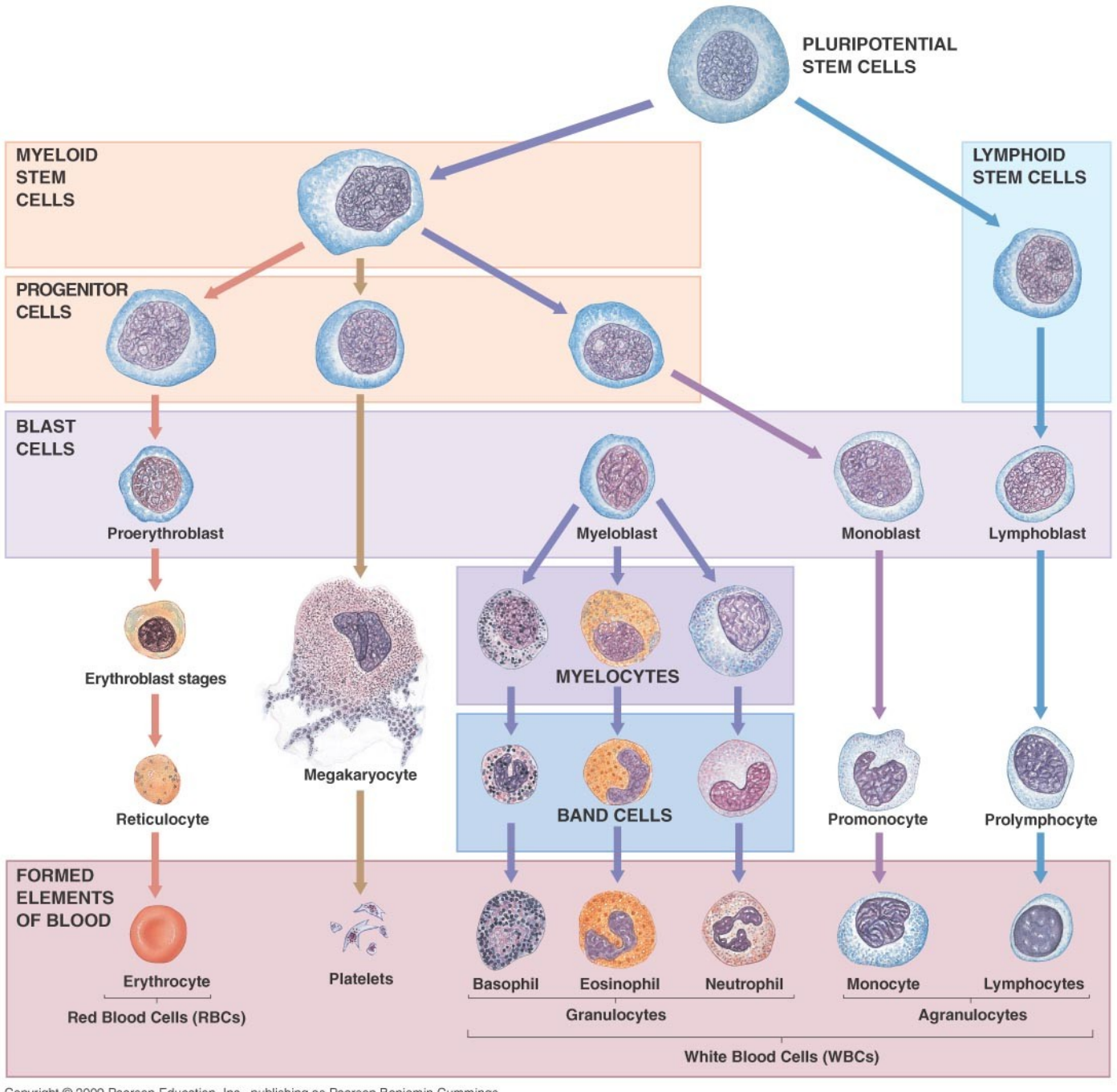




- A) Granulocytes
  - Granules, lobed nuclei
  - All phagocytic
  - Neutrophil: Nuclei of 2-6 lobes
  - Eosinophil: Nuclei bi lobed
  - Basophil: Dark purple granules
- D) lymphocyte
  - Large nucleus
  - T, B lineage
  - NK
- E) monocyte diff. into MØ

Hematopoiesis

- Formation of blood cells
- Occurs mostly in red bone marrow
- All cells arise from same pluripotent hematopoietic stem cells
- MSCs form fat cells, osteoblasts, chondrocytes, fibroblasts and muscle cells



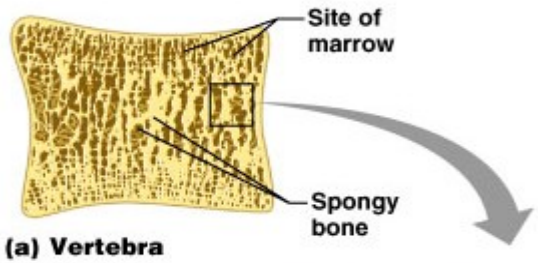
Not shown are mast cells, osteoclasts, dendritic cells

Bone marrow

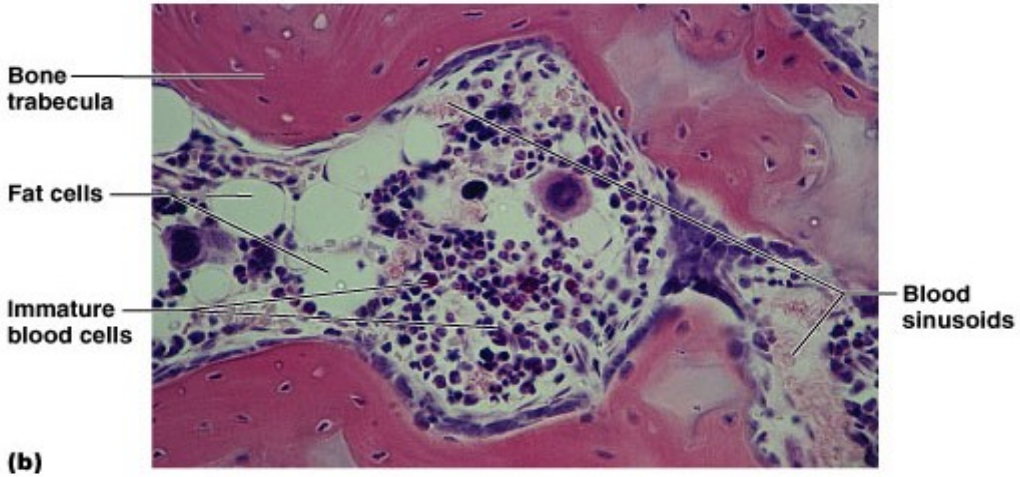
- Red marrow (medulla ossium rubra)
  - Consists mainly of haematopoietic tissue
  - Site of haematopoiesis (red and white blood cells, platelets)
- Yellow marrow (medulla ossium flava)
  - Made up of fat cells
- With age more red BM is converted to yellow BM
- BM stroma
  - Creates a microenvironment
    - Fibroblasts, MØ, adipocytes
    - Osteoblasts, osteoclasts
    - Endothelial cells
- Mesenchymal Stem cells (MSC)
  - Pluripotent stem cells that can differentiate *in vitro* and *in vivo* into a number of cell types incl. osteoblasts, chondrocytes, myocytes, adipocytes

Examination of bone marrow

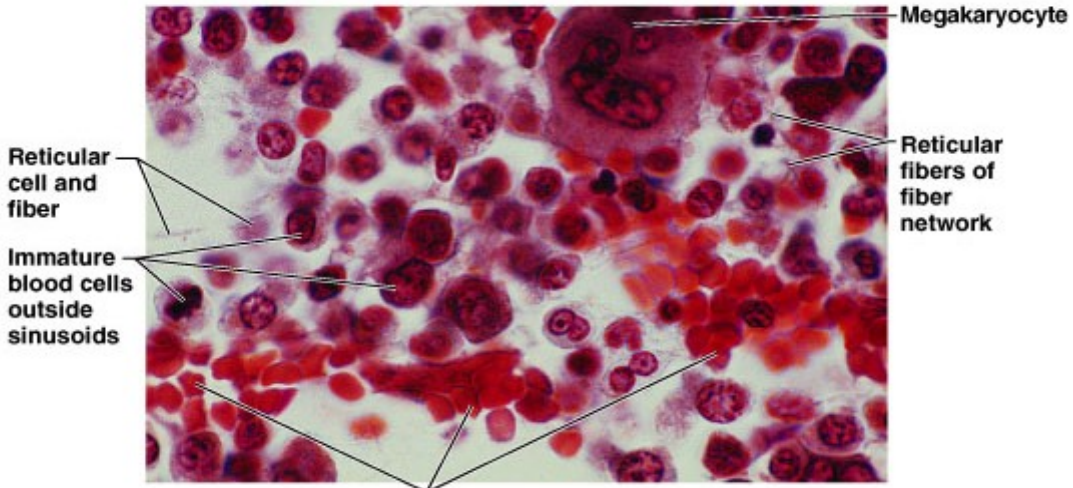
- BM sample obtained via biopsy or aspiration
- Used to newly diagnose & confirm suspected pathology
- To examine haematopoiesis
- Parallel to analysis of PB drawn from a vein by a phlebotomist
- Invasive procedure, not a routine



(a) Vertebra



(b)



(c)

Bone marrow harvest for transplantation

- BM is collected (pelvis under general anesthesia) and infused back:
  - Autologous Tx - same patient
  - Allogeneic Tx
    - Matched sibling
    - Matched Unrelated Donor (MUD)
  - Donor – recipient compatibility (MHC/HLA alleles)
  - Donor registers around the world

Cell storage for transplantation

- Cells frozen in 5-10% DMSO/human serum
  - DMSO, Dimethyl sulfoxid
  - Prevents the formation of ice crystals during the freezing process
- Stored at liquid nitrogen (-196 °C) for months/years
- Decreasing the temperature as 1°C per minute over night at -80°C in the Mr Frostie containing isopropyl alcohol

The Anthony Nolan Trust

- http://www.anthonynolan.org/
  - story of Anthony Nolan (1971-1979)
  - born with a rare Wiskott-Aldrich syndrome
  - only cure was Tx but no donor available
  - Shirley Nolan (1942-2002) and her legacy to start a donor register
  - Currently over 500 000 donors fully typed
  - Important charity – please log-in & donate
  - Research Institute & project Allostem
    - major EU grant involved 13 countries including CZ (Prof. Bartunkova, Prague)
  - Essential contribution to EBMT

MHC proteins

- Major Histocompatibility Complex, chr. 6
- HLA, human leukocyte antigens
  - Transplant antigens to prevent graft rejection
- HLA I. class (HLA-A, B, C)
  - Expressed on all nucleated cells
- HLA II. class (HLA-DP, DQ, DR)
  - Expressed on cells of IS
- MHC III. class
  - complement
- Prof. S Marsh at ANRI, President of the European Federation for Immunogenetics
- Allele frequencies vary in different populations and ethnic groups

Haematopoietic stem cell transplantation

- Progenitor stem cell transplantation derived from:
  - BM
  - peripheral blood
  - cord blood
- Autologous Tx
  - Requires extraction/apheresis of stem cells (HSC)
  - Stored in the liq nitrogen
  - Patient undergoes high-dose chemo ± radiotherapy
  - Established as the second-line treatment for lymphoma (not for AML)
- Allogeneic Tx
  - HLA matching
  - Recipient's immunosuppression
  - full ablative vs Reduced intensity conditioning (RIC)
  - RIC pioneered by Prof Stephen Mackinnon at University College London
  - Numerous clinical trials ongoing

Post HSCT

- Cytokine storm
- Graft-versus-host disease (GvHD) as a major complication post SCT
  - T cells present in the transplant recognize the host's (recipient's) cells as foreign
  - Minor histocompatibility antigens
  - Acute within 100 days as major challenge to transplant mortality and morbidity (grade 1-4)
  - Chronic as moderate to severe
  - Skin, liver, gut and GI tract, lung
  - Donor T cells mediate graft -versus-tumour effect (versus leukaemia, lymphoma or myeloma)

Graft – versus - tumour effect

- GvL (versus leukaemia)
  - Most prominent in CML patients, (also in ALL)
- GvM (myeloma)

Cytotherapy, 2012; 14: 1110–1118

informa
healthcare

Human Vdelta1 gamma-delta T cells exert potent specific cytotoxicity against primary multiple myeloma cells

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Abstract

Background aims. Human gamma-delta (γδ) T cells are potent effector lymphocytes of innate immunity involved in anti-tumor immune surveillance. However, the Vδ1 γδ T-cell subset targeting multiple myeloma (MM) has not previously been investigated. *Methods.* Vδ1 T cells were purified from peripheral blood mononuclear cells of healthy donors and patients with MM by immunomagnetic sorting and expanded with phytohemagglutinin (PHA) together with interleukin (IL)-2 in the presence of allogeneic feeders. Vδ1 T cells were phenotyped by flow cytometry and used in a 4-h flow cytometric cytotoxicity assay. Cytokine release and blocking studies were performed. Primary myeloma cells were purified from MM patients' bone marrow aspirates. *Results.* Vδ1 T cells expanded from healthy donors displayed prominent cytotoxicity by specific lysis against patients' $CD38^+$ $CD138^+$ bone marrow-derived plasma cells. Vδ1 T cells isolated from MM patients showed equally significant killing of myeloma cells as Vδ1 T cells from normal donors. Vδ1 T cells showed similarly potent cytotoxicity against myeloma cell lines U266 and RPMI8226 and plasma cell leukemia ARH77 in a dose-dependent manner. The interferon (IFN)-γ secretion and Vδ1 T-cell cytotoxicity against myeloma cells was mediated in part through the T-cell receptor (TCR) in addition to involvement of Natural killer-G2D molecule (NKG2D), DNAX accessory molecule-1 (DNAM-1), intracellular cell adhesion molecule (ICAM)-1, CD3 and CD2 receptors. In addition, Vδ1 T cells were shown to exert anti-myeloma activity equal to that of Vδ2 T cells. *Conclusions.* We have shown for the first time that Vδ1 T cells are highly myeloma-reactive and have therefore established Vδ1 γδ T cells as a potential candidate for a novel tumor immunotherapy.

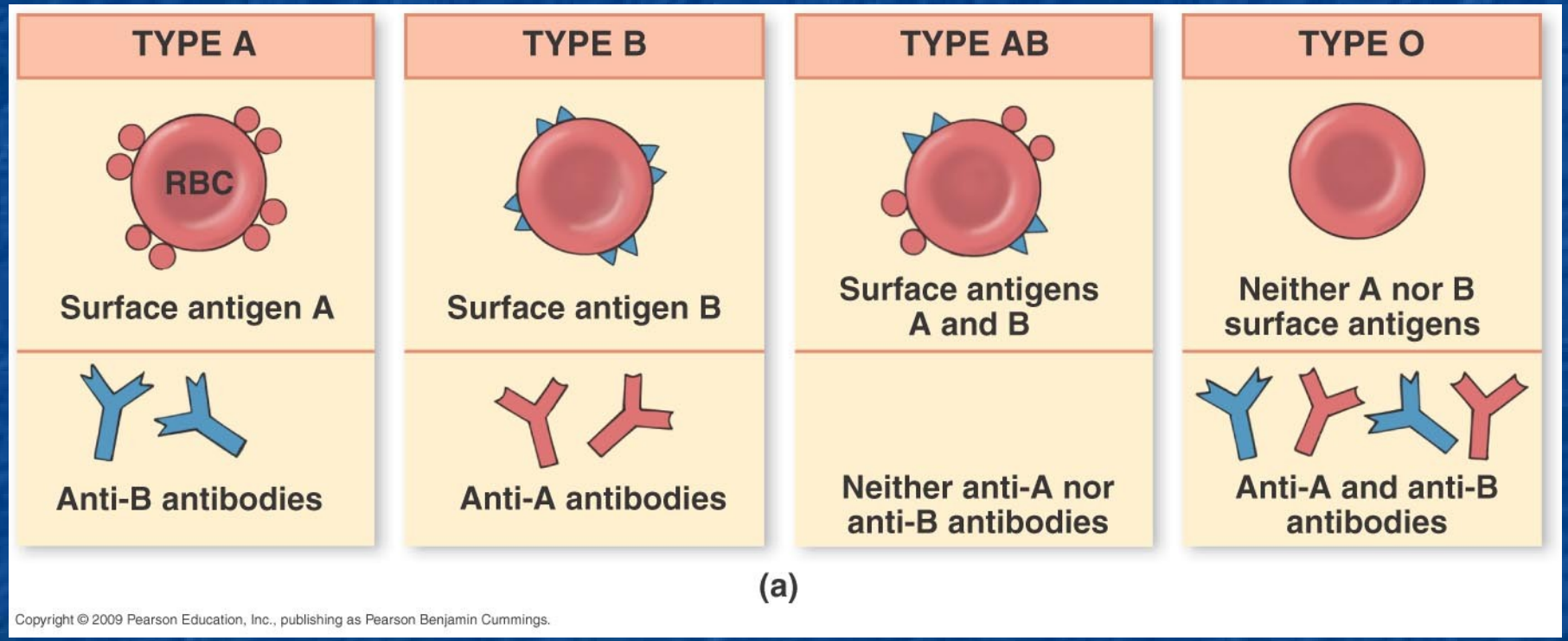
SCT and CMV

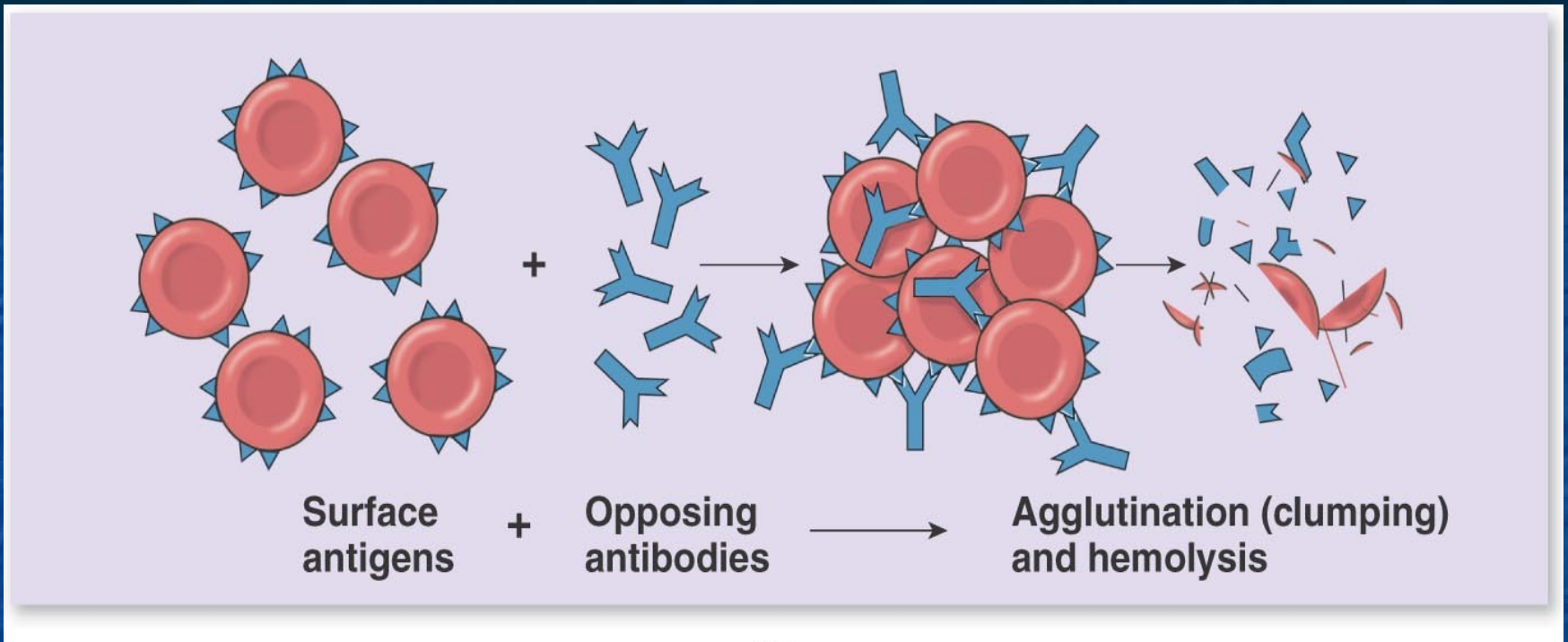
- HCMV cytomegalovirus
- Common beta-herpes virus (HHV5)
- Primary infection followed by a latent infection
- Vigorous immune response, persistent suppression of viral replication
- CMV seropositivity associated with immune senescence of virus-specific CD4+ and CD8+ T cells (Prof. Paul Moss, Graham Pawelec, Mark Wills)
- Multiple strategies to evade the host immune system
- Immunocompetent vs immunocompromised host
  - Donor+ Recipient+
  - D+ R-
  - D- R+
  - D- R-

Blood transfusion

- process of receiving blood intravenously
- to replace a lost blood component (red blood cells, plasma, platelets or clotting factors)
- donated blood processed/separated by centrifugation
- tested for infections (HIV 1, 2, HTLV 1, 2, Hep B, C , syphilis and CMV)
- stored in Blood Bank
- compatibility testing between D and R
- typing of recipient's blood determines the ABO blood groups and Rh status
- sample tested for any alloantibodies that may react with donor blood

ABO blood groups





- If a blood transfusion is given to a person who has antibodies to that type of blood, then the transfused blood will be attacked and destroyed (transfusion reaction)

ABO blood group types

- Europe:
  - A 45%
  - B 16%
  - AB 6%
  - O 33%

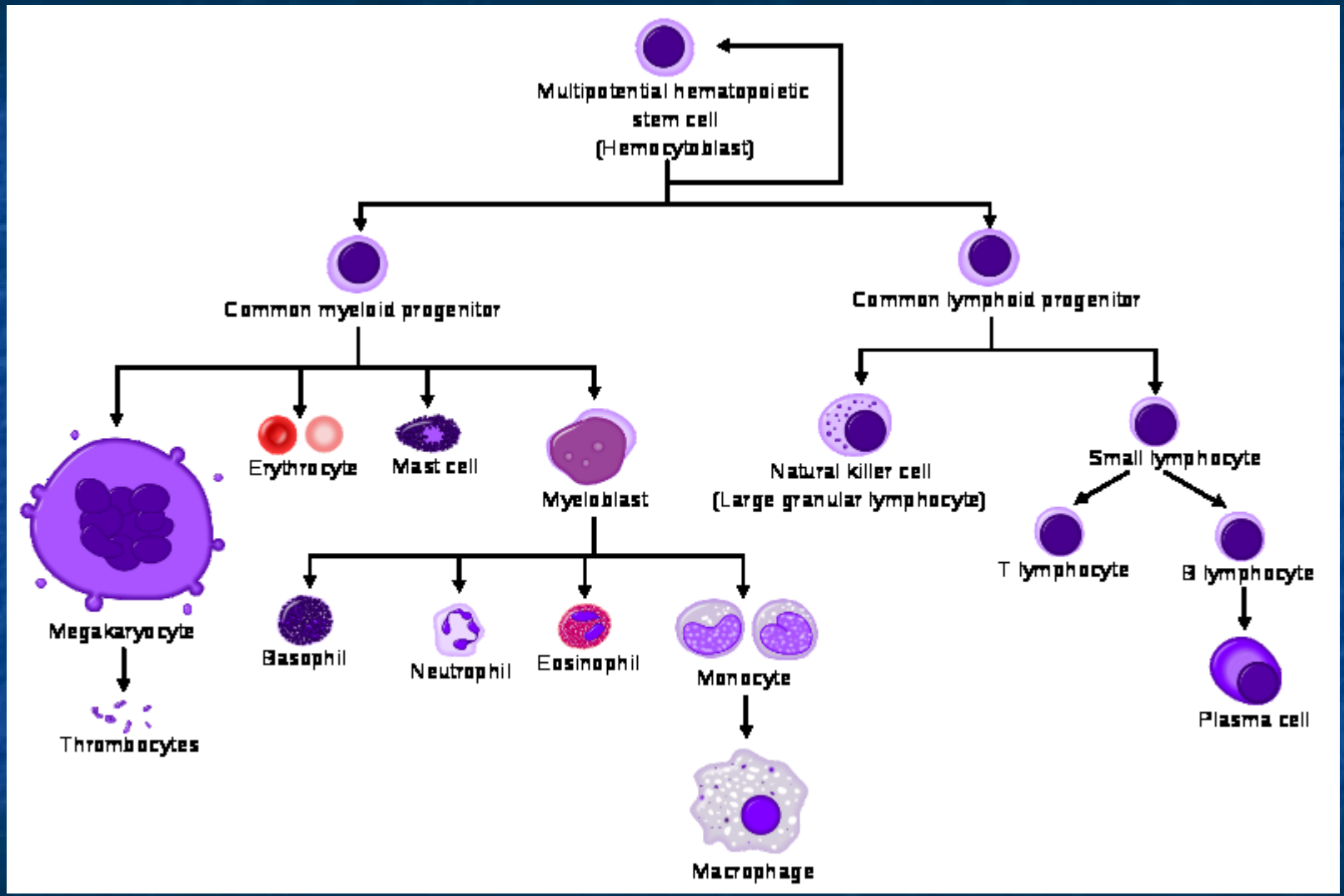
TABLE 20.4 Differences in Blood Group Distribution

Population	Percentage with Each Blood Type				
	O	A	B	AB	Rh^+
U. S. (average)	46	40	10	4	85
Caucasian	45	40	11	4	85
African-American	49	27	20	4	95
Chinese	42	27	25	6	100
Japanese	31	39	21	10	100
Korean	32	28	30	10	100
Filipino	44	22	29	6	100
Hawaiian	46	46	5	3	100
Native North American	79	16	4	<1	100
Native South American	100	0	0	0	100
Australian Aborigines	44	56	0	0	100

Rh blood group system

- consists of 50 defined blood-group antigents
- The commonly used terms *Rh factor*
  - *Rh positive* (85%)
  - *Rh negative* (15%) refer only to the *D antigen*
- We either have or don't have it on the surface of red cells
- Condition of hemolytic disease of the newborn
  - Incompatibility between mother and the fetus

Haematological disorders


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
Thrombocytes
Plasma cell
Macrophage

Disorders of Erythrocytes

- Polycythemia: too many cells
- Anaemia: not enough cells

Anaemia - symptoms

- Among over 400 types of anaemia
- Defined as a condition that develops when:
  - Decrease in the total number of red blood cells (RBC)
  - Decrease of the amount of haemoglobin and/or its reduced ability to carry oxygen
- Symptoms:
  - fatigue and tiredness, pain, shortness of breath, fast heartbeat, cold hands
  - bone deformities (found in thalassemia major)
  - leg ulcers (sickle cell anaemia)
  - enlarged spleen
  - in children - poor performance at school
  - in elderly, in patients

Anaemia - causes

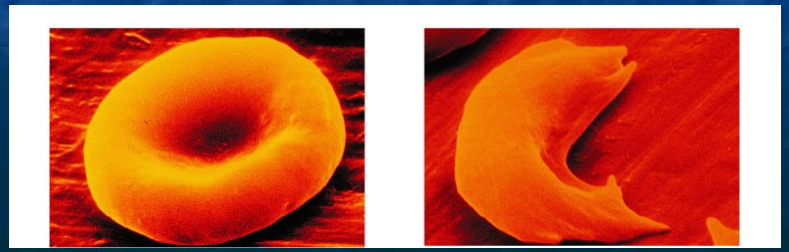
- **Blood loss**
  - Common in women, pregnancy
  - Acute: trauma and surgery
  - Chronic: many types of cancers (colon, bladder carcinomas), IBD patients
- **Decreased production of RBC - result of BM failure & differentiation of stem cells**
  - Pure red blood cell aplasia (PRCA)
  - Aplastic anaemia; along with exposure to chemicals, radiation, drugs, viral infection
  - Fanconi anaemia; 22x identified genes involved in DNA repair
- **Impaired/ faulty production of RBC and maturation of erythroblasts**
  - Deficiency of Vitamin B12; Pernicious anaemia
  - Iron deficiency anaemia; deficient heme synthesis
  - Severe type Myelophthisis; displacement of BM by malignant tumours or fibrosis
  - Myelodysplastic syndrome (MDS)
- **Increased destruction of RBC**

Increased destruction of RBC classified as hemolytic anaemias

- **Hereditary spherocytosis**
  - defect in RBC cell membrane caused by mutations in gene relating to proteins that allow RBC to change shape; then destroyed by the spleen
- **Antibody mediated**
  - primarily by IgG attacking the RBCs, leaving their Fc exposed to monocytes and MΦ, destroyed as spherocytes in the spleen
  - Warm autoimmune hemolytic anaemia (WAIHA) at 37°C
  - Cold antibody induced anaemia (at 28-31°C)

Disorders of Erythrocytes - Hemoglobinopathies

- are inherited single-gene disorders
- characterized by decreased and/or unstable haemoglobin
  - Thalassemia
    - usually results in underproduction of normal globin proteins often through mutations in regulatory genes
    - Beta; subtypes major (both beta globin genes missing) and intermedia
    - Alpha; subtypes Hb H and hydropsis fetalis
    - Minor; either alpha or beta globin gene missing
  - Sickle cell disease
    - Estimated that 7% of world's population (~420 million) are carriers
    - Inheritance of two abnormal B-globin gene (chr 11)
    - The gene defect is a SNP (single nucleotide polymorphism) where GAG changes to GTG and results in glutamic acid being substituted by valine (E6V)



G6PD Deficiency

- Glucose-6-phosphate dehydrogenase deficiency
  - enzyme involved in the pentose phosphate pathway
  - important in red blood cell metabolism
- Perhaps most common, world-wide congenital abnormality
  - > 300 variants identified
  - X-linked inheritance
- Common G6PD deficient variants are associated with an acute intermittent hemolysis and anemia
- vast majority never symptomatic!
- Mediterranean and others: may hemolyze with fava beans

Anaemia - diagnosis and treatment

- Diagnosis
  - Blood test, physical exam, symptoms and medical history
  - Complete blood count (CBC) to determine the number, size and volume of Hb
  - Blood iron level to indicate of body's iron stores
  - Vitamin B12 levels
  - Detection of rare types, RBC fragility, reticulocyte count, bilirubin
- Treatment
  - Iron and folic acid supplements
  - Blood transfusion

Disorders of Platelets

- Thrombocytopenia
  - normal platelet count ranges from 150,000 - 450,000 per µL
  - platelet count below 50,000 per µL
  - occasional bruising, nosebleeds, bleeding gums
  - !! internal bleeding
  - many causes: decreased production or increased destruction (SLE, HIV)
    - Vitamin B12 or folic acid deficiency
    - Leukaemia, MDS
    - Decreased production of trombopoietin by the liver in liver failure
    - Bacterial, viral infections, sepsis
    - Hereditary: Fanconi anemia
  - Treatment depending on the cause
    - Corticosteroids
    - Platelet transfusion

Disease of the bone marrow

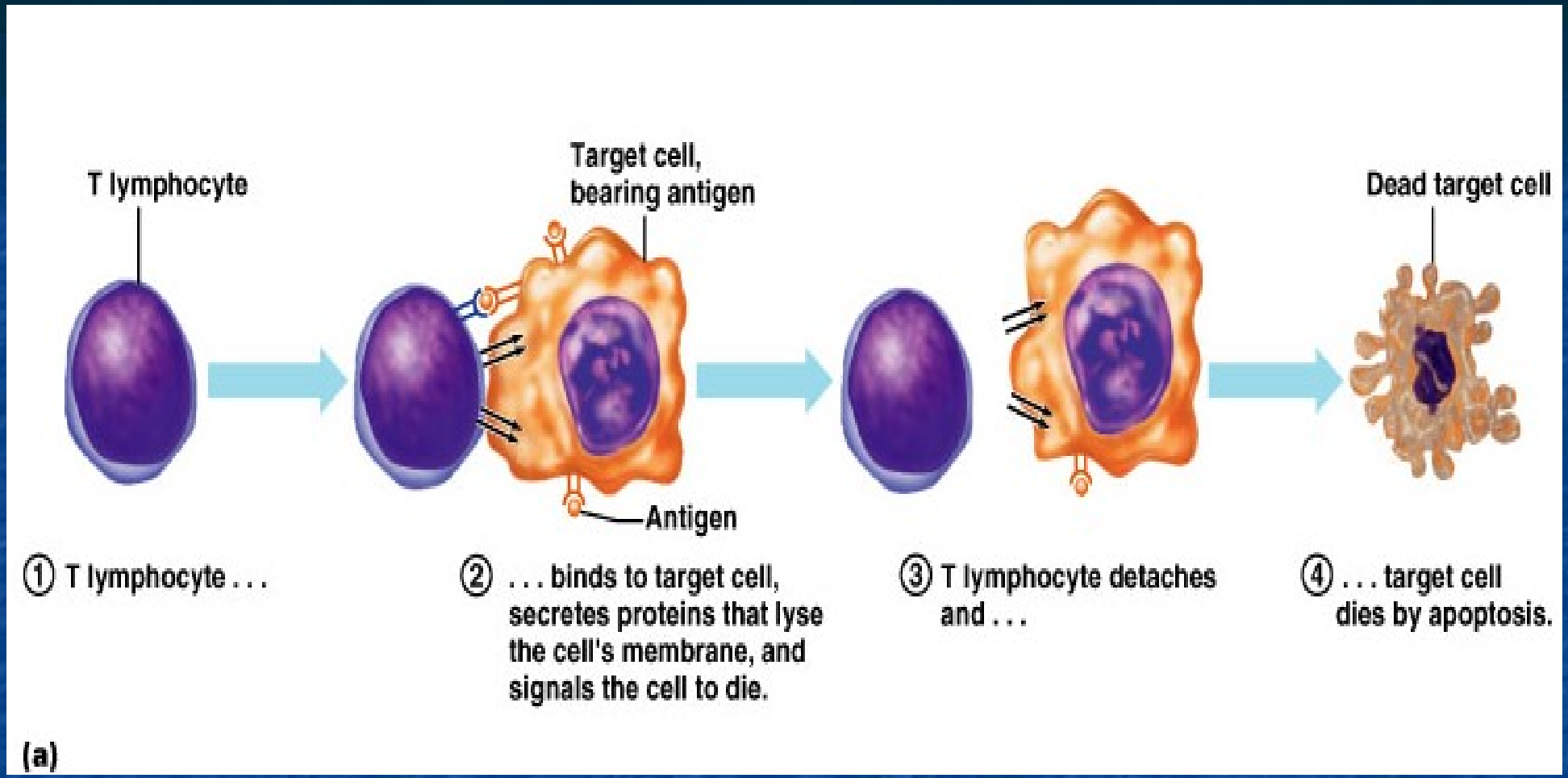
- Congenital defects
- Aplastic anemia
- Malignancies
  - Leukaemia
  - Lymphoma
  - Multiple myeloma

Congenital defects

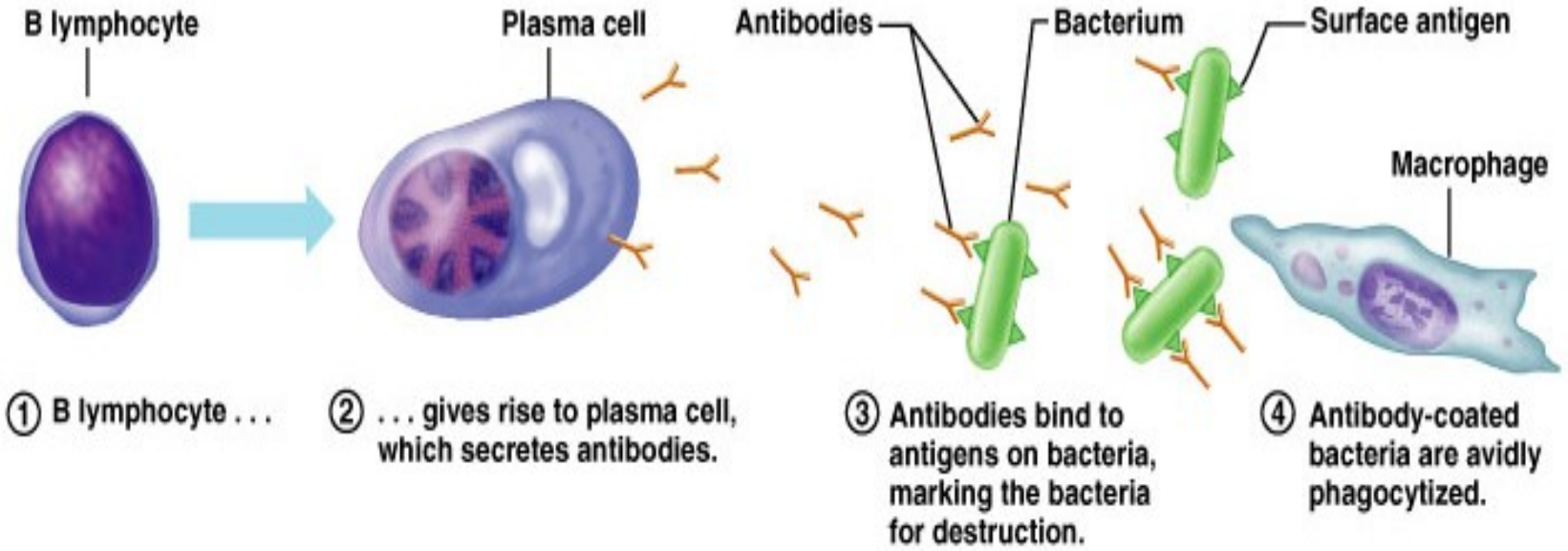
- Dyskeratosis congenita (DKC)
  - is a rare progressive congenital disorder resembling premature aging
  - Essen. bone marrow failure syndrome
  - DKC typically develop between ages 5-15 years
  - is a result of one or more mutations in the long arm of the chr X in the gene DKC1
  - Heiss NS, Knight SW, Vulliamy TJ, et al." May 1998, *Nat. Genet.* **19** (1): 32–38

Haematological Malignancies - Objectives

- Define the disease: acute vs chronic leukemia…
- Classify leukemia
- Understand the pathogenesis
  - Genetic alterations including translocations, mutations (leukaemogenesis)
- Understand the pathophysiology
- Able to list down the laboratory investigations required for diagnosis
- Understand the basic management of leukemia patients



Stress ligands
shedding
trogocytosis


B lymphocyte
Plasma cell
Antibodies
Bacterium
Surface antigen
Macrophage
① B lymphocyte . . .
② . . . gives rise to plasma cell, which secretes antibodies.
③ Antibodies bind to antigens on bacteria, marking the bacteria for destruction.
④ Antibody-coated bacteria are avidly phagocytized.
(b)

Leukaemia I.

- Definition:
- heterogenous group of malignant disorders which is characterised by uncontrolled clonal and accumulation of blasts cells in the bone marrow and body tissues
- Excessive production of WBC
- Often non fully differentiated cells called “blasts”
- WBC have abnormal function
  - Resistant to apoptosis
  - Excessive proliferation
  - Tumour microenvironment in the bone marrow
- Disruption of normal haematopoesis in bone marrow

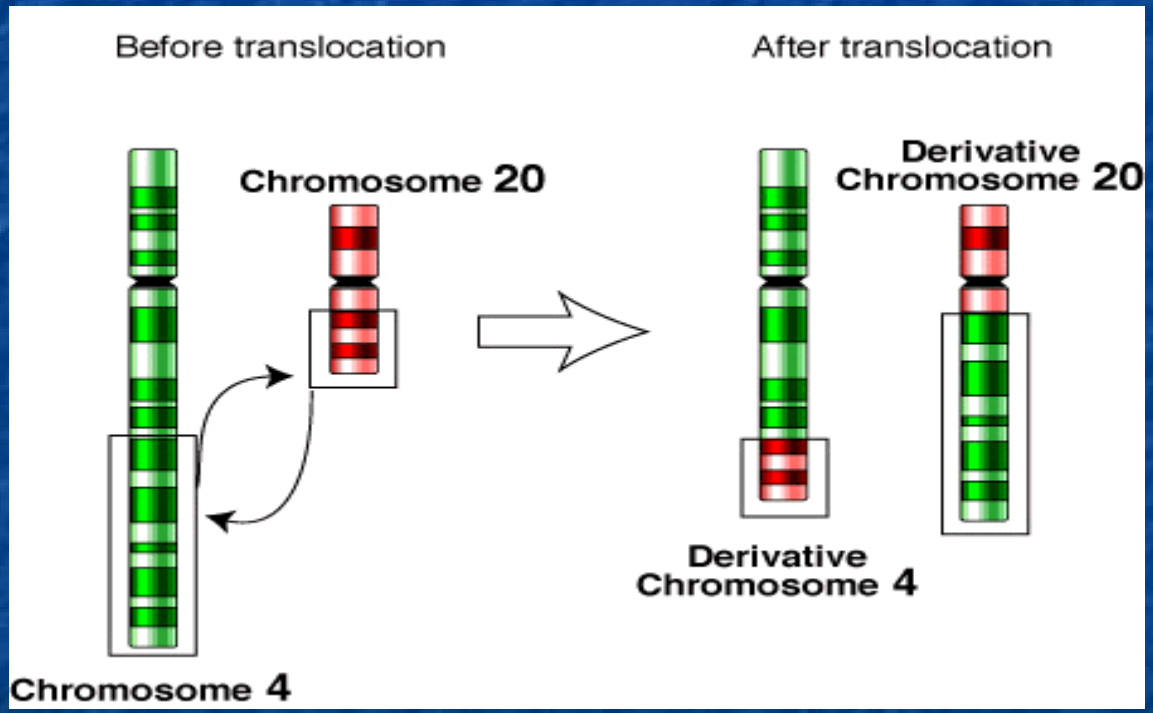
Leukaemia II.

- Classification
  - Acute
    - Acute lymphoblastic leukemia (T-ALL & B-ALL)
    - Acute myeloid leukemia
  - Chronic
    - Chronic myeloid leukemia
    - Chronic lymphocytic leukemia

Leukaemogenesis

- Develops as a result of a genetic alteration within single cell in the bone marrow
  - Hereditary factors (Fanconi, Down sy)
  - Radiation, chemicals, drugs
  - Virus related
  - Oncogenes, tumour suppressor genes
  - Retrovirus mediated (HTLV-1, EBV)
  - Age related

Leukaemia and chromosomal translocations



Mechanisms of Translocation

- Ionising radiation can caused breakage of the phosphodiester backbone of both strands of DNA
- Double-strand breaks are very efficiently repaired
  - Potential loss of genetic material
  - Double-strand ends recognised as “foreign” DNA and destroyed
- If Double-strand breaks occur in two different chromosomes then possibility for incorrect repair taking place

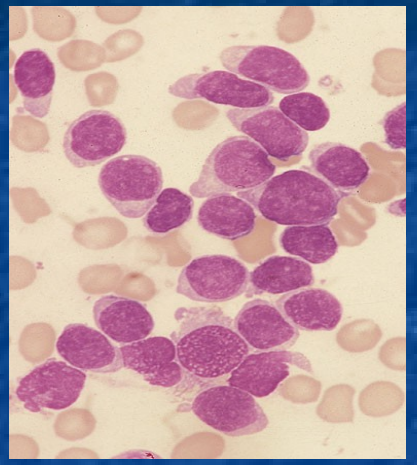
Frequent translocations

B-ALL	t(1;19)	5%
B-ALL (in children)	t(12;21)	22%
T-ALL	t(5;14)	20%
T-ALL	1p32 deletion	25%
AML	t(15;17)	13%
AML	t(8;21)	7%
CML	t(9;22)	99%

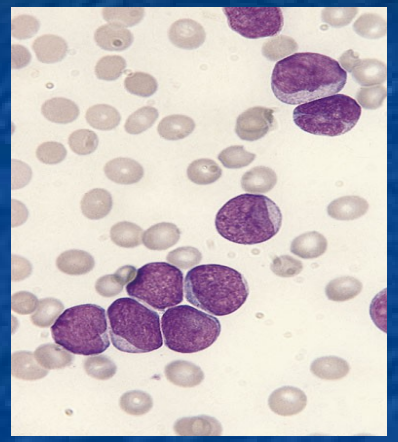
Techniques used in Molecular Diagnosis

- Morphology
- Flow Cytometry
- PCR
- FISH

AML morphology



m0



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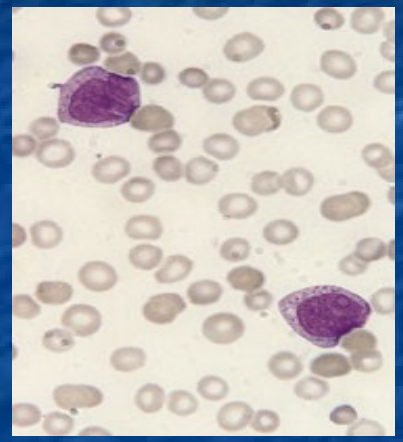

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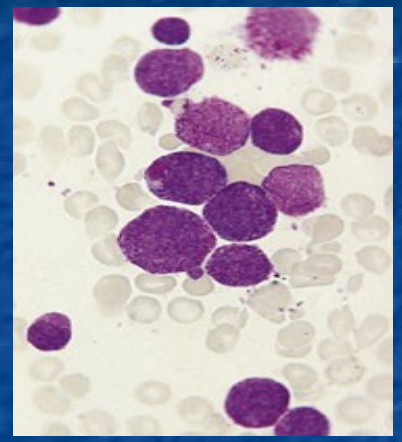

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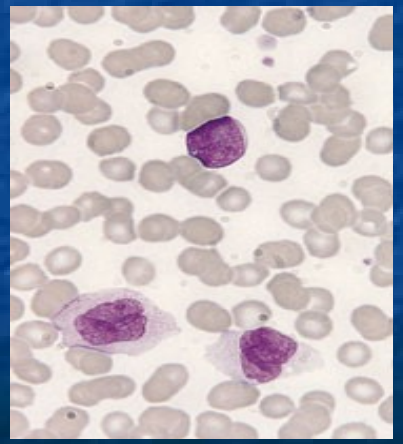

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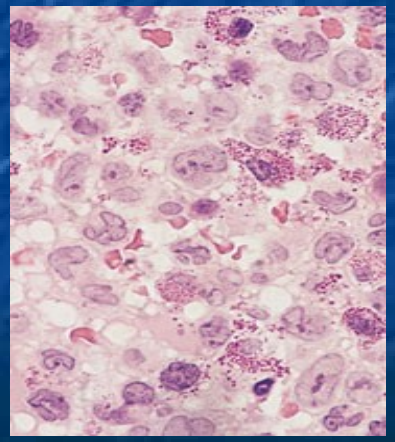

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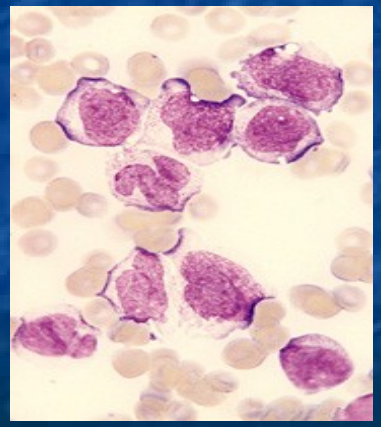

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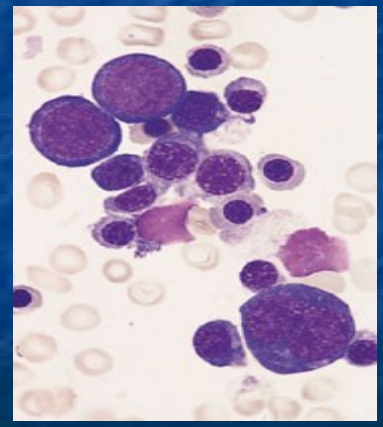

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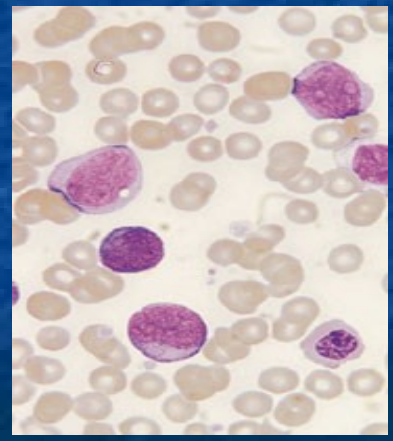


m7

From lecture by Dr NJ Dodd BS967-7-SP: Session 6 *courses.essex.ac.uk/bs/**bs967**/restricted/NJD%20Leukaemia.ppt*

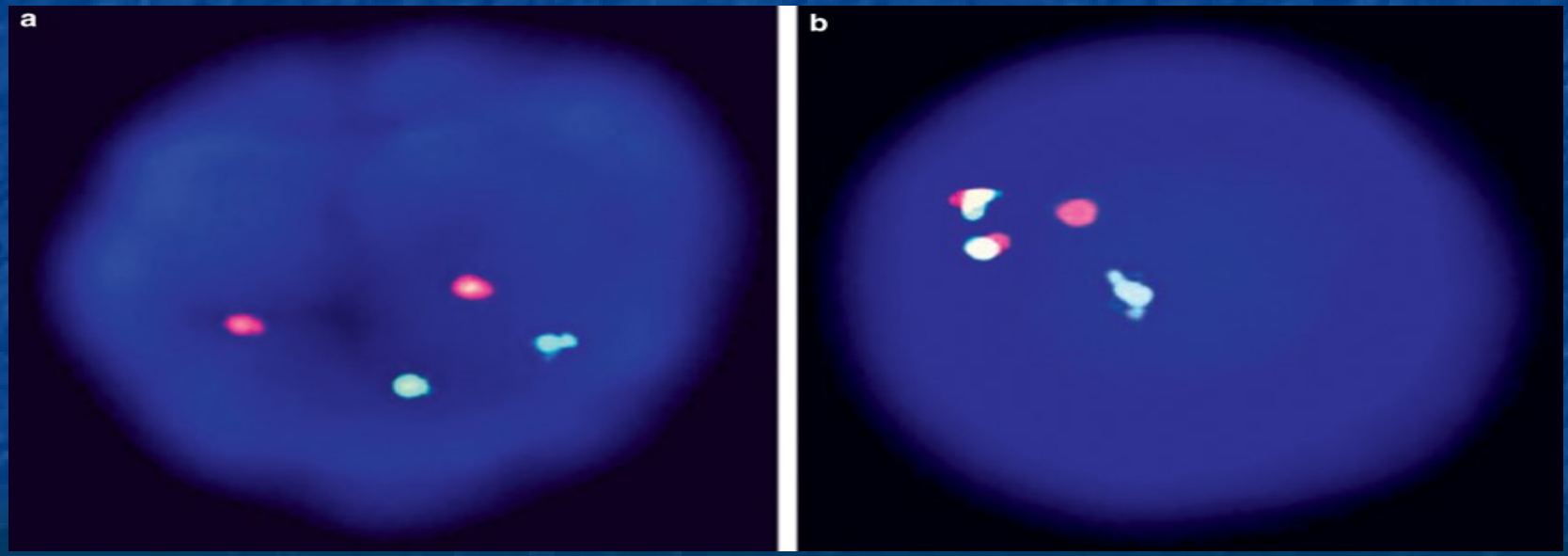
FAB classification of AML

M0 Undifferentiated blasts
M1 AML without maturation
M2 AML with maturation
M3 Acute promyelocytic leukemia
M4 Acute myelomonocytic leukemia
M5 Acute monocytic leukemia
M6 Acute erythroblastic leukemia
M7 Acute megakaryoblastic leukemia

Fluorescence *in situ* hybridization (FISH)

- Use of fluorescently-labeled DNA probes to hybridize onto metaphase spread of chromosomes
- Allows for the location of the probe on the chromosome to be identified

t(15;17)



Fluorescence *in situ* hybridization investigation of cutaneous lesions in acute promyelocytic leukemia
Wrede et al. *Modern Pathology* (2005) **18,** 1569–1576.

Acute Leukaemogenesis

Oncogene can be activated by :

- **chromosomal translocation**
- **point mutations**
- **inactivation**

- In general, several genes have to be altered to effect neoplastic transformation

Pathophysiology

- Acute leukaemia cause morbidity and mortality through:
  - **Deficiency in blood cell number and function**
  - **Invasion of vital organs**
  - **Systemic disturbances by metabolic imbalance**

Acute Lymphoblastic Leukaemia

- Cancer of the blood affecting the white blood cell *LYMPHOCYTES*
- Commonest in the age 2-10 years
- Peak at 3-4 years.
- Incidence decreases with age, and a secondary rise after 40 years.
- In children - most common malignant disease
- 85% of childhood leukaemia

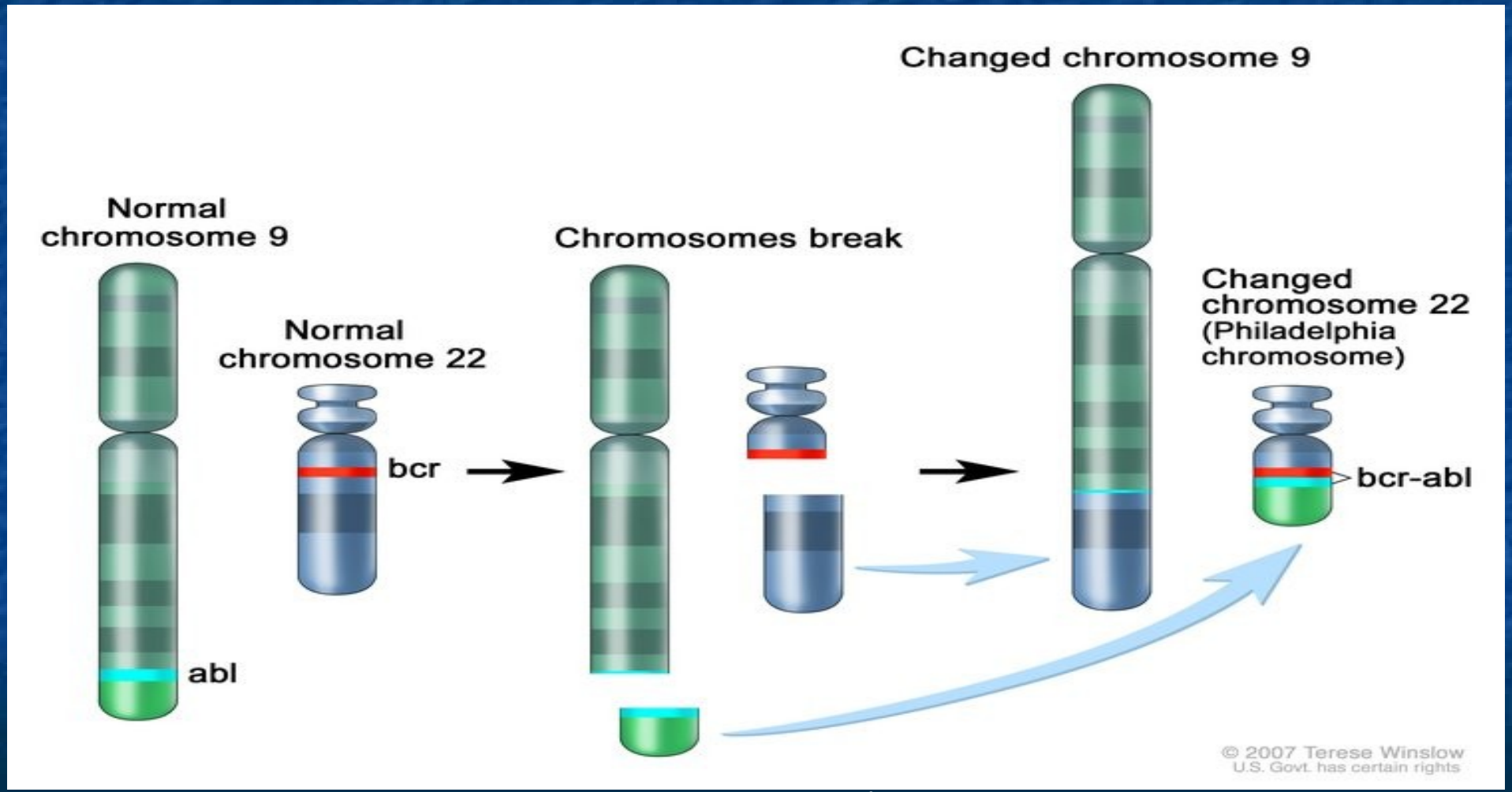
Acute Myeloid Leukaemia

- Arise from the malignant transformation of a myeloid precursor
- Rare in childhood (10%-15%)
- The incidence increases with age
- 80% in adults

Molecular biology of CML

- 90% of patients have Philadelphia Chromosome (Ph)
- t(9;22) balanced translocation
- disruption of the ABL (Chr 9) and BCR (Chr 22) genes
- formation of two hybrid genes
  - 5'BCR/3'ABL
  - 5'ABL/3'BCR
- Only the BCR/ABL hybrid gene is active
  - BCR/ABL mRNA
  - p210 'fusion' protein

t(9;22) translocation in CML



visualsonline.cancer.gov/addlb.cfm?imageid=7153

Why is p210 (BCR/ABL) protein oncogenic?

- ABL
  - protein tyrosine kinase
  - interacts with the adaptor protein CRKL
- BCR
  - interacts with the adaptor protein Grb2
- CRKL and GRB2 activates the RAS growth stimulation signalling pathway
  - Inactivation of pro-apoptotic protein (BAD)
  - Up-regulation of the anti-apoptotic protein (BCL_{XL})
- p210 is a constitutively active tyrosine kinase resulting in the permanent activation of the RAS pathway

New CML Treatment

- Design compounds that specifically target the p210 protein
- p210 is CML specific
- imatinib (Gleevec, Glivec, STI571)
- specifically inhibits the ABL kinase
  - imatinib inhibits the growth of CML cells in culture
  - Progression-free survival at 24 months is 87%
- Prof John Goldman Hammersmith Hospital London
- Prof John Barret NIH Washington
- Prof Francois Mahon Bordeaux
- Prof Ráčil, Prof Mayer FN Brno

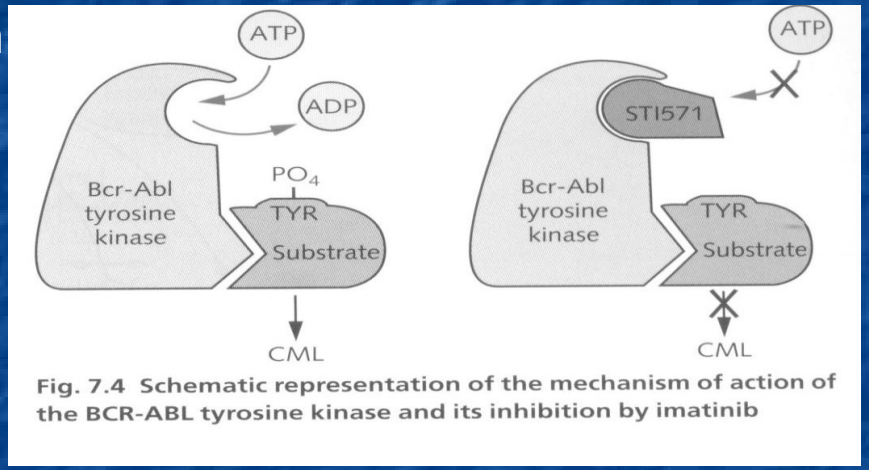


Fig. 7.4 Schematic representation of the mechanism of action of the BCR-ABL tyrosine kinase and its inhibition by imatinib

Molecular Haematology Provan & Gribben

Chronic lymphocytic leukaemia

- Most common leukaemia in the Western countries
  - lymphocytosis of > 5000 cells/μl for
  - > 3 months
- Flow cytometry of peripheral blood (phenotype CD19, CD5, CD23)
- Bone marrow biopsy
- Staging according to Rai (I-IV)
- Mutated IgVH
- Del11q (ATM)
- Del17p
- Del13q (RB1)
- +12
- TP53
- Prof Michael Doubek, IHOK, FN Brno

Multiple Myeloma

- B cell maligancy of plasma cells CD38+CD138+ in the bone marrow
- Pre-malignant stage:
  - MGUS – monoclonal gammopathy of undetermined significance
  - Progression of 1% per annum
- Bone marrow biopsy
- Therapy (IMIDS)
- Prof. Roman Hájek FN Ostrava

Questions