BONE MARROW AND SPLEEN EXAMINATION

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HISTOLOGY OF BONE MARROW

Overview

Bone Marrow

- Bone marrow sample
- Bone marrow overview
- Bone marrow histology in a core biopsy
- Myeloid cells
- Monocytic and dendritic cells
- Erythroid cells
- Megakaryocytes
- Lymphoid histology
- Bone

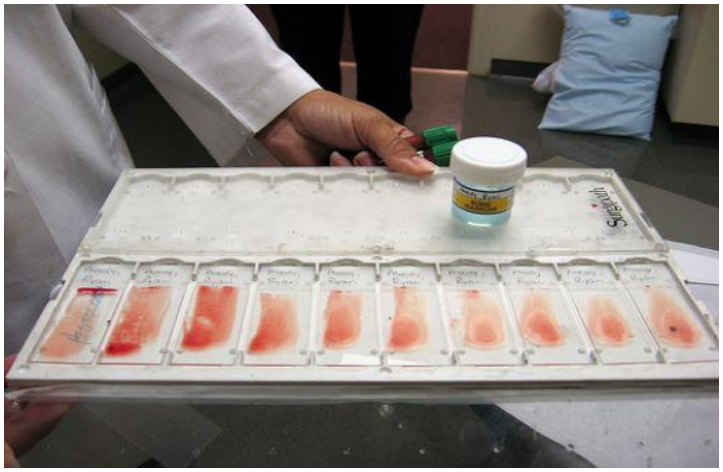
Spleen

- An abbreviated version

Bone Marrow in The Pathology Lab

Usual Sample

- Bone marrow smear
- Bone marrow clot
- Bone marrow biopsy (decalcified)

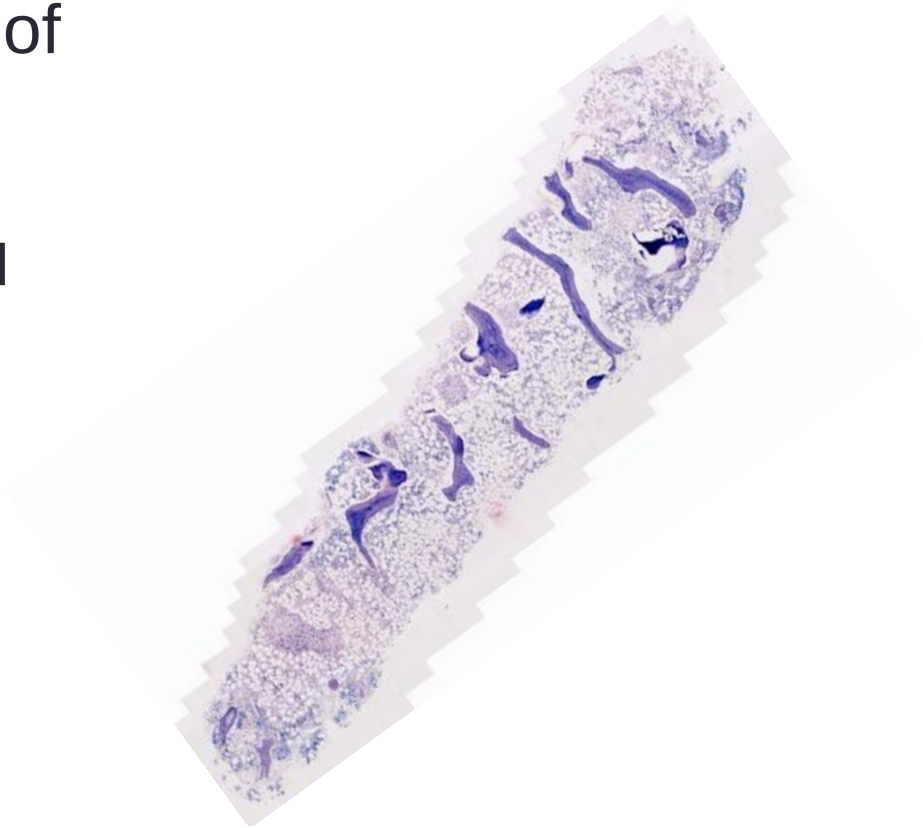


Always add information from

- Clinical history
- Labs (specially CBC)
- Flow cytometry
- Iron stain

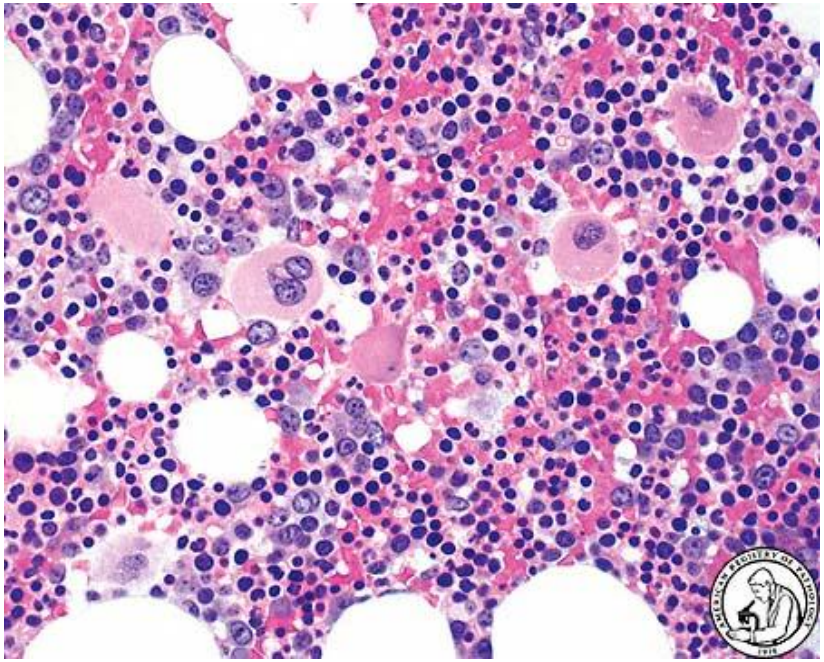
Bone Marrow Biopsy

- The biopsy is used for quantitative evaluation of the marrow
  - Cellularity
  - M:E ratio, it is considered the best estimation (vs differential from smear)
  - Numbers of megakaryocytes
  - Presence of tumors, granulomas, infiltrates
  - Presence of fibrosis, necrosis



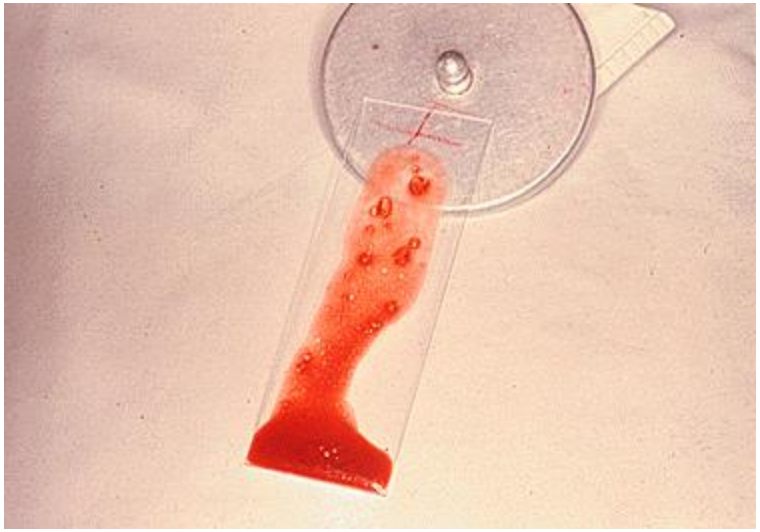
Bone Marrow Clot Section

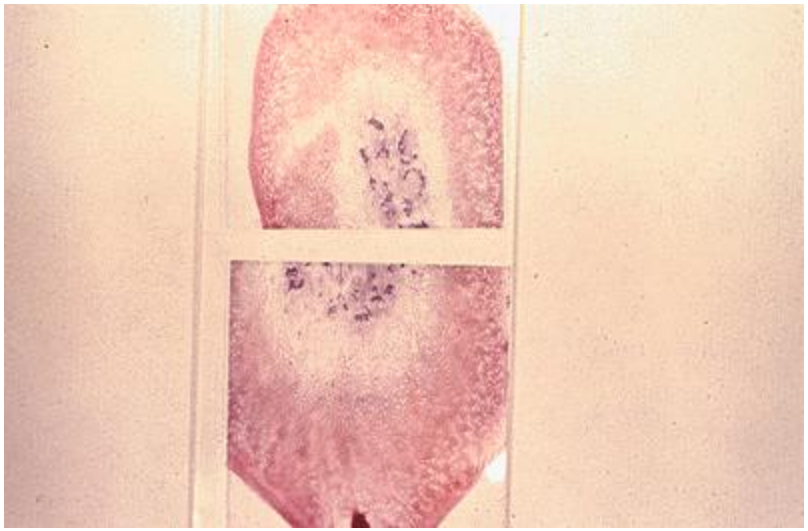
- May also be used for quantitative evaluation, especially if the biopsy is suboptimal
- May give a better evaluation of erythropoiesis, since there is no bone present in the specimen, it can be cut thinner, aiding in the evaluation of red cell precursors
- **The best for immunos… if representative**



Bone Marrow Smears

- The smears are used for qualitative evaluation of the marrow
  - cell identification
  - dyspoiesis
  - maturation
  - cytologic abnormalities





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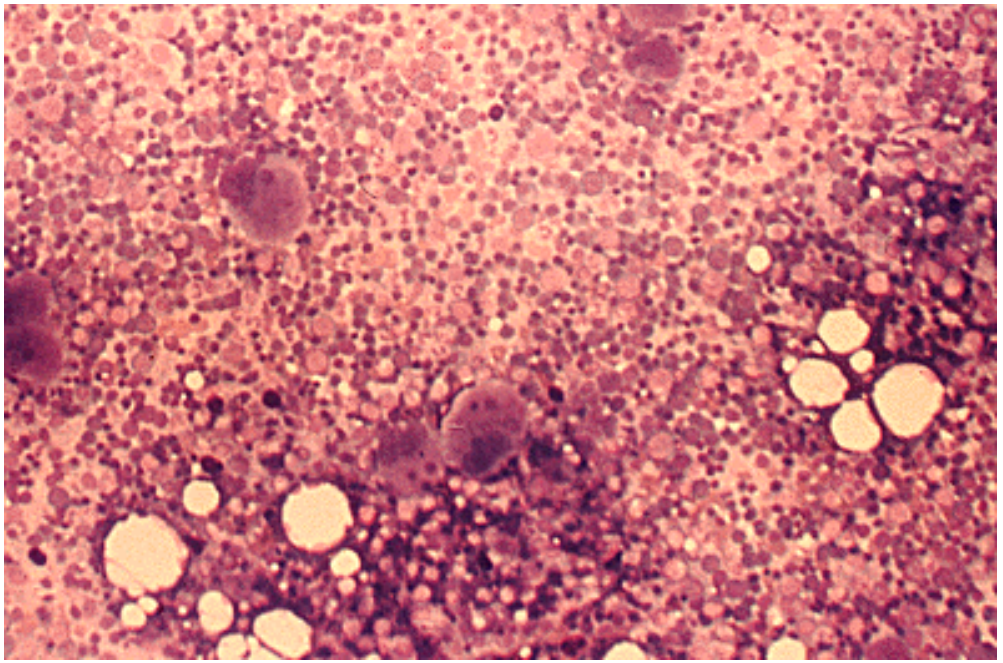
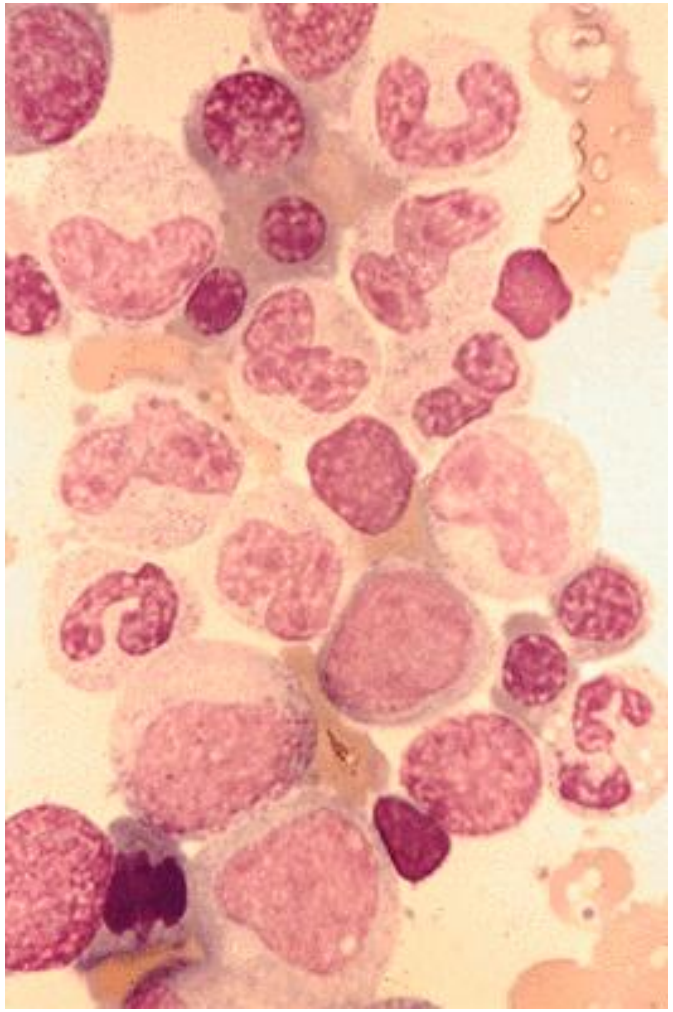


This bone marrow is from a patient with chronic myelocytic leukemia.
It is hypercellular and contains only a small amount of fat. There are a number of megakaryocytes, which are the largest cells of the bone marrow. The number of **megakaryocytes** is estimated in the thickest part of the particle and not in the sinusoidal blood. The normal number is 3-10 per low-power field. **More than 15 in one field in the particle is considered increased**. If you have to search for them and find only **1-2 per particle, they are decreased**.

Bone Marrow Smear – The differential

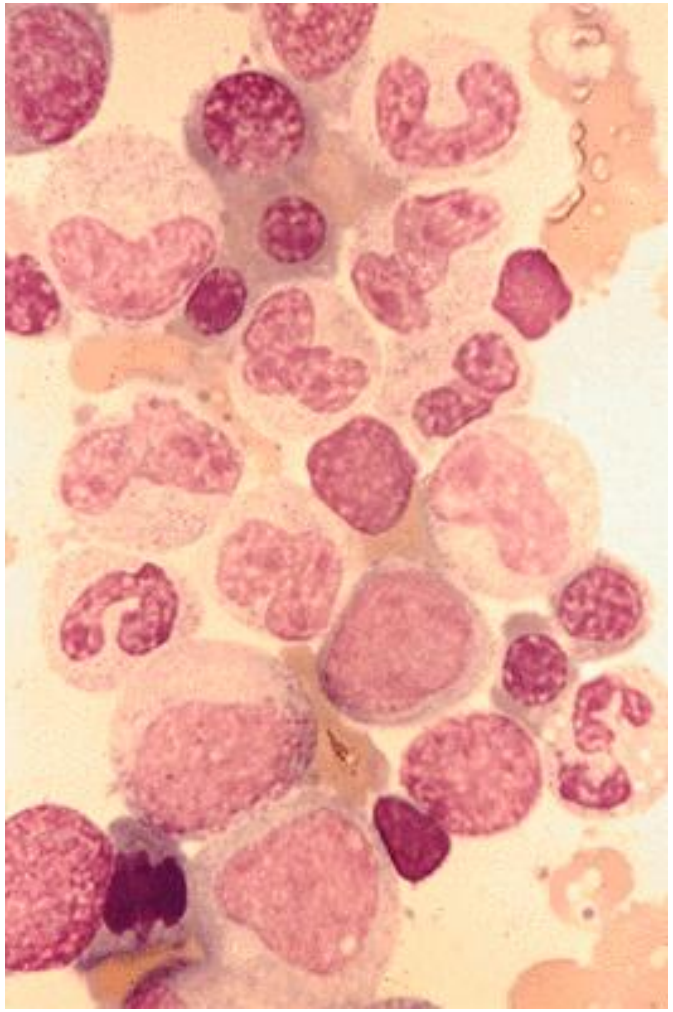
Practical advices

- Always do your differential with 1,000x magnification.
-
- Count at least 200-300 nucleated cells
- .
- Between 40-60 cells can be counted in one field with oil immersion.
- It becomes difficult with 100 or more cells, as we tend to skip or to count the same cells twice.
- Fewer than 10 cells per field indicates sinusoidal blood rather than actual bone marrow particles I divide the field into imaginary quarters and start at the 12-o'clock position and count clockwise.
- Beginners find it easier to count all the granulocytes in the field and then to go back over it and count the lymphocytes, nucleated red cells, plasma cells, and the other types in the same fashion.



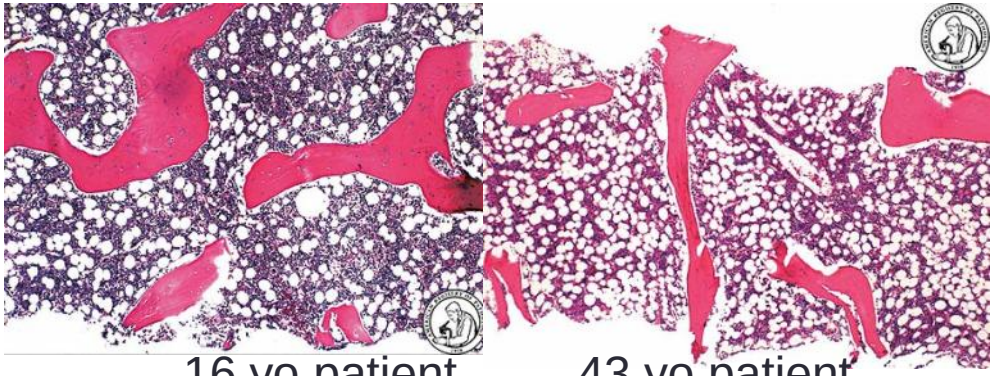
Bone Marrow Smear – The differential

- There are 13 granulocytes:
  - two segs
  - four bands
  - four metamyelocytes
  - two myelocytes
  - one promyelocyte (some promyelocytes do not contain granules)
- Four normoblasts (E5)
- Two late erythroblasts (E4) one of which is in mitosis.
  - There are also three naked nuclei, which are disregarded



Basic approach in the biopsy

- Cellularity
  - Depending on Pt age
- Myeloid to Erythroid ratio (ME ratio)
  - 1:6 in first week of life
  - 2.5-4:1 (adult physiologic range [3:1])



16 yo patient

43 yo patient

- Increase in either component is reported as myeloid/erythroid “predominant” in the presence of a normal fat:cell ratio, and “hyperplasia” when the cellularity of the bone marrow exceeds 70%.

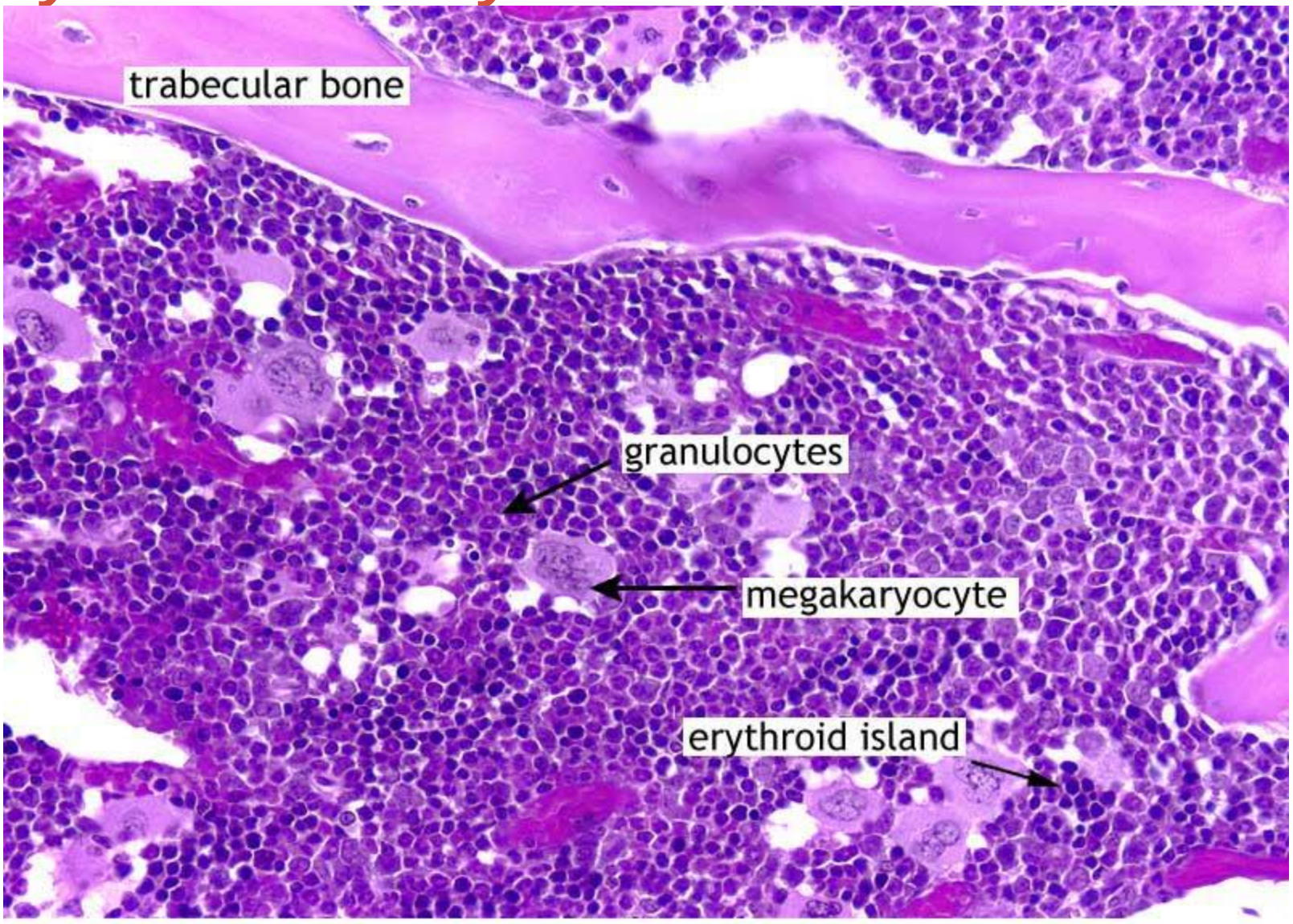
How to tell which ones are which?

- Erythroid cells are seen as round dark nuclei without much cytoplasm - “black dots” usually with a halo around them

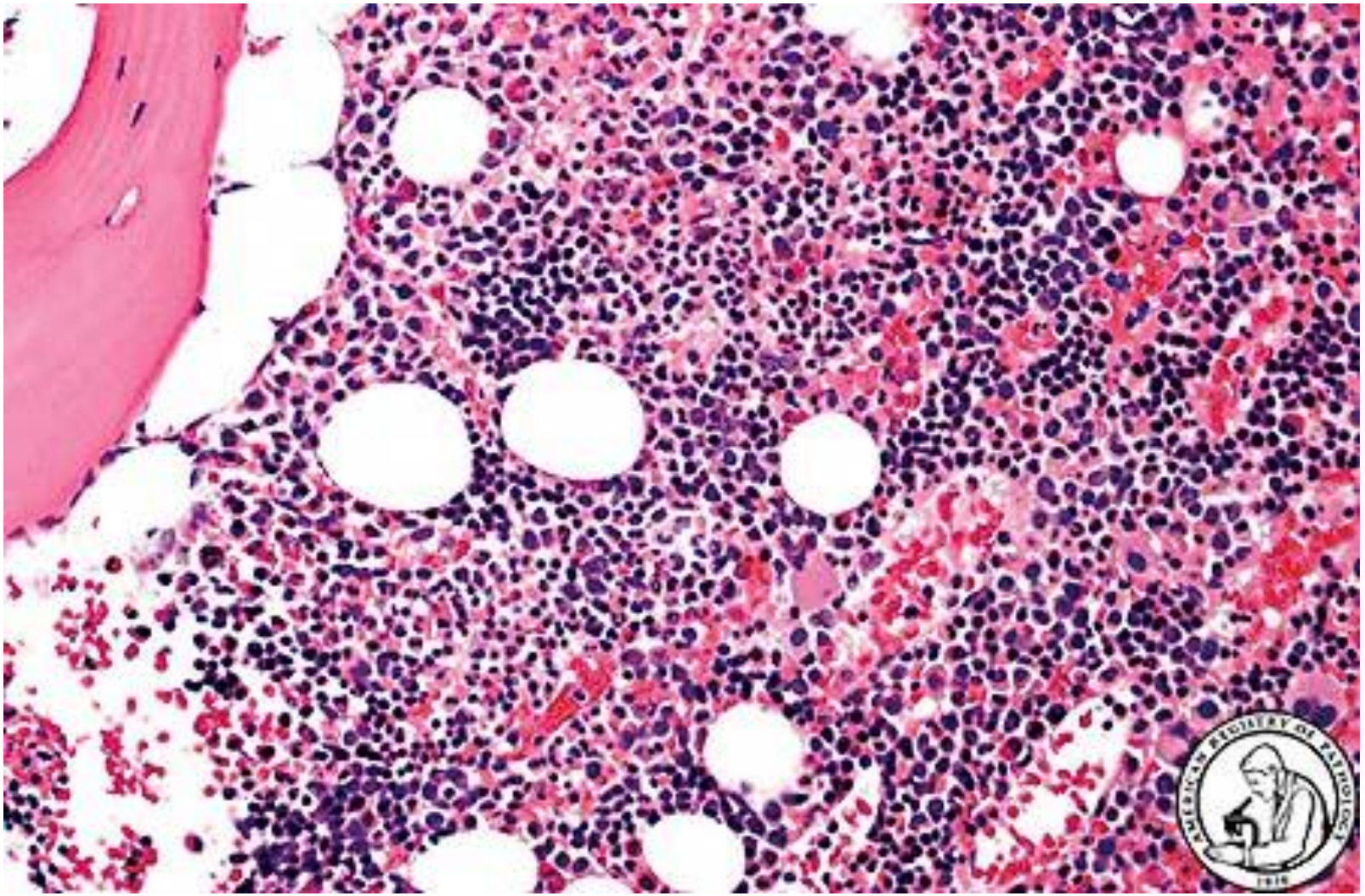
- Myeloid cells have lighter staining nuclei and pink cytoplasm, with maturation you can identify polys and bands **(paratrabecular distribution)**

- Megakaryocytes are large with multilobated nuclei (perisinusoidal)

Myeloid vs. Erythroid



Erythroid hyperplasia



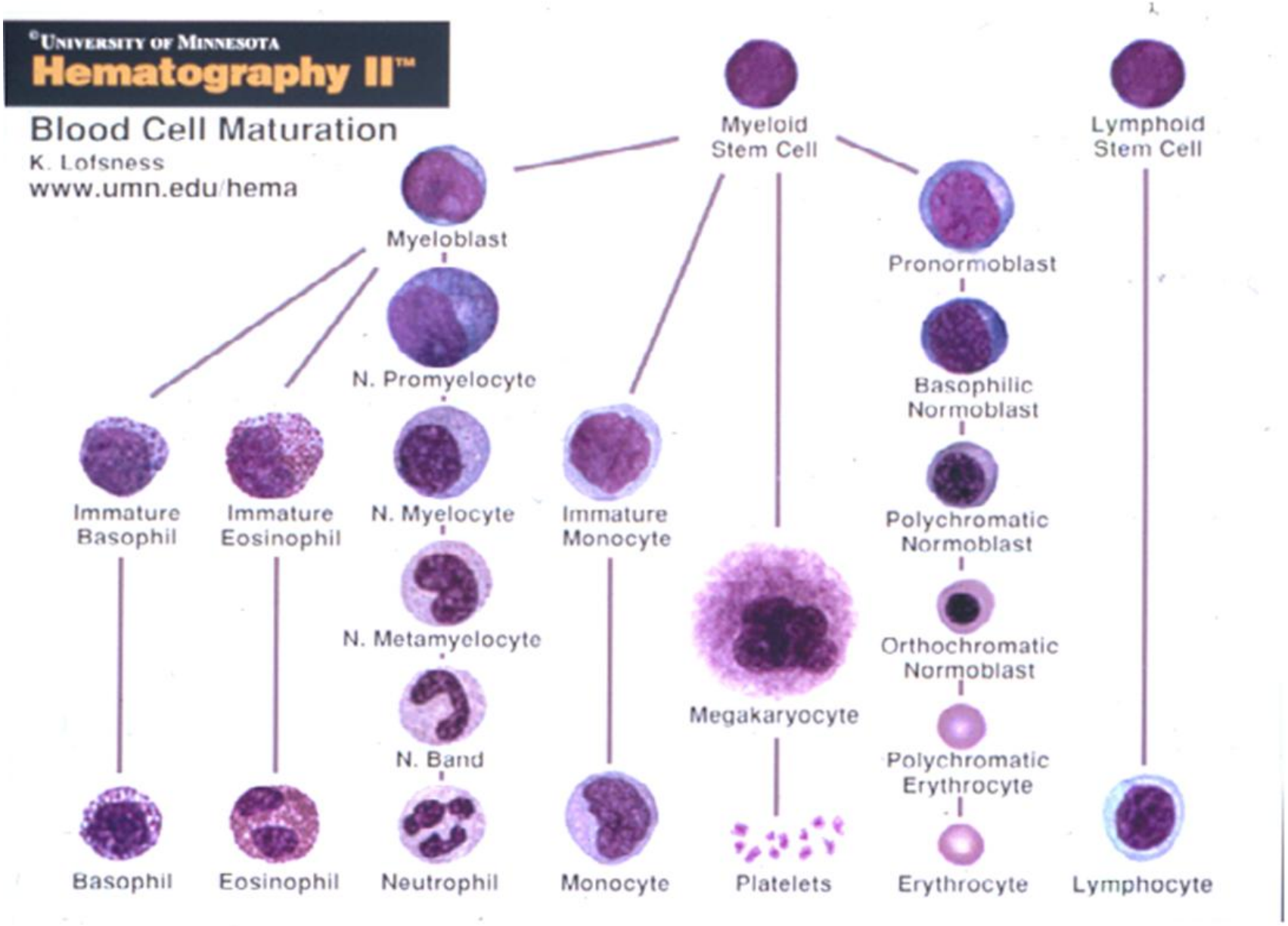
Bone marrow lexicon

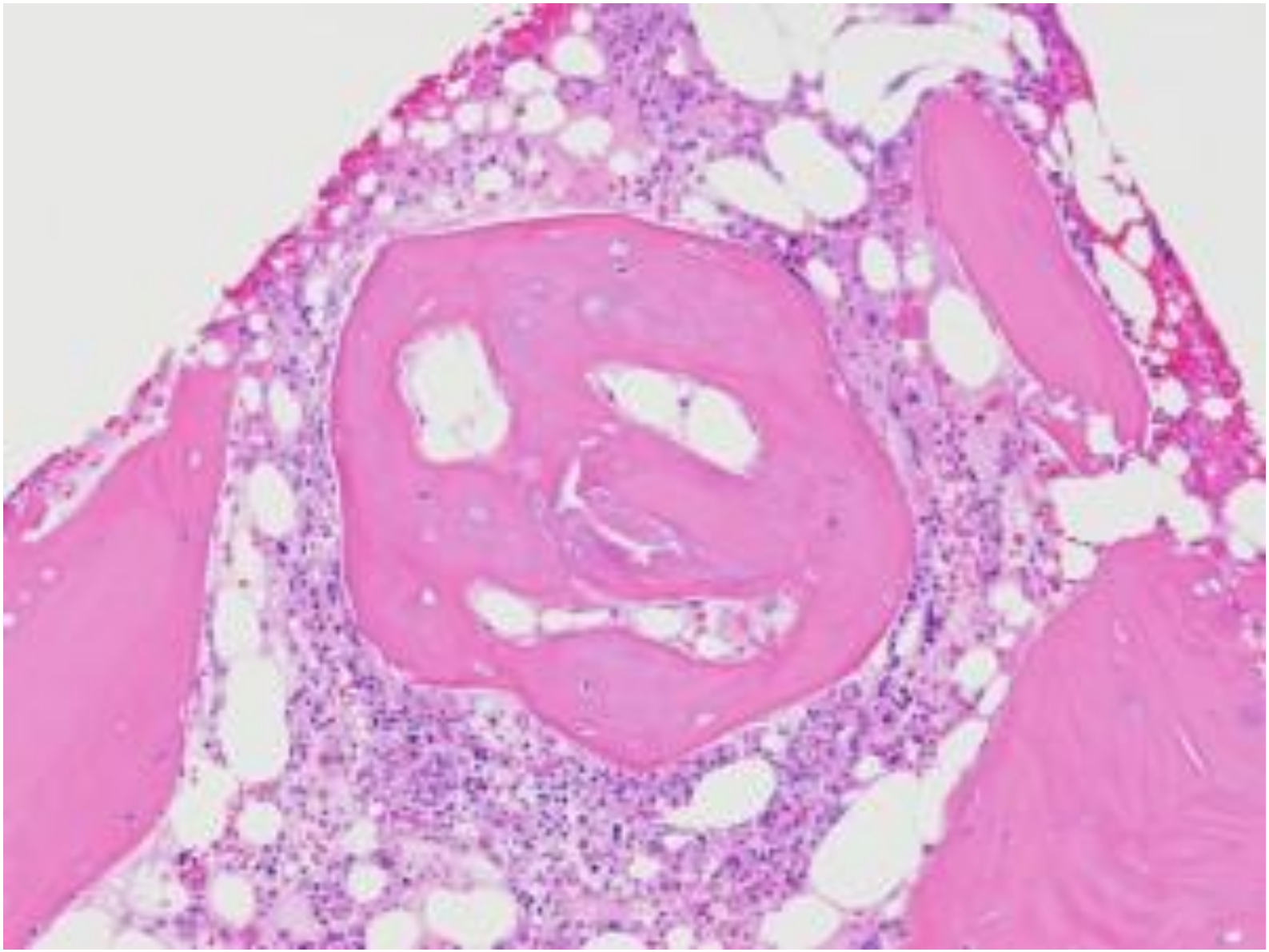
- Blast → least differentiated
- Cyte → more differentiated
- Pro → 2nd cell in maturation sequence
- Meta → 4th cell in maturation sequence (if 4 maturation stages)

Table 4—Nomenclature

PREFIX	SUFFIX	MYELOCYTIC SERIES	MONOCYTIC SERIES	MEGAKARYOCYTIC SERIES	ERYTHROCYTIC (RUBRICYTIC) SERIES	LYMPHOCYTIC SERIES	PLASMOCYTIC SERIES
	___blast	myeloblast	monoblast	megakaryoblast	rubriblast	lymphoblast	plasmoblast
pro___	___cyte	promyelocyte	promonocyte	promegakaryocyte	prorubricyte	prolymphocyte	proplasmocyte
	___cyte	myelocyte	monocyte	megakaryocyte	rubricyte	lymphocyte	plasmocyte
meta___	___cyte	metamyelocyte	_____	metamegakaryocyte	metarubricyte	_____	_____

Bone marrow, differentiation and cytological features



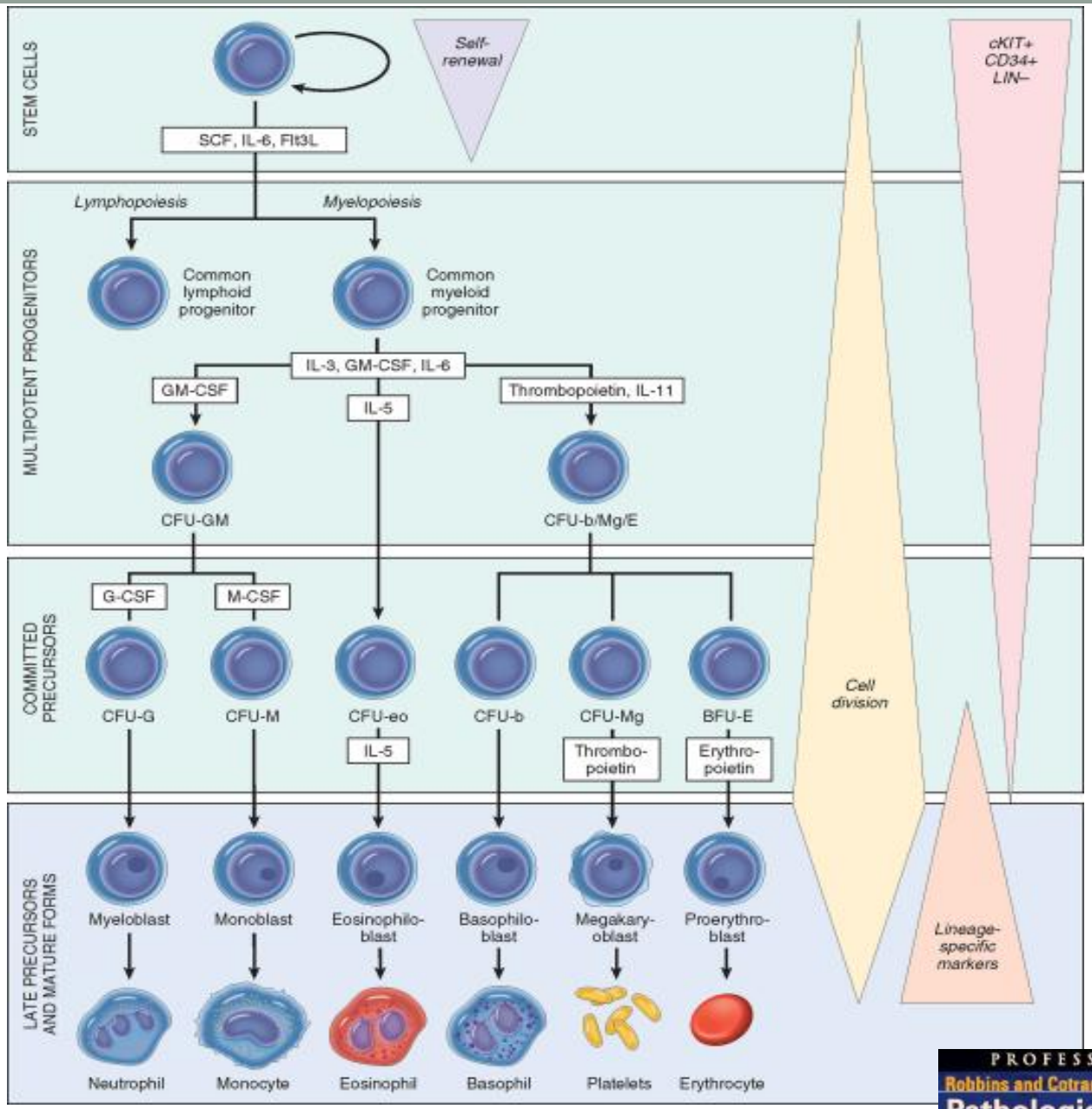


Fetal hematopoiesis

- First detected in the yolk sac (2nd to 3rd week), and exclusively produces nucleated red blood cells
- Then dorsal aorta, liver (6th week), spleen and bone marrow (14th weeks)
- Hematopoietic stem cells (CD34, c-kit, Thy1) and CD34 receptors
- The mesenchymal component (adipose tissue, bone and fibroblast) comes from primitive mesenchymal stem cells.

Microenvironment Controls Differentiation

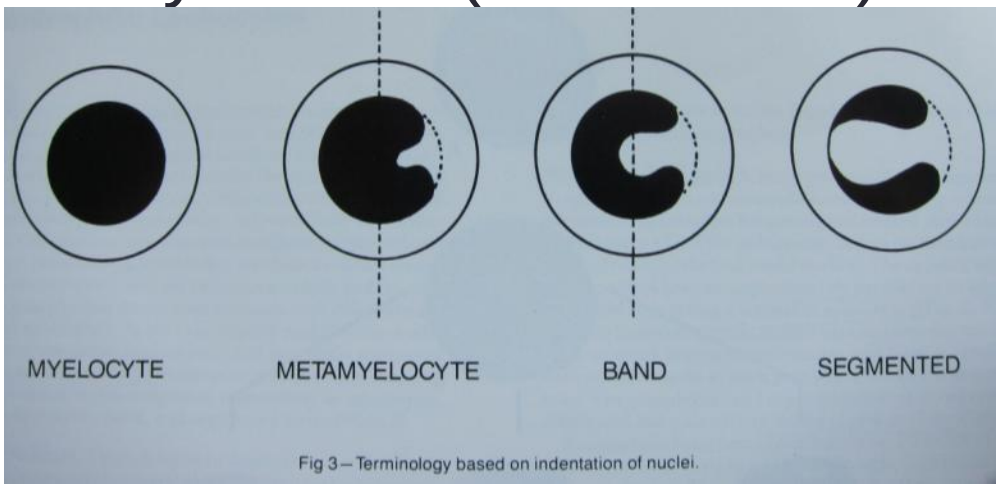
- Immature granulocytic cells have paratrabecular arrangement.
- T-cells and macrophages (IL-6, G-CSF, M-CSF) produce several cytokines that regulate the microenvironment
- Specific cytokines promote lineage specific proliferation:
  - G-CSF → Granulocytes
  - M- CSF → Monocytes/macrocytic lineage
  - IL-5 → Eosinophil/basophil production

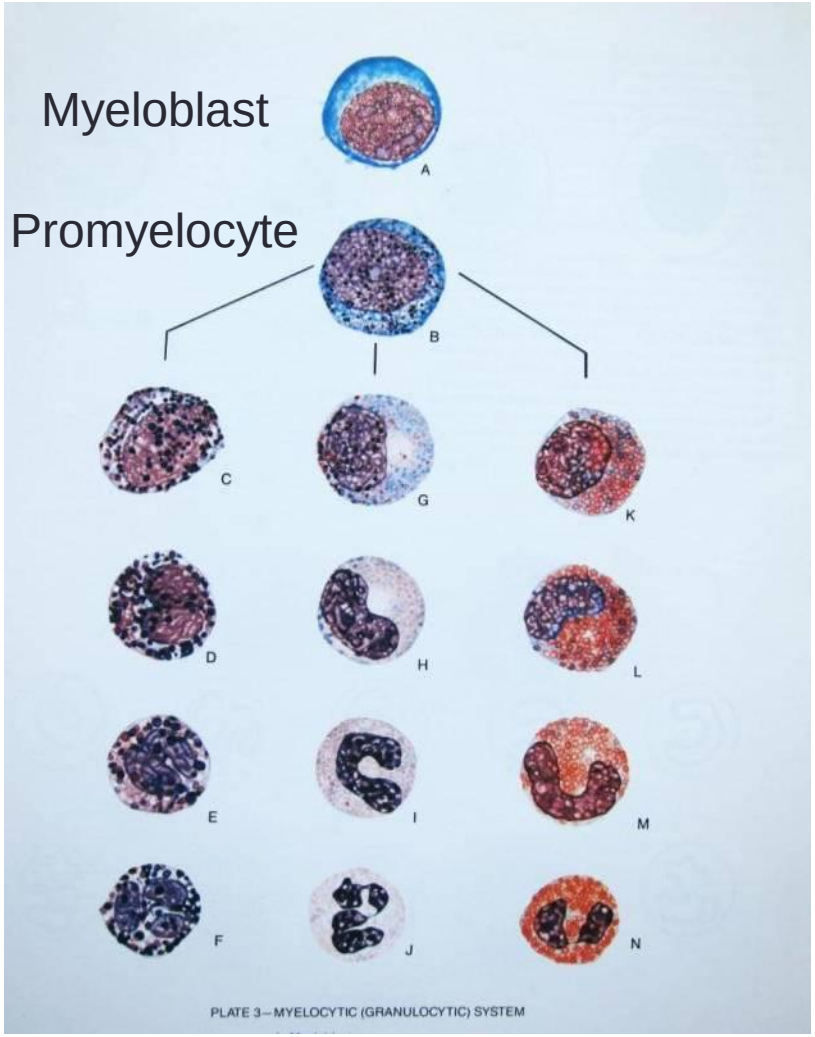


PROFESSIONAL EDITION
Robbins and Cotran EIGHTH EDITION
Pathologic Basis of Disease
KUMAR • ABBAS • FAUSTO • ASTER

Lineage development: Granulocytic cells

- Granulopoietic cycle within the bone marrow takes 10 to 14 days but can be accelerated by cytokines (G/GM-CSF)





Lineage development: Granulocytic cells

TABLE 2. *Types of myeloid elements and their normal range in the bone marrow*

Cell type	Range
Myeloblasts	0% to 2%
Promyelocytes	2% to 5%
Myelocytes (neutrophilic)	9% to 16%
Metamyelocytes	7% to 23%
Band Forms	8% to 15%
Neutrophils	4% to 10%
Myelocytes (eosinophilic)	0% to 2%
Band	0% to 2%
Mature	0% to 2%
Monocytes/macrophages	0% to 3%
Basophils	0% to 1%
Mast Cells	0% to 2%

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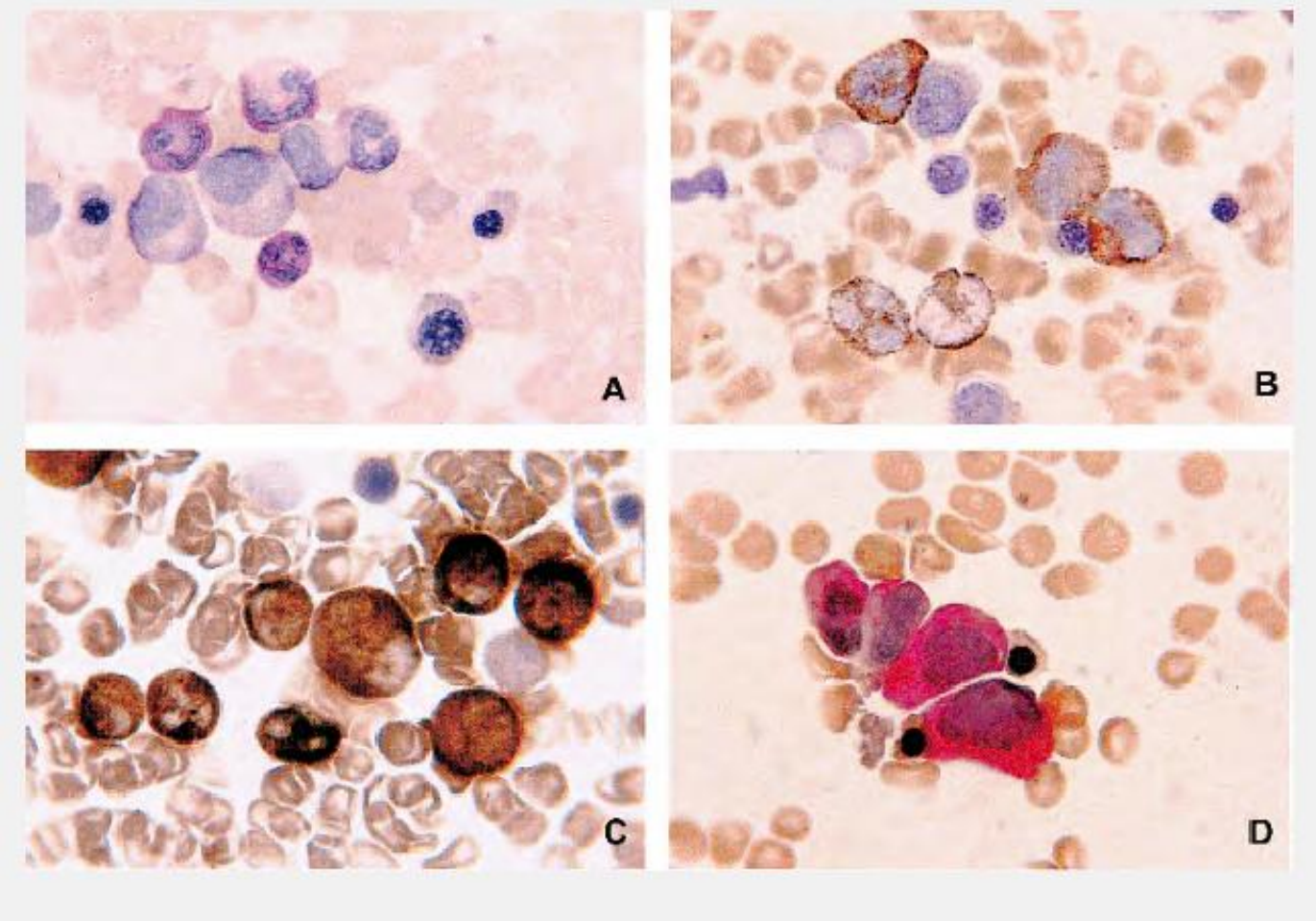
Lineage development: Granulocytic cells



A. PAS, B. Sudan black, C. Myeloperoxidase, D. Alpha-naphthol AS-D chloroacetate

Table 1-2

GRANULOPOIESIS[a]

Stage of Maturation	Morphology	Cytochemical/Immunophenotypic Properties
Myeloblast	High nuclear to cytoplasmic ratio Blastic, dispersed chromatin Agranular, minimally granular cytoplasm	Myeloperoxidase + or – Most myeloblasts are CD34+, HLA-DR+, and coexpress myeloid+ lineage antigen such as CD33
Promyelocyte	Eccentric nucleus with prominent paranuclear hof (pale zone) Sparse, concentrated azurophilic granules	Myeloperoxidase + Typically CD34 –, HLA-DR –, and myeloid antigen + (e.g., CD33, CD13)
Neutrophilic myelocyte	Round nucleus with condensed chromatin Moderate to abundant secondary (specific) granules which give the cytoplasm a finely granular pink appearance	Myeloperoxidase +, leukocyte alkaline phosphatase + Myeloid antigen + (CD34 –, HLA-DR –)
Neutrophilic metamyelocyte	Indented nucleus, condensed chromatin Cytoplasm packed with granules with predominance of secondary granules	Myeloperoxidase +, leukocyte alkaline phosphatase + Myeloid antigen + (CD34-, HLA-DR-)
Band neutrophil	Horseshoe-shaped mature nucleus lacking discrete indentations Cytoplasm packed with granules with predominance of secondary granules; gelatinous granules also present	Myeloperoxidase +, leukocyte alkaline phosphatase + Myeloid antigen + (CD34 – HLA-DR –)
Neutrophil	3-5 discrete nuclear lobes Highly condensed chromatin Cytoplasm packed with granules with predominance of secondary granules; gelatinous granules also present	Myeloperoxidase+, leukocyte alkaline phosphatase + Myeloid antigen + (CD34 –, HLA-DR –)

[a]Data from references 39, 46, and 62.

Lineage development: Granulocytic cells

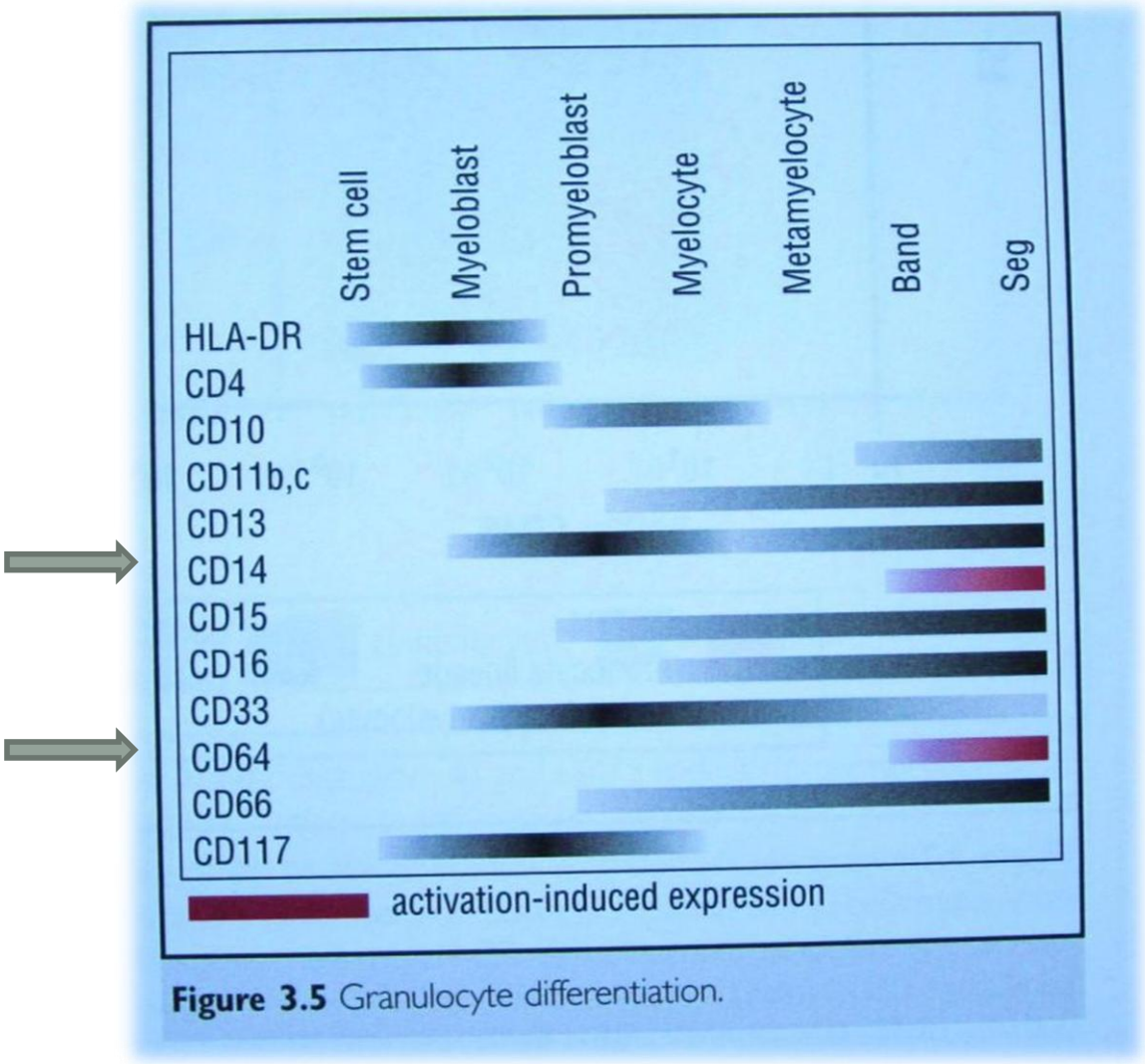


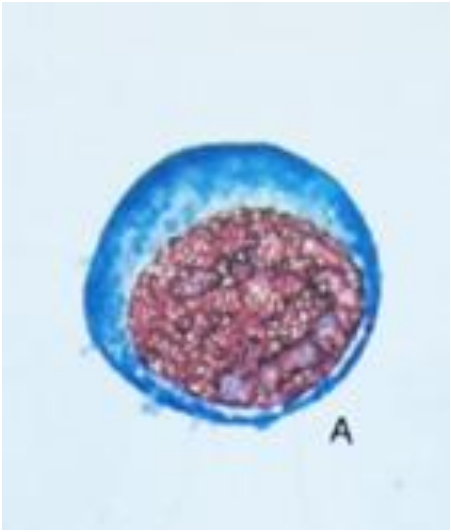
Figure 3.5 Granulocyte differentiation.

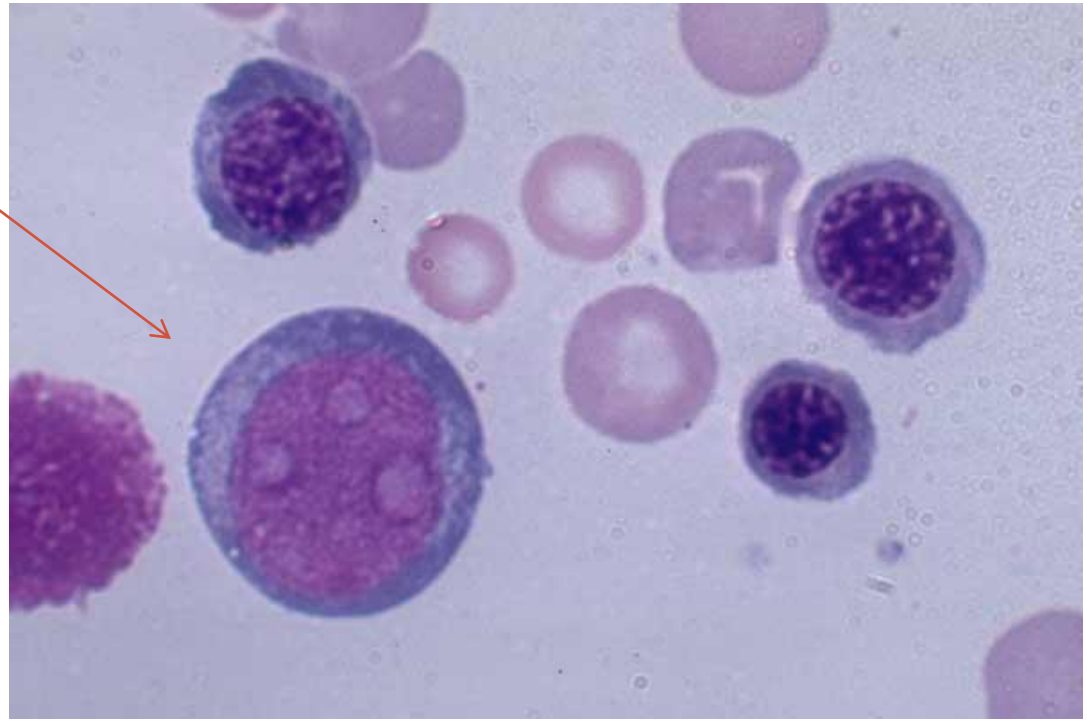
Neutrophilic Leukocytes

- Maturation is characterized by development of dark-blue (primary) granules that later on is replaced by secondary granules that differ in their size and staining pattern
  - Neutrophil small granules that stain with light blue and pink
    - Also: Gelatinase (tertiary) granule, which lacks both myeloperoxidase and lactoferrin, but contains gelatinase, acetyltransferase, and lysozyme
  - Basophil large basophilic granules
  - Eosinophil

Myeloblast

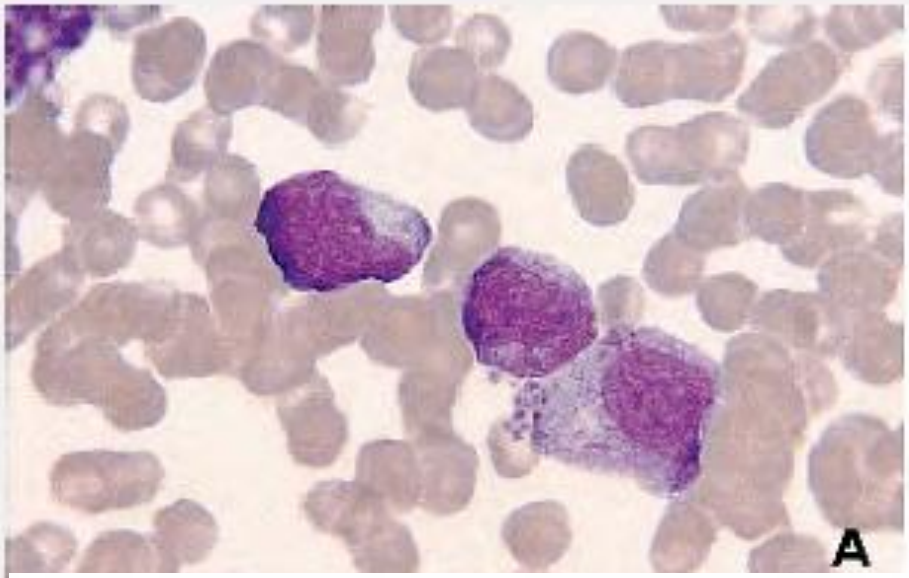
- Nuclei
  - Round
  - Evenly staining
  - 2-3 nucleoli
- Cytoplasm
  - No granules*
  - Unevenly staining
  - Perinuclear clearing



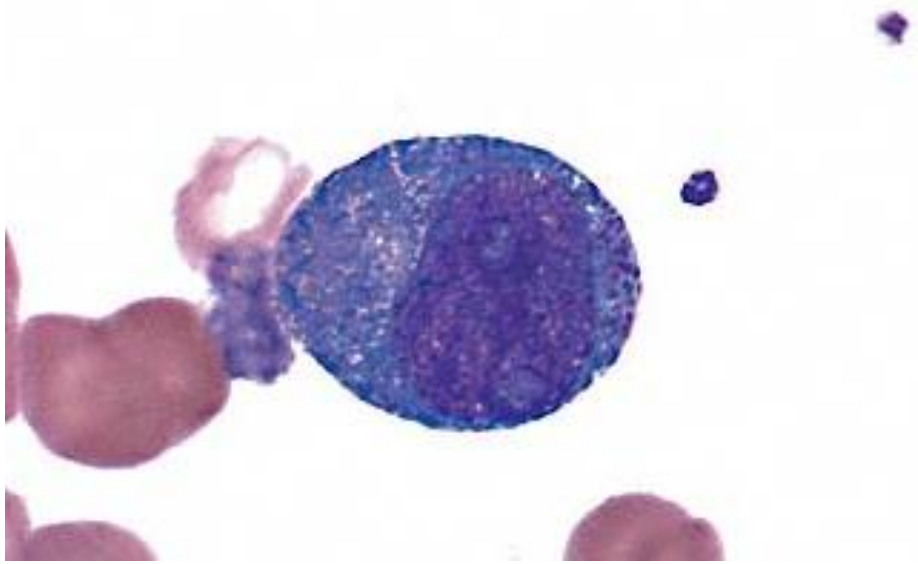


Myeloblast

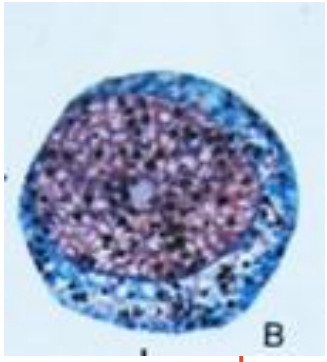
- CD34+, CD117+, CD38+, HLA-DR+



- Also as myeloid cells:
  - CD13+
  - CD33+

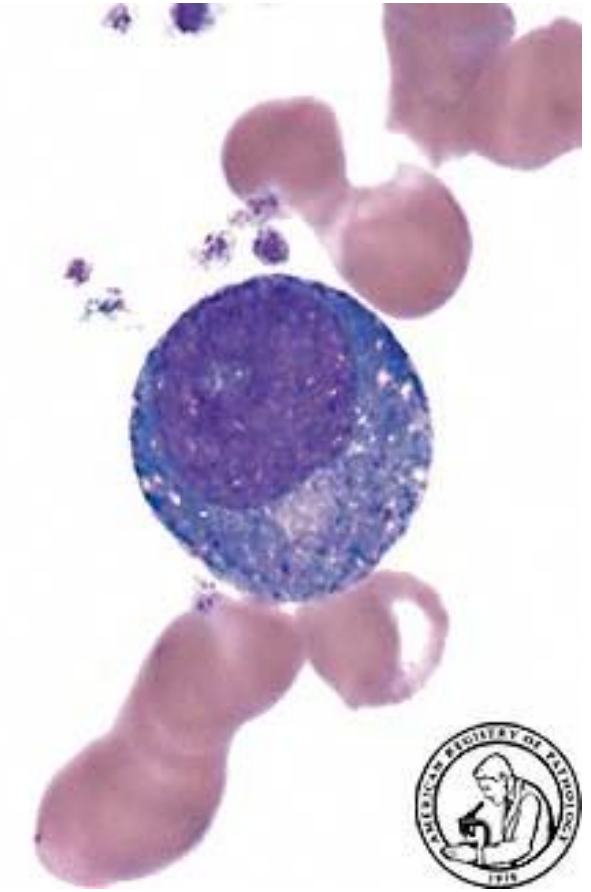


Promyelocyte



- A myeloblast when develop distinctive granules
- Nucleous
  - Chromatin is coarser than myeloblast
  - Indistinct nucleoli
  - Oval round nuclei
- Cytoplasm
  - **Primary granules are dark-blue or reddish-blue**

Becomes a **myelocyte** once you identify the granules as basophilic, eosinophilic or neutrophilic



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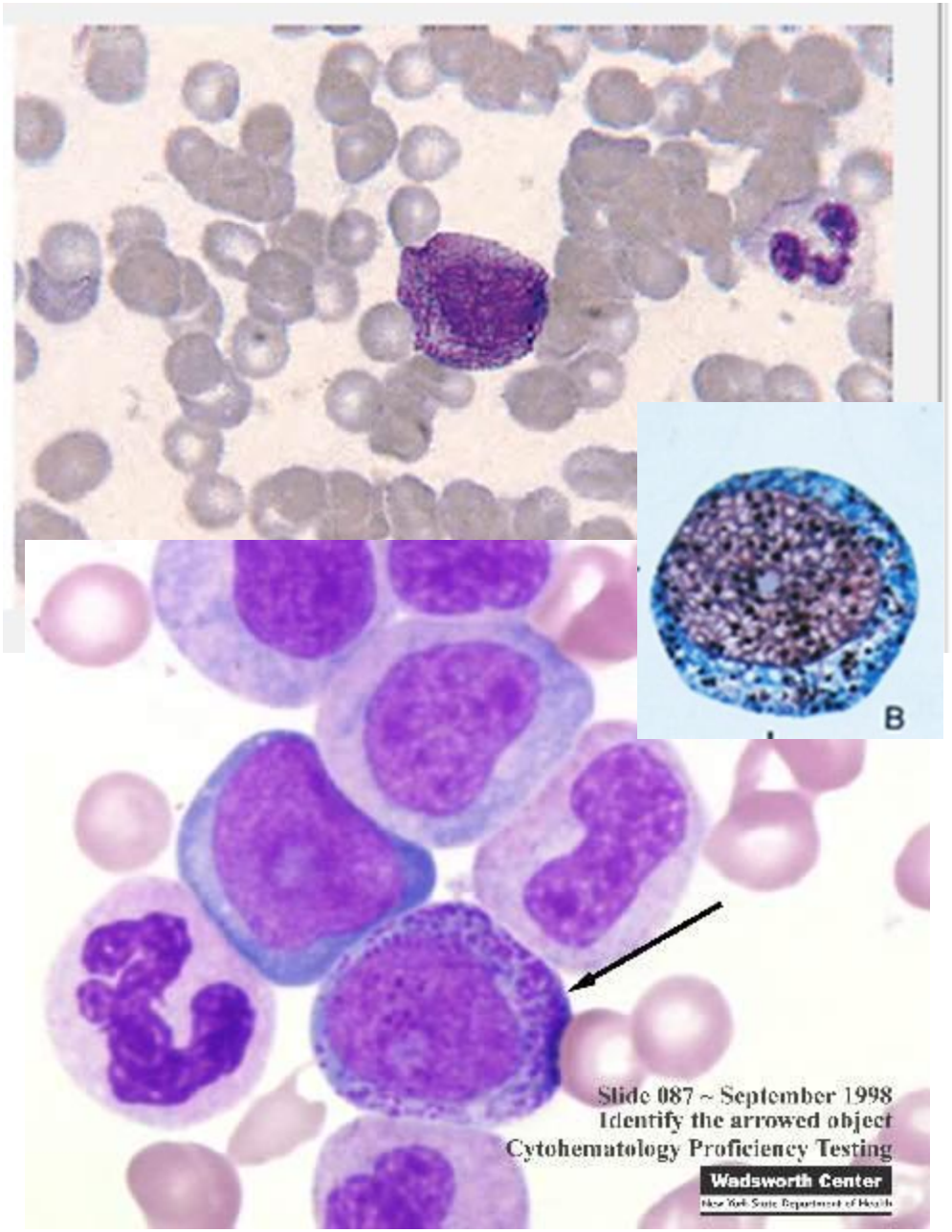
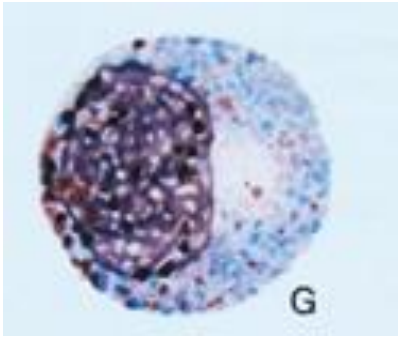
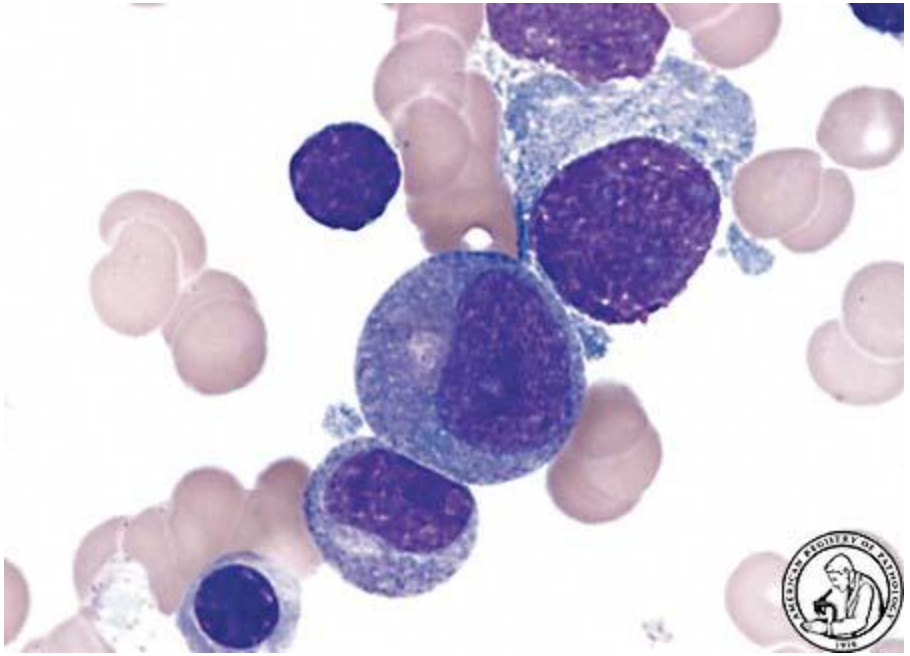


Neutrophilic Myelocyte



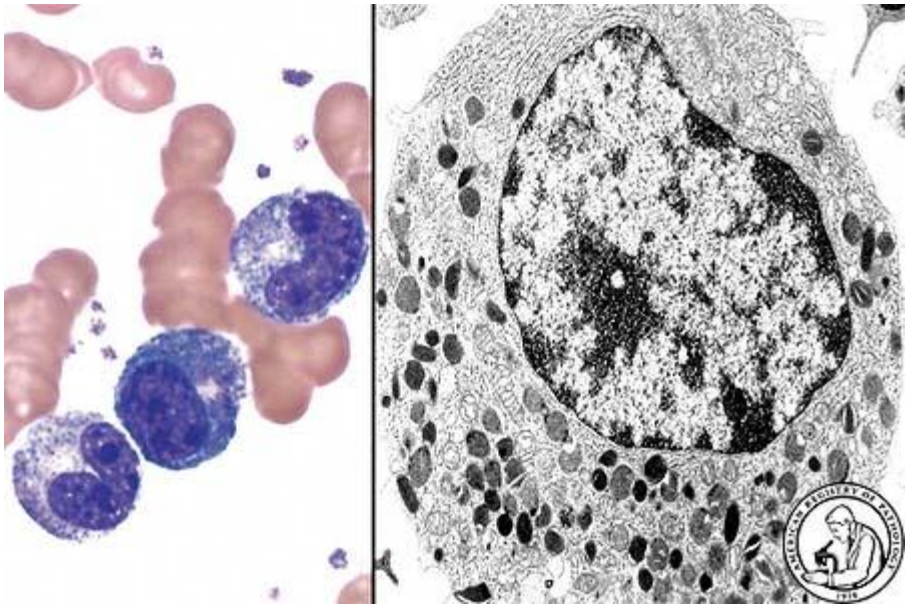
- Nuclei
  - Round to oval, flattened in one side
- Cytoplasm
  - Initially a perinuclear island of ill-defined reddish granules so neutrophilic differentiation



Secondary granule formation begins in the Golgi region highlighted by the paranuclear hof in this early neutrophilic myelocyte (bone marrow aspirate; Wright stain).

Neutrophilic metamyelocyte

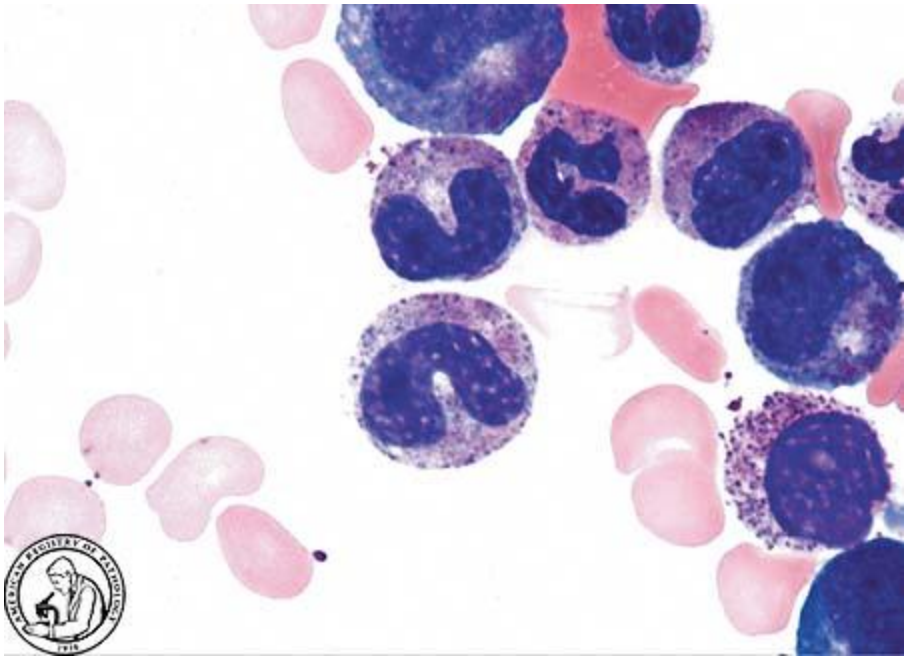
- Nuclei
  - **Slightly indented** nuclei
- Cytoplasm
  - Small pinkish granules
- May be seen normally on PB but also indicates myeloid hyperplasia



Composite of a neutrophilic myelocyte (center), neutrophilic metamyelocyte (top), and band neutrophil (bottom) in a bone marrow aspirate smear shows the progression of maturation changes of the nucleus and cytoplasm. Right: Electron micrograph of a myelocyte shows primary and secondary granules (bone marrow aspirate; Wright stain).

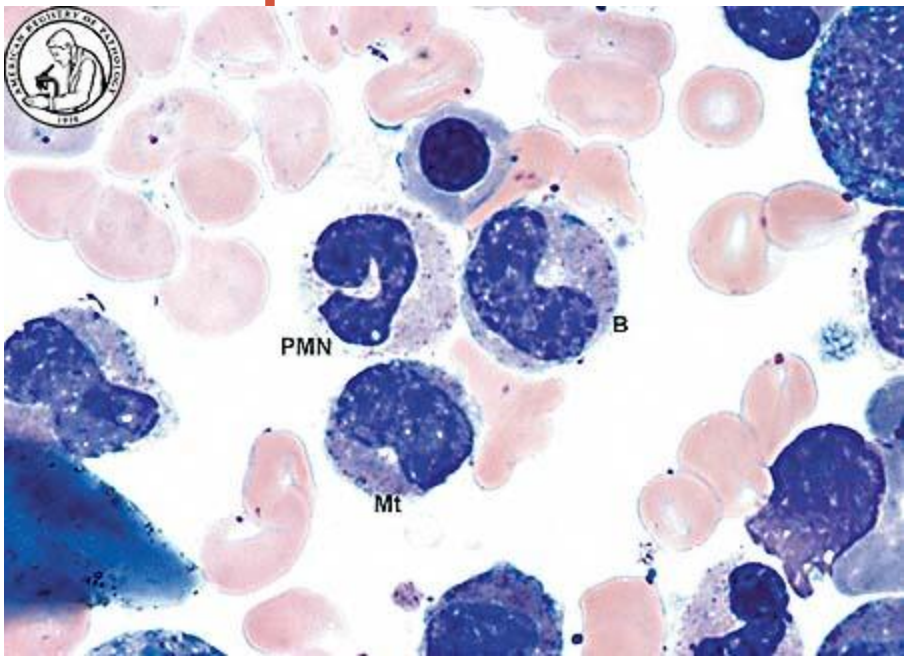
Neutrophilic Band

- Nucleus
  - Characteristic horseshoe nucleus
  - Indentation is greater than half of the hypothetical round nucleus
- Cytoplasm
  - Evenly distributed granules that stain shades of pink and blue
- 1-5% of PB in healthy individuals, if increased is called “shift to the left”



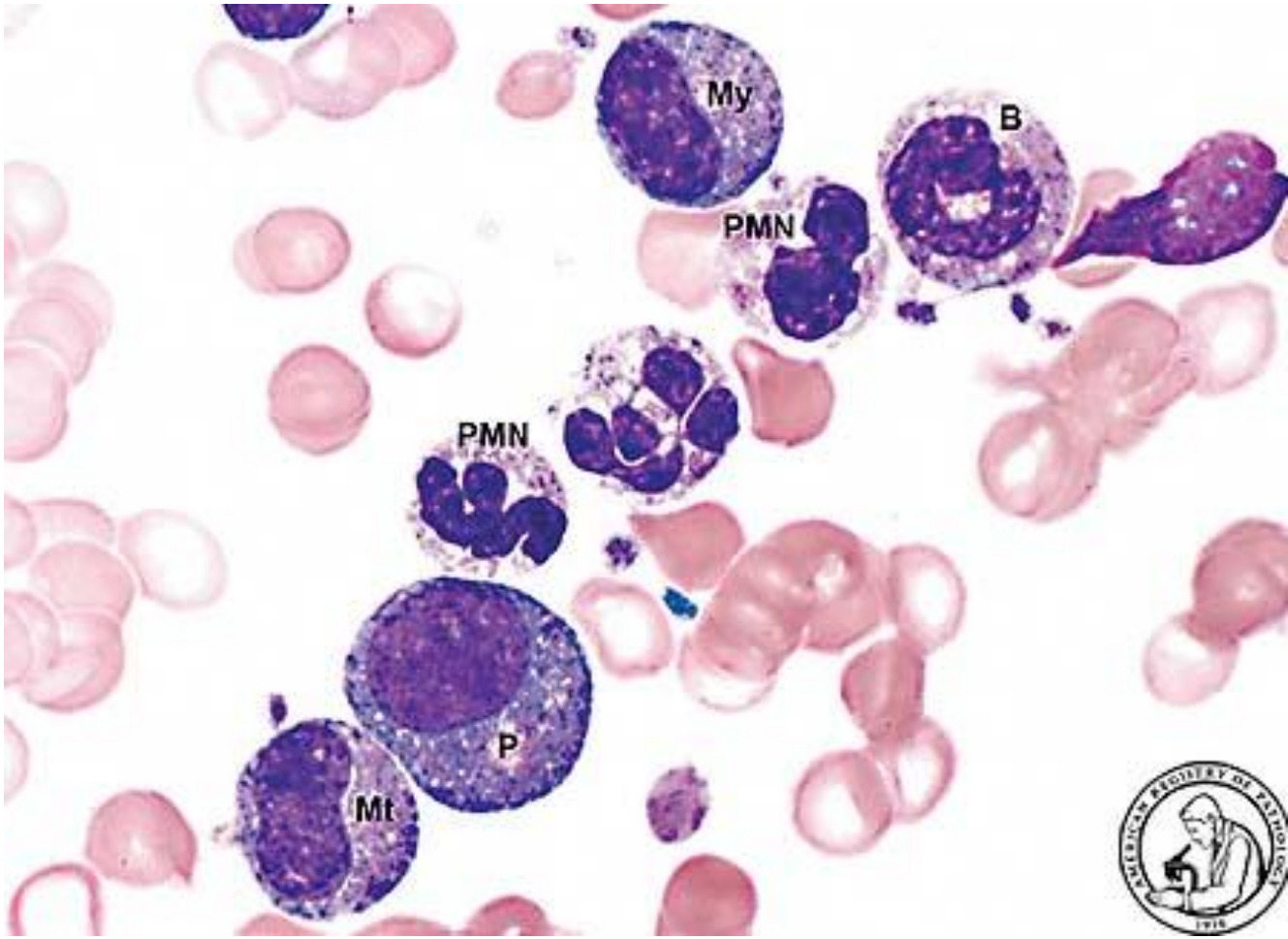
And finally… The Neutrophil

- Nuclei
  - Typically lobulated
  - 35% 2 lobes
  - 41% 3 lobes
  - Hyperlobulation →pernicious anemia (6 or more lobes)
  - Hypolobulation →Pelger-Huet anomaly (2 round lobes connected with a short filament [pince-nez form])
- 50-70% of WBC



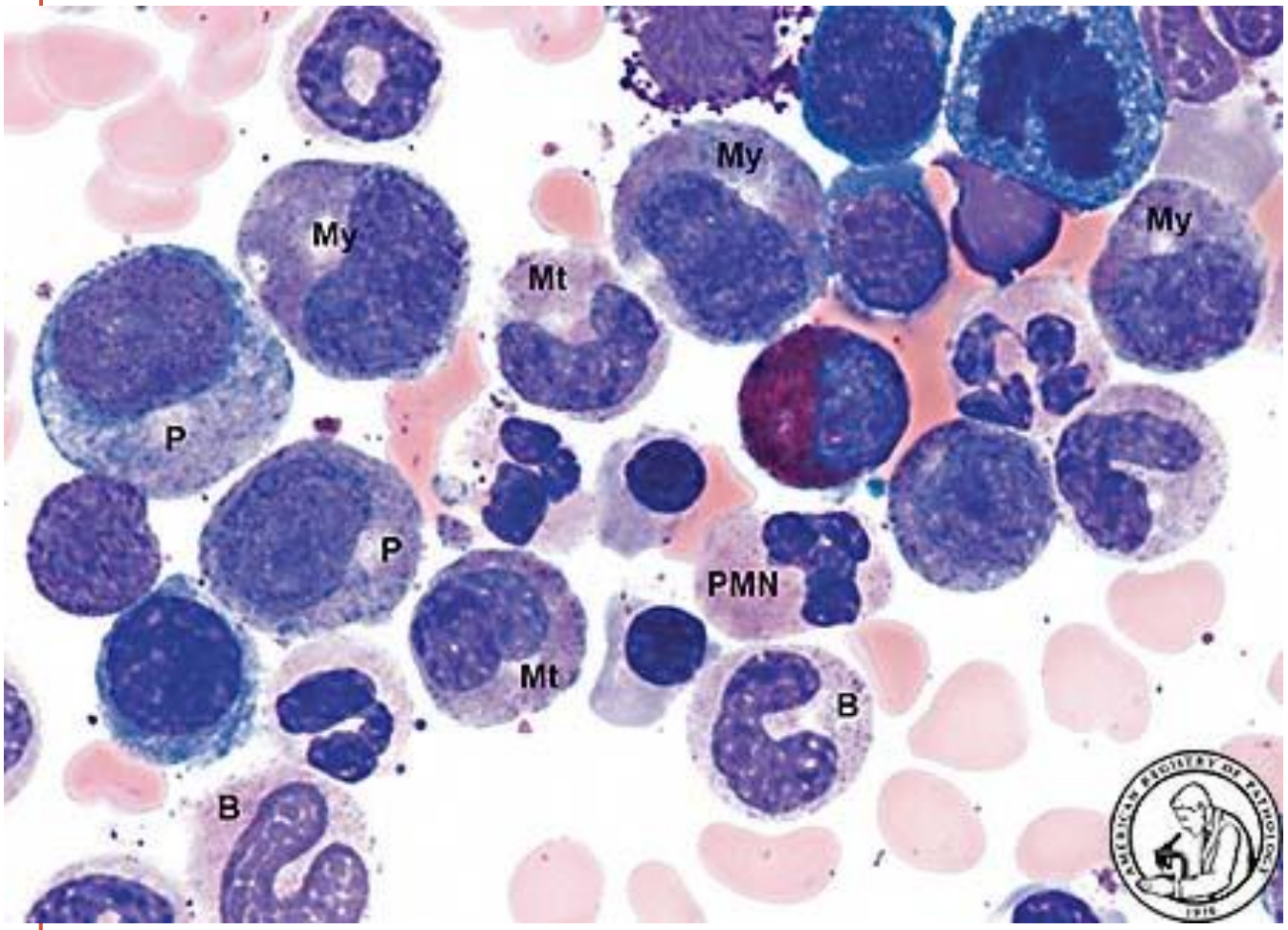
The neutrophilic metamyelocyte (Mt), band neutrophil (B), and segmented neutrophil (PMN) evident in the center of this bone marrow aspirate smear highlight the nuclear and cytoplasmic features of maturation (Wright stain).

Progressive nuclear maturation from the round eccentric nucleus of a promyelocyte (P) (lower left) through the myelocyte (My), metamyelocyte (Mt), band (B), and segmented neutrophil (PMN) (bone marrow aspirate smear; Wright stain).



STAGES OF NEUTROPHILIC MATURATION

Early promyelocyte (P), neutrophilic myelocyte (My), neutrophilic metamyelocyte (Mt), band neutrophil (B), and segmented neutrophil (PMN). The progression from basophilic to eosinophilic cytoplasm and the acquisition of first, primary, and then, secondary granules in conjunction with gradual and progressive nuclear segmentation and condensation of the nuclear chromatin are evident .

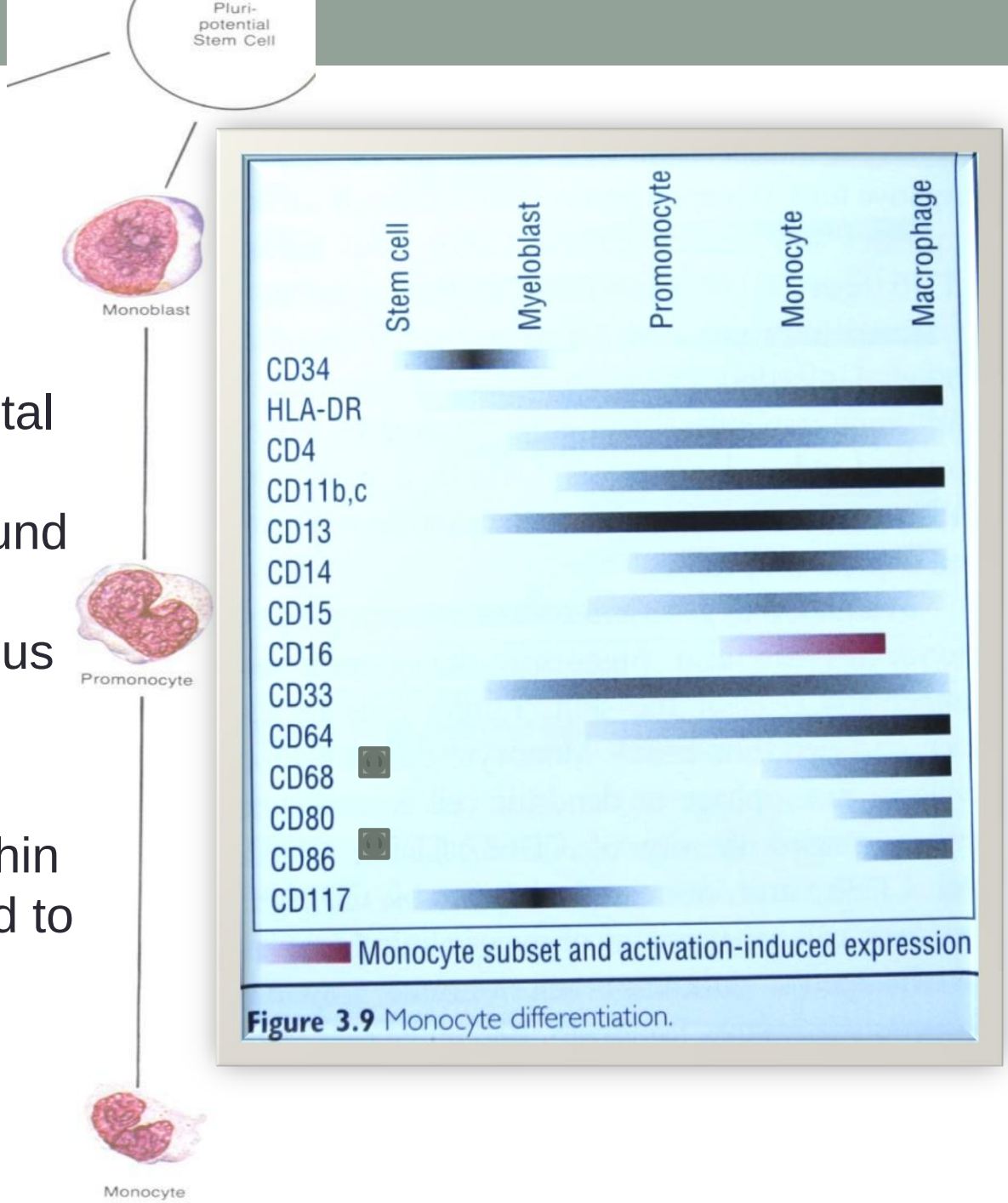


Morphological abnormalities

- Toxic granules: prominent blue-black to purplish granules that resemble primary granules. Mean asynchrony between maturation of nuclei and lysosomes
- Dohle bodies: pale sky-blue cytoplasmic inclusions (Rough endoplasmic reticulum), acute phase reaction and May-Hegglin anomaly

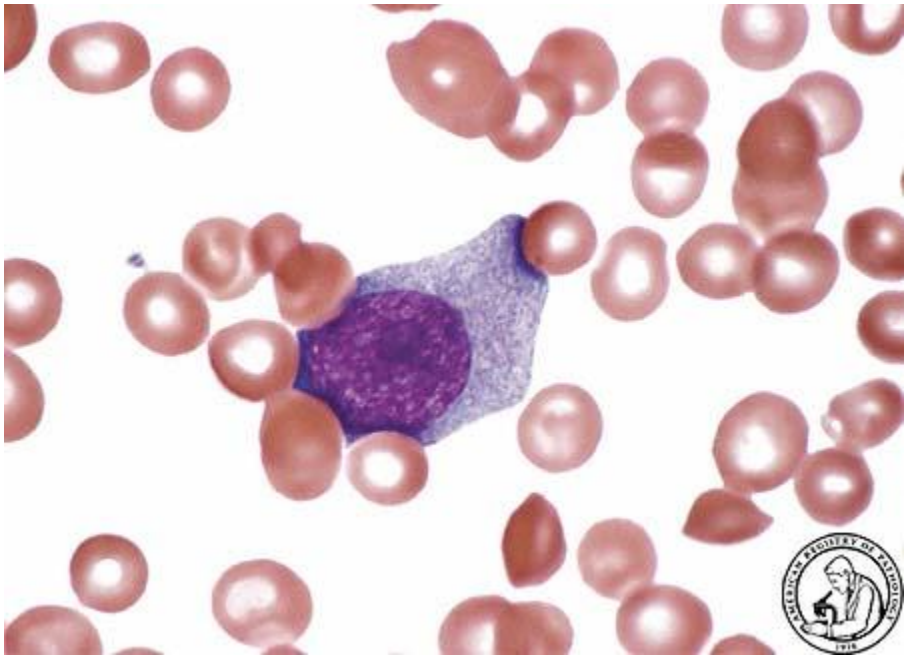
Monocytes

- Monocytes and related dendritic cells play a pivotal role in host defense from microbial pathogens, wound healing, angiogenesis, hematopoiesis, and various inflammatory reactions
- Monocytic production within bone marrow is estimated to take about 2 to 3 days



Monocytes

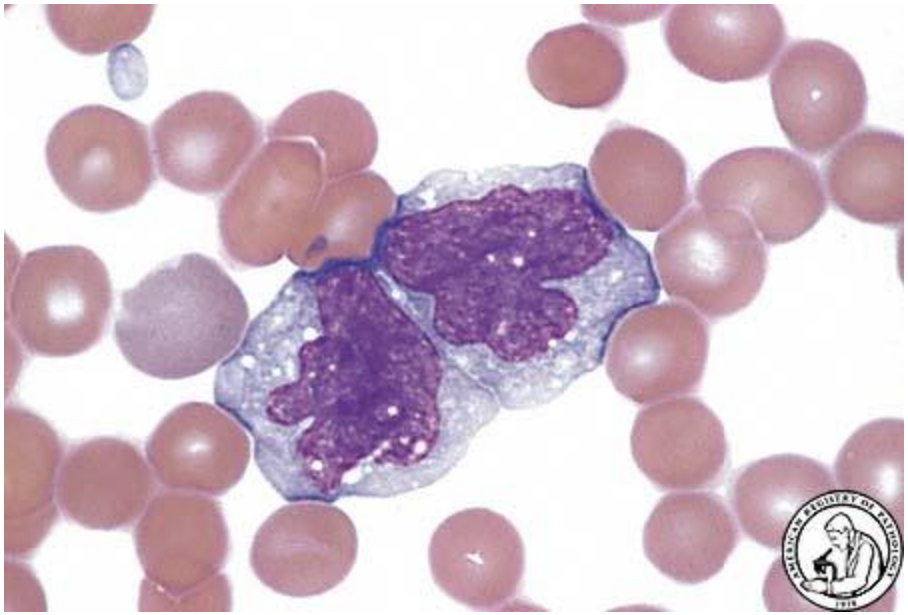
- Neither monoblasts nor promonocytes are typically evident in normal bone marrow
- Monoblasts:
  - Nuclei: round to oval with dispersed, blastic nuclear chromatin
  - Cytoplasm: abundant and pale blue, with either agranular or subtle, finely granular cytoplasm
-



Leukemic monoblast has voluminous, slate blue-gray, finely granular cytoplasm and an immature round nucleus

Monocytes

- **Promonocytes:**
  - Nuclei: folded nuclear configuration with a typically prominent nucleolus and fairly dispersed nuclear chromatin
  - Cytoplasm: abundant and pale blue, with either agranular or subtle, finely granular cytoplasm

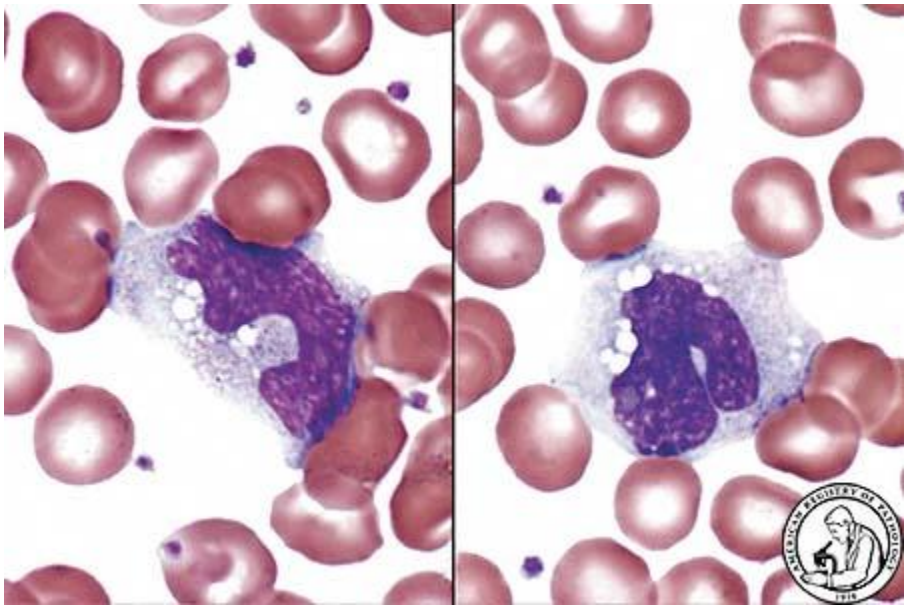


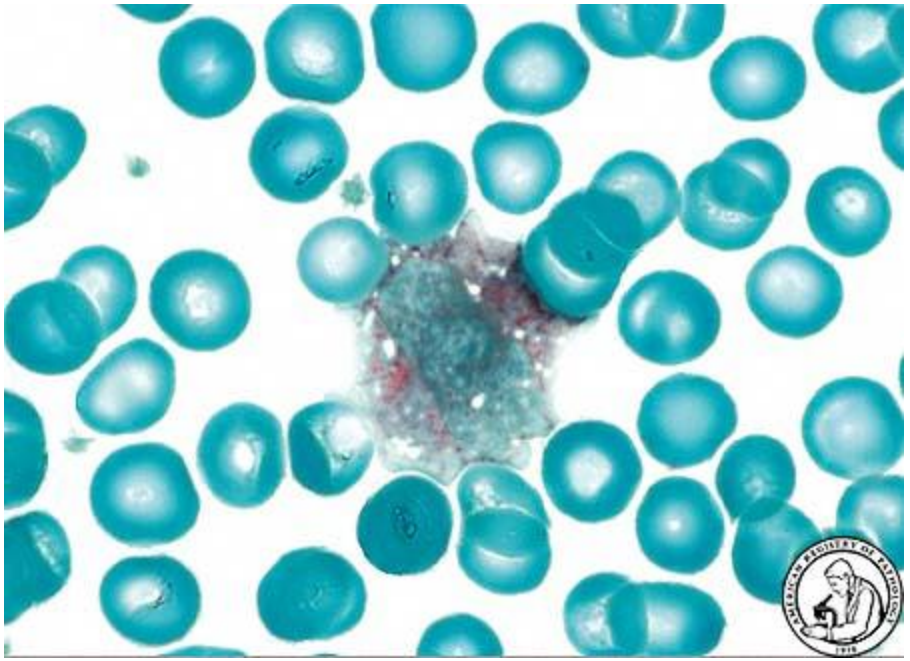
Leukemic promonocytes have abundant cytoplasm and folded, immature nuclei

Monocytes/Macrophages

- Diffuse cytoplasmic positivity for **alpha-naphthyl butyrate** and **alpha-naphthyl acetate** esterase (non-specific esterases) in all monocyte stages

- IHC include:
  - **Lysozyme, CD68** (KP1 or PG-M1 epitopes), and CD163



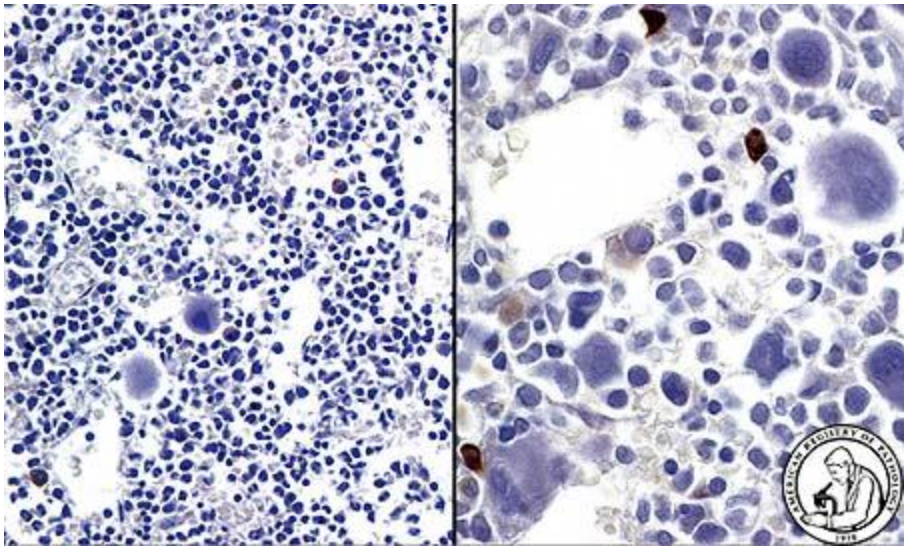


Dendritic cells

- Dendritic cells are defined more by their functional activities than by specific morphologic features, although immunophenotypic subsets are well described
- Dendritic cells varies by the specific cell type, and differences in phenotype are based on derivation from **either** myeloid or lymphoid progenitor cells

Dendritic cells

- Dendritic cells are infrequent in bone marrow, and immunohistochemical techniques (a profile consisting of CD68, CD123-, and CD43-positive, myeloperoxidase-negative cells) are generally required for cell identification

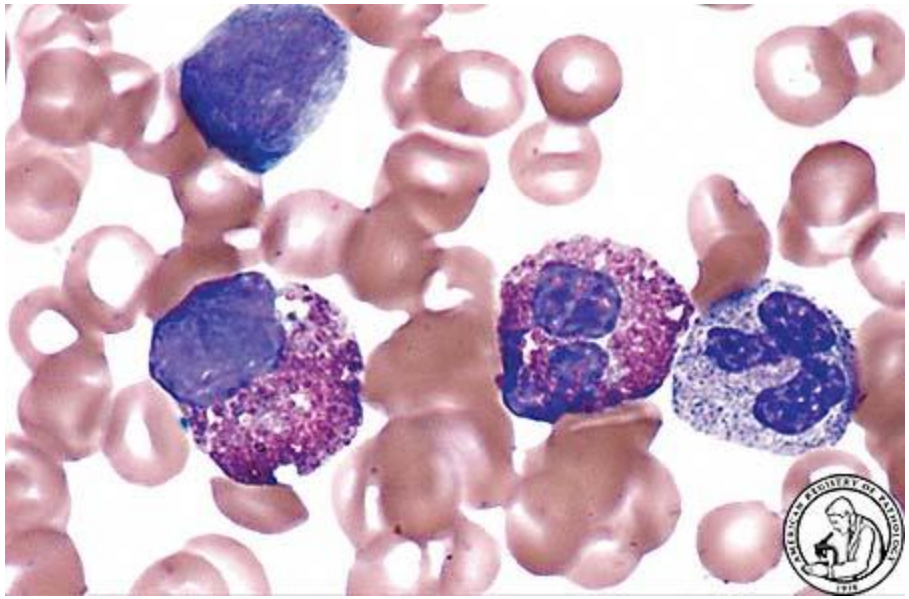


- This composite shows rare CD1a-positive cells (left) and slightly more numerous S-100 protein-positive cells (right) in a normal bone marrow core biopsy from 1-year-old female

Other myeloid components

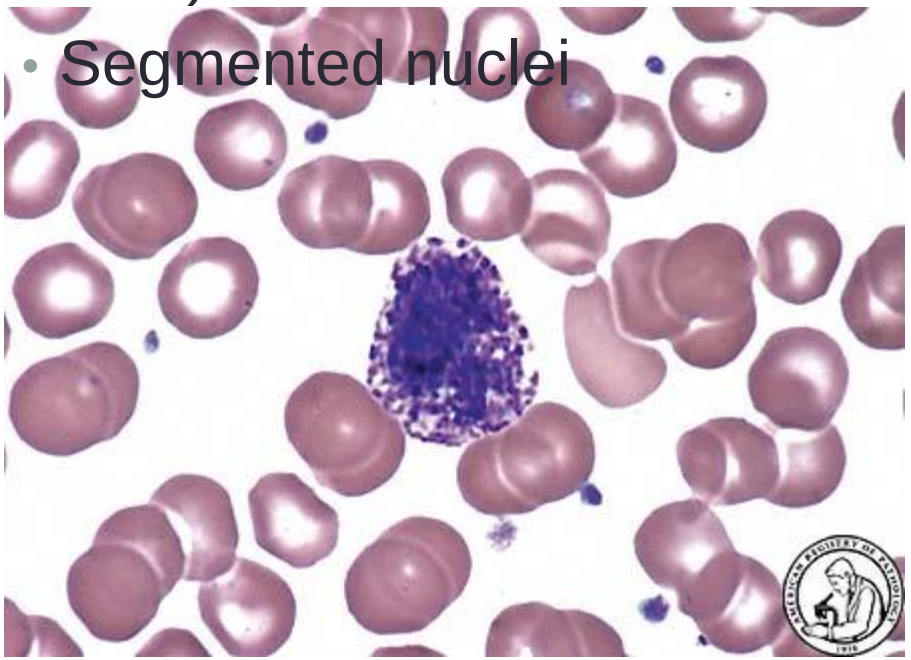
Eosinophilic myelocyte and mature eosinophil

- Eosinophil granules are large and **refractile**, and contain major basic protein, eosinophil peroxidase



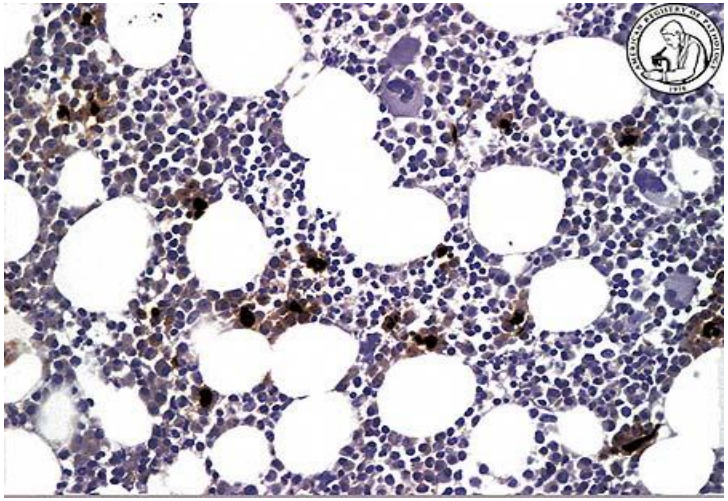
Mature segmented basophil

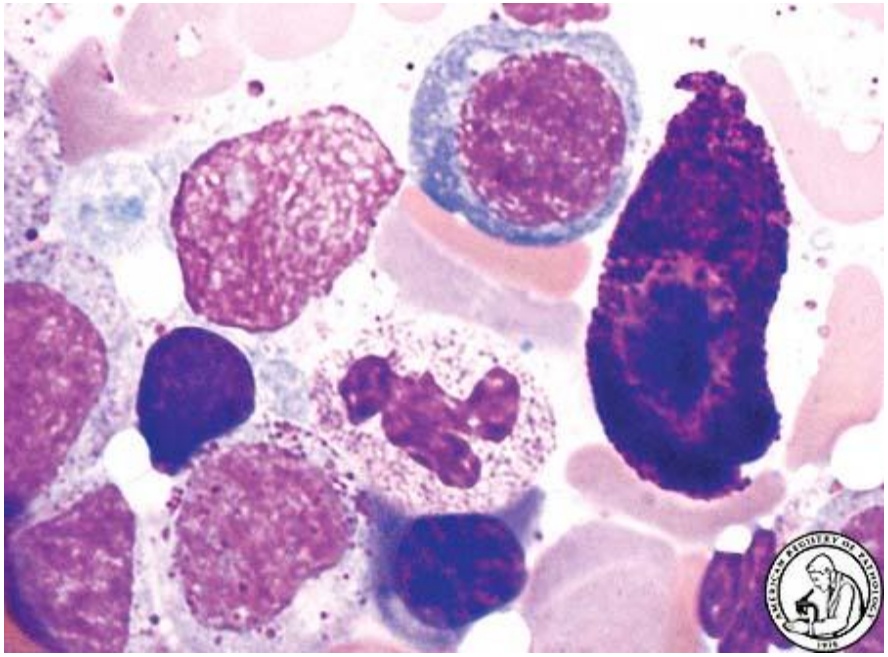
- Granules have histamine and heparin (like mast cells)
- Segmented nuclei

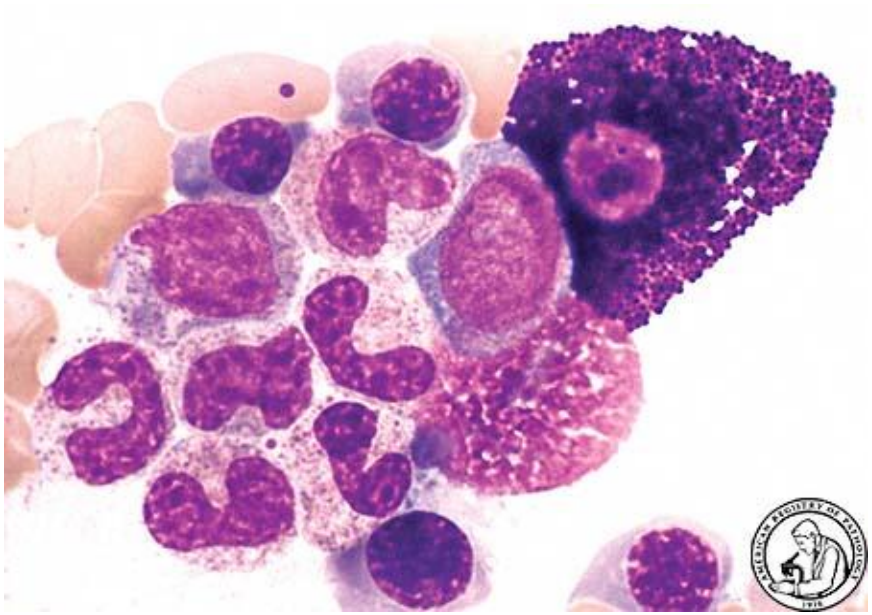


Mast Cells

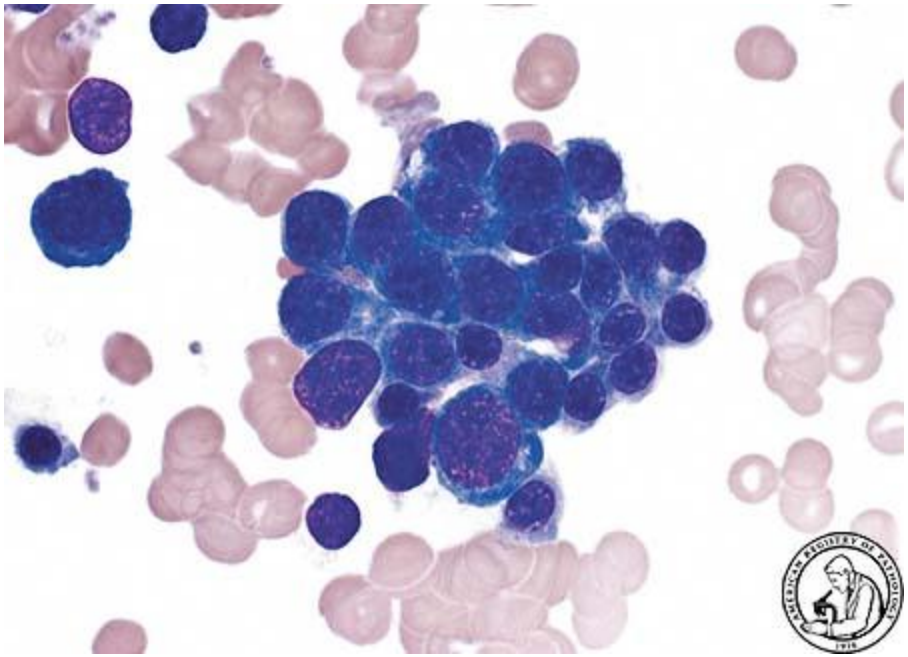
- Round to oval nuclei
- Granules have histamine and heparin
- Tryptase or CD117, highlight perivascular distribution in bone marrow



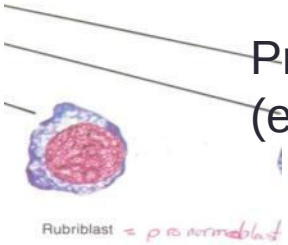




Erythroid cells



Erythropoiesis occurs in discrete colonies within the hematopoietic cavity
Basophilic RNA-rich cytoplasm in immature forms
Production takes 5 to 7 days



Pronormoblast (erythroblast)

Basophilic normoblast

Polychromatophilic normoblast

Orthochromatic normoblast

Basophilic erythrocyte

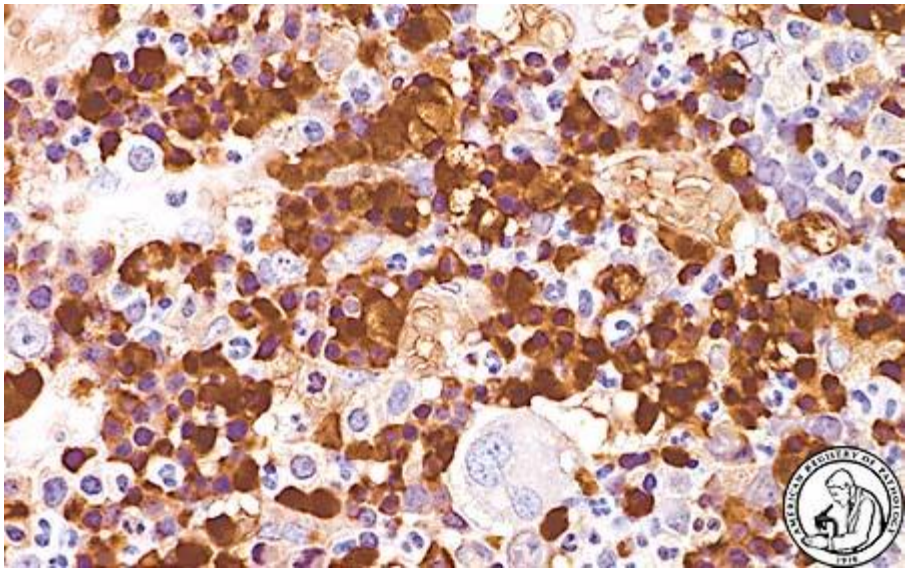
Reticulocyte

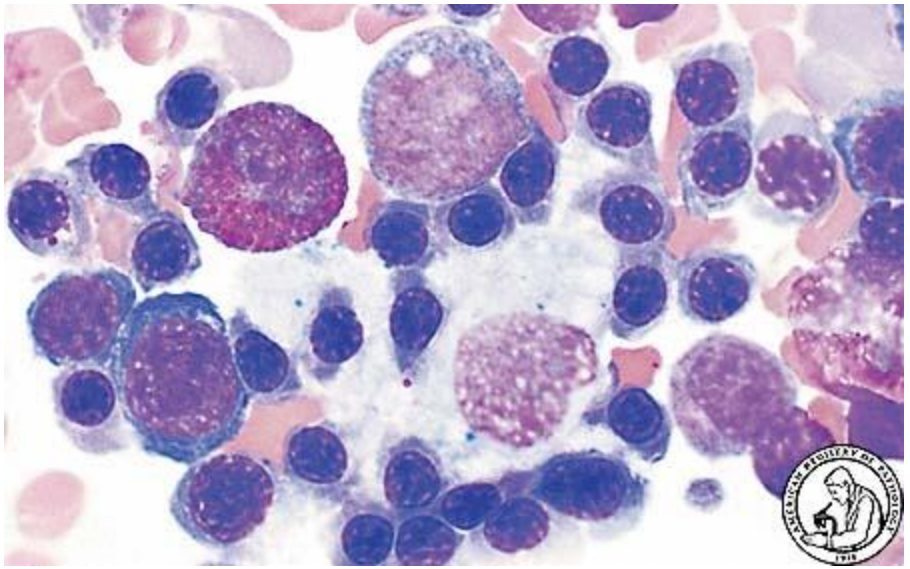
Erythrocyte

Erythroid cells

- Erythropoietin is the dominant lineage specific growth factor
- Controls survival and proliferation
- Binds to a receptor that activates *JAK2*, initiating the downstream signaling pathways resulting in phosphorylation and activation of *GATA1*

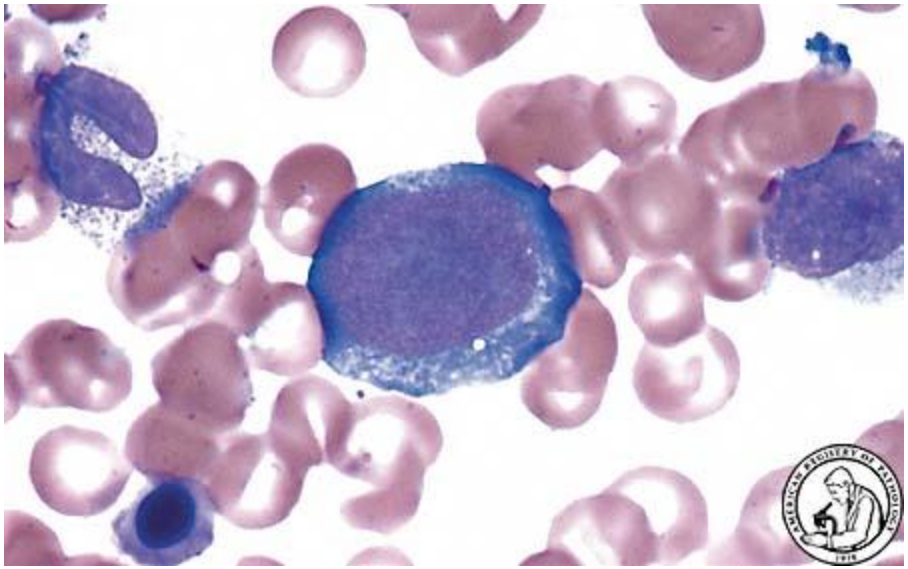
IHC for HgbA

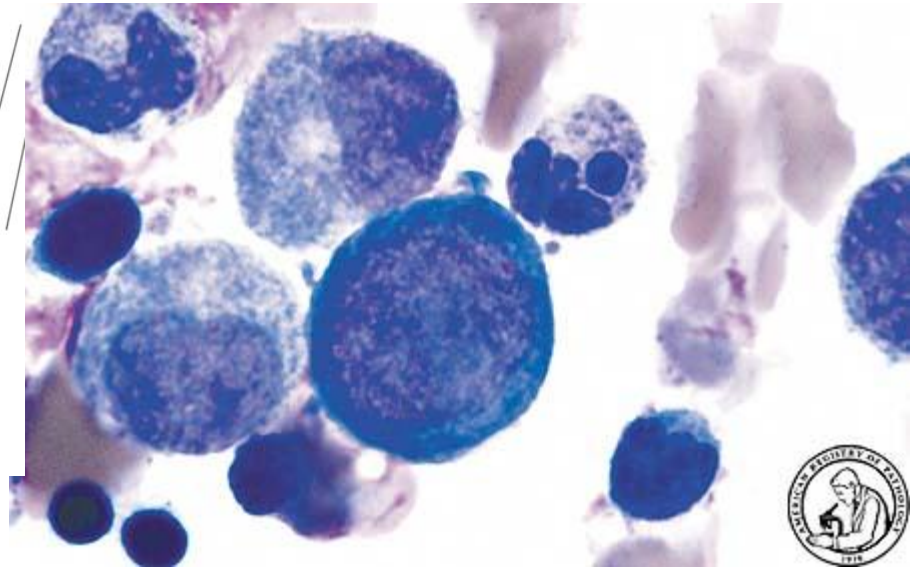




Erythroid cells

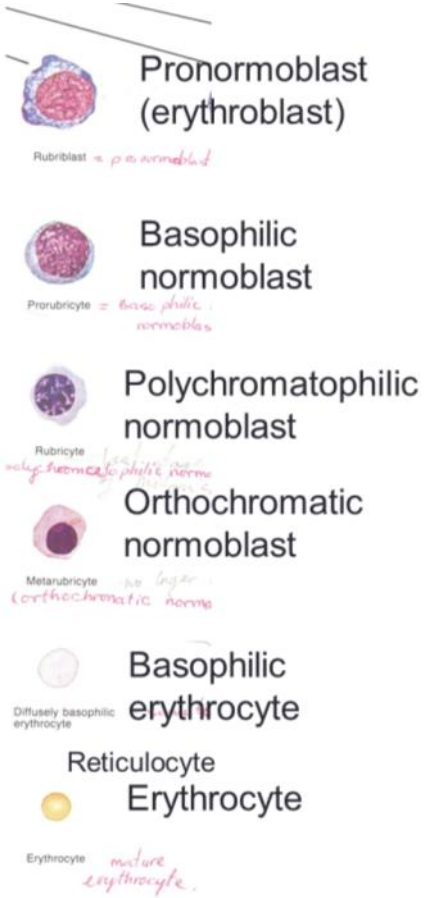
- erythroblast has deeply basophilic cytoplasm and a round nuclear contour

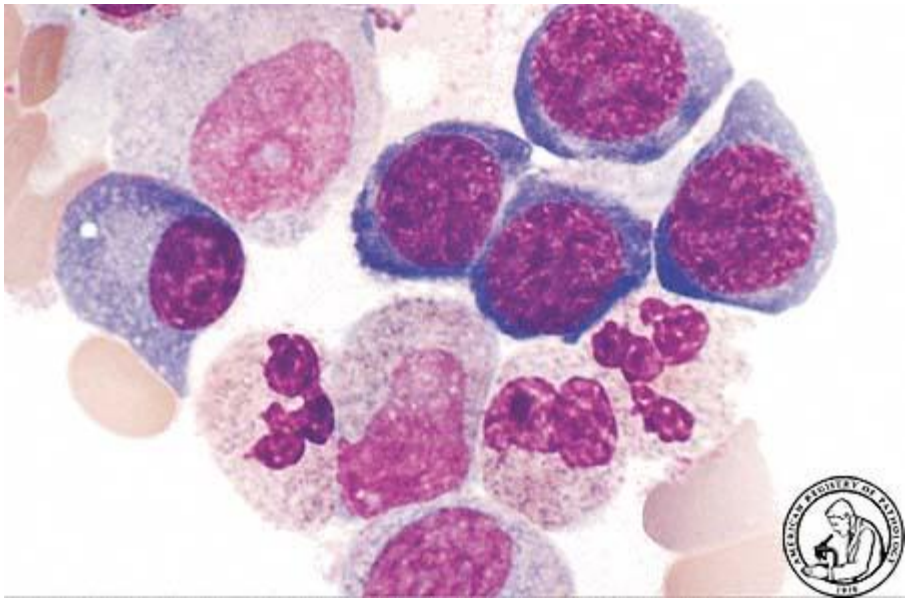


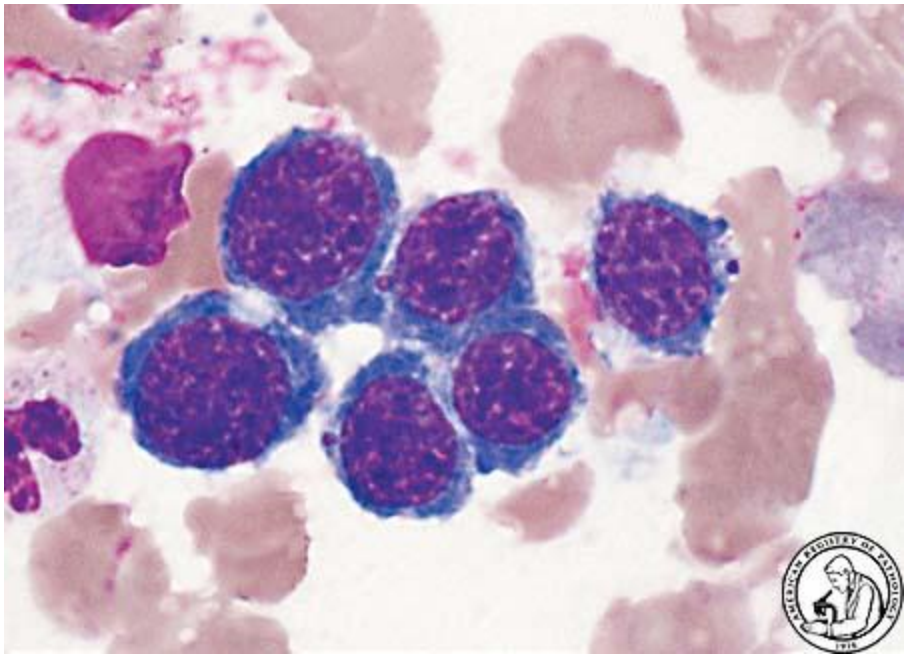


Erythroid cells

- Basophilic normoblasts

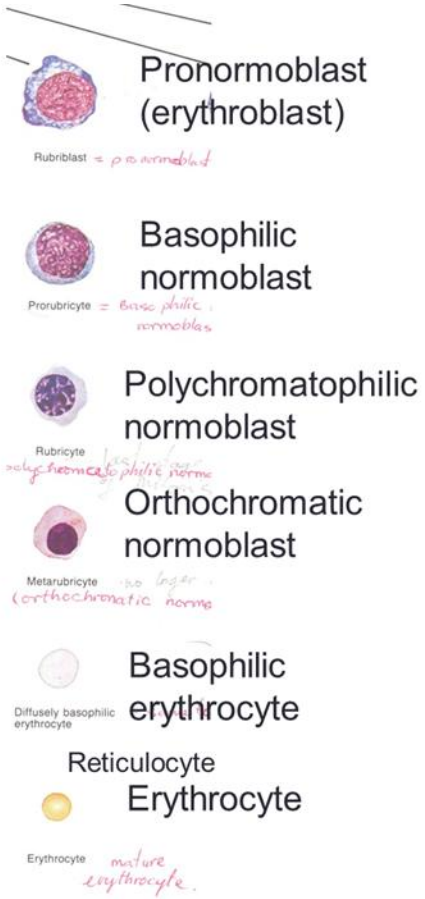


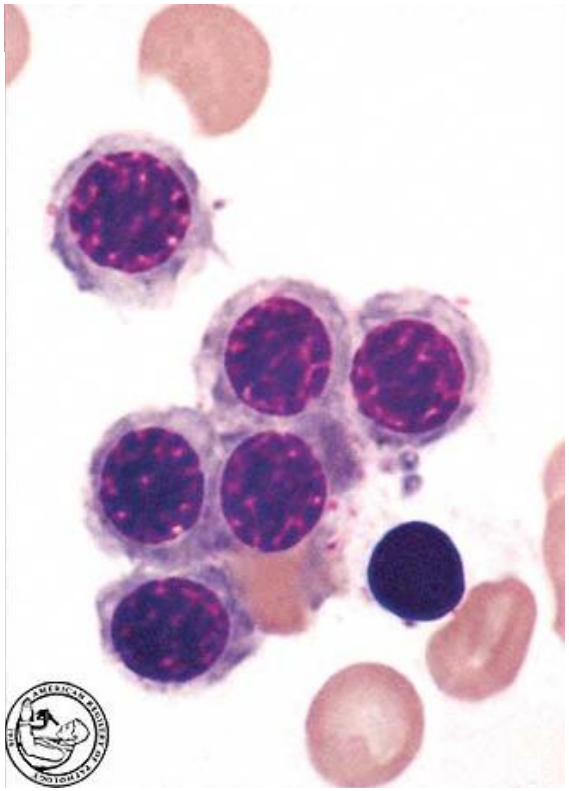




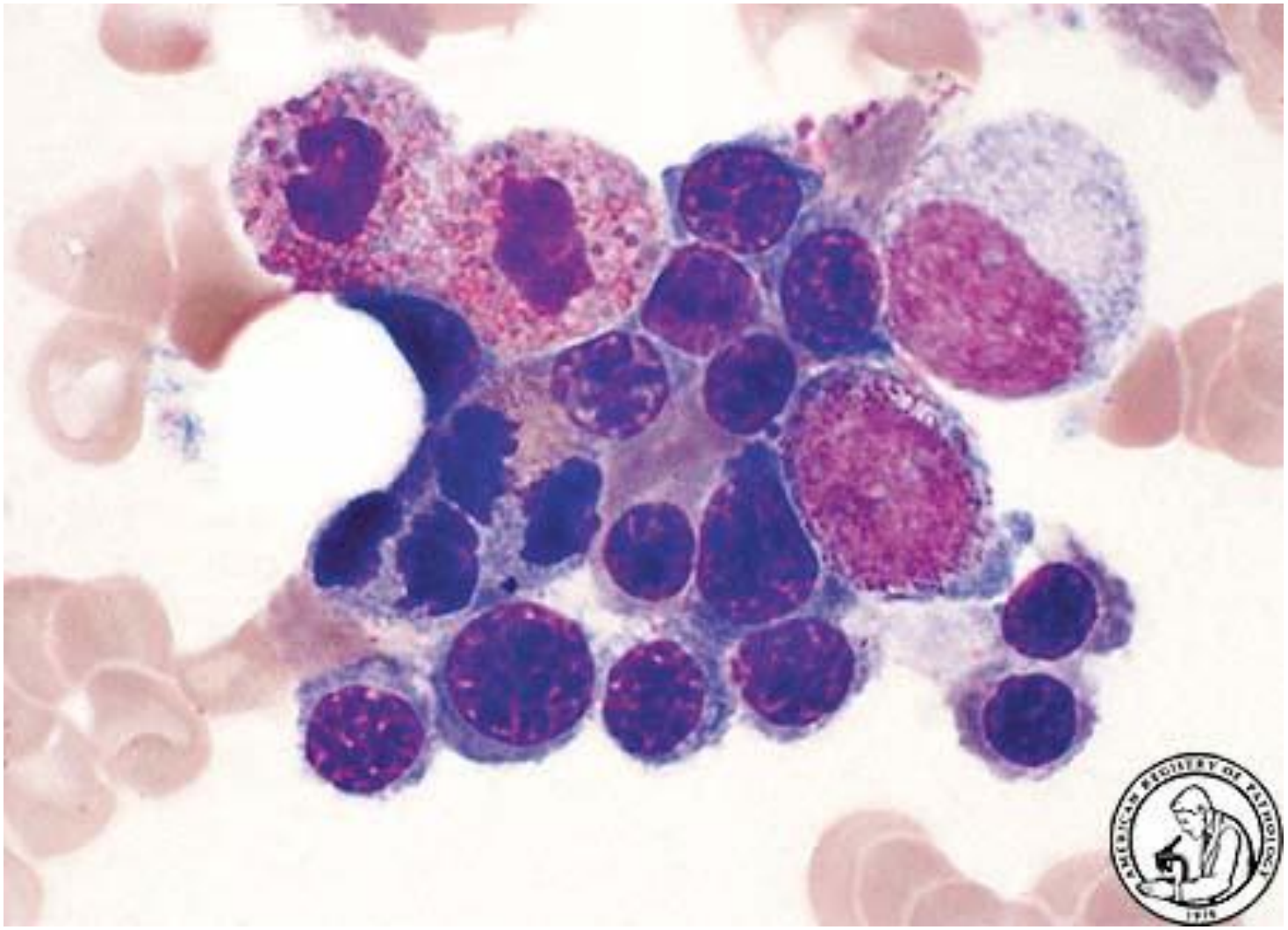
Erythroid cells

- Polychromatophilic normoblasts
  - Last stage of cellular division

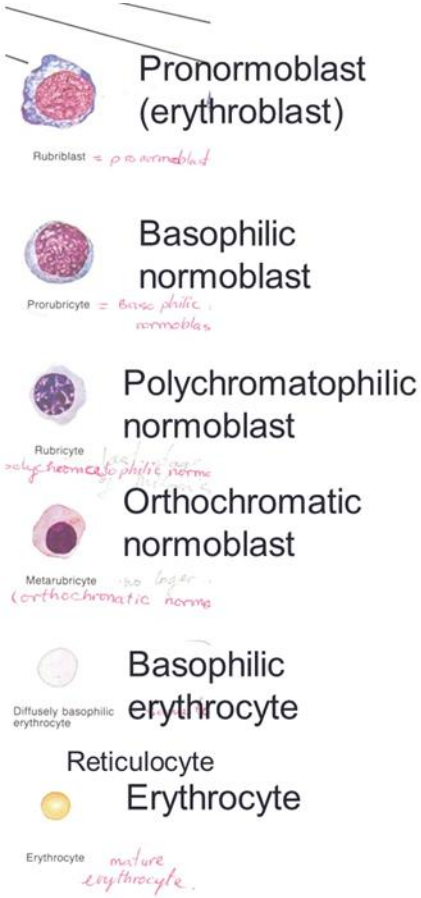


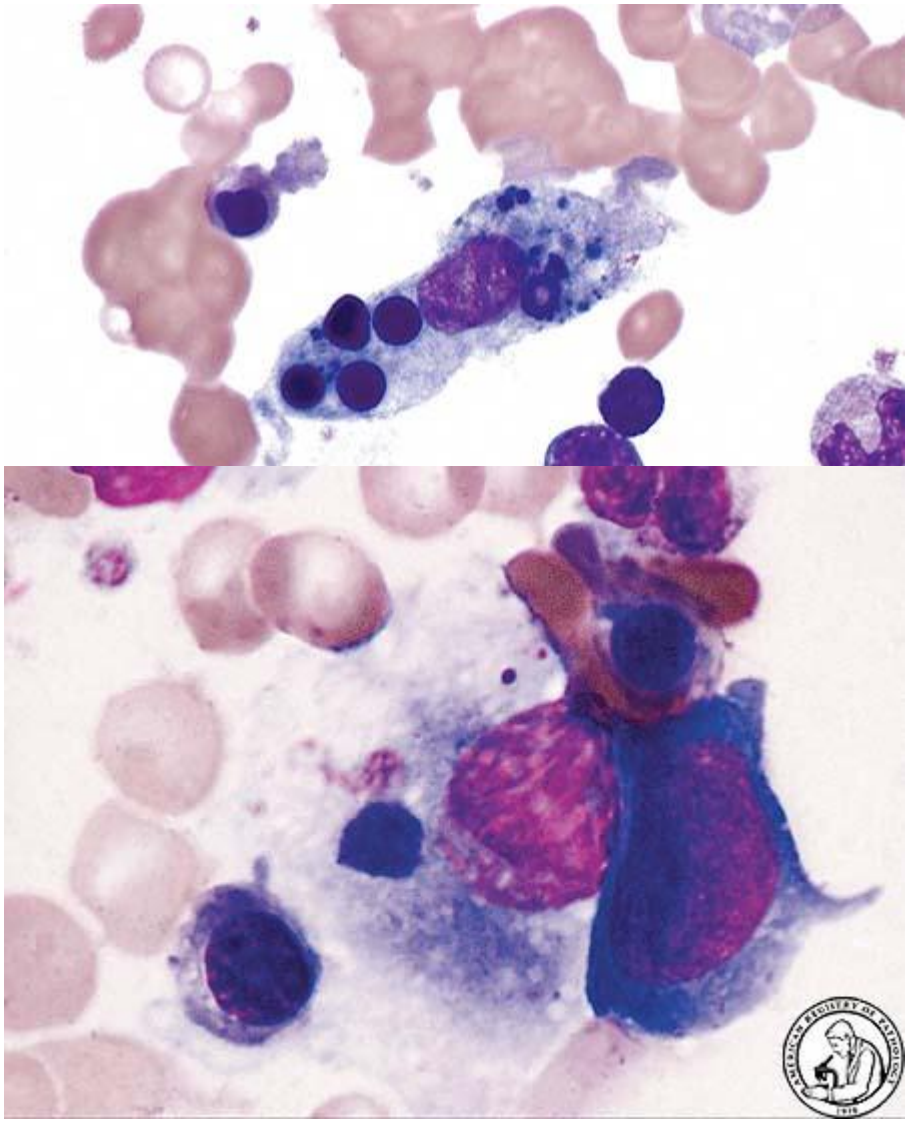


Stages of Erythroid Maturation



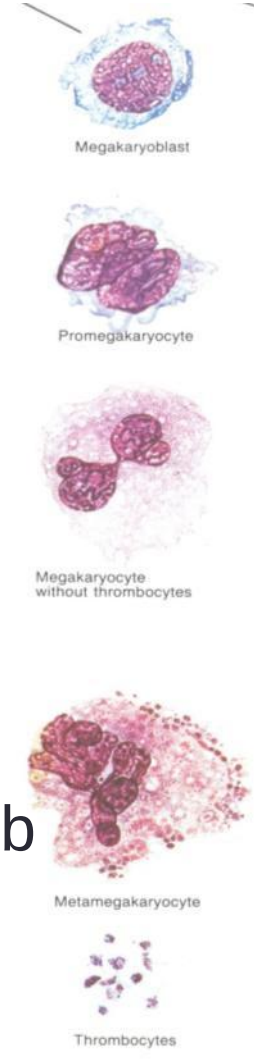
Monocytes/macrophages

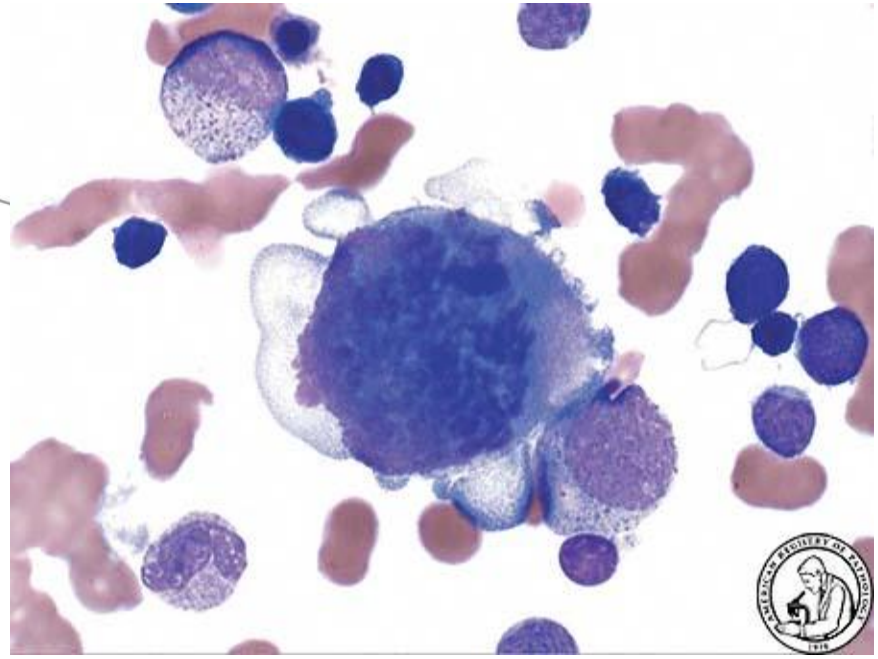


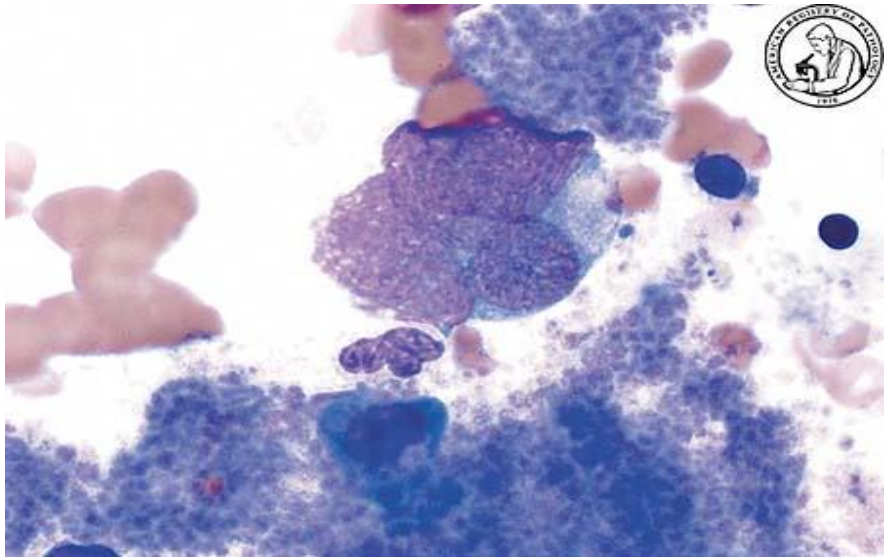


Megakaryocytes

- Megakaryoblast
- Promegakaryocyte
- Platelet-shedding megakaryocyte
- IHC:
- CD61, CD41, CD42b

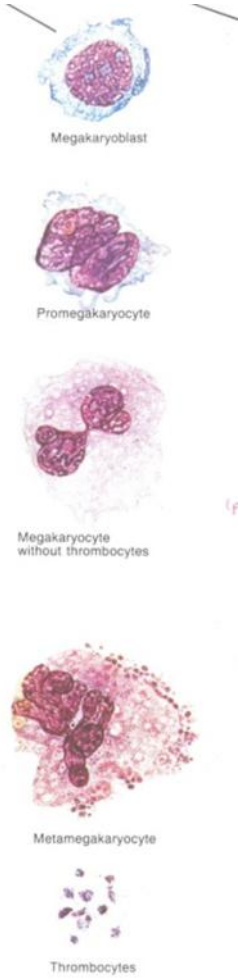


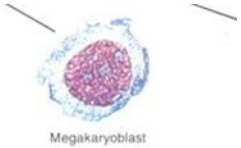


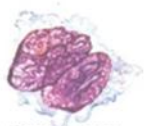


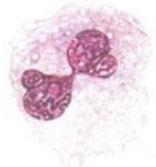
Megakaryocyte Morphology

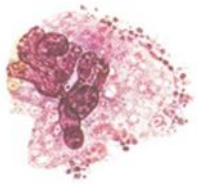
Stage of Maturation	Morphology
Megakaryoblast	Difficult to recognize by morphology alone, but tends to be large blast with a high nuclear to cytoplasmic ratio, basophilic cytoplasm, and variable cytoplasmic blebbing
Promegakaryocyte	Spectrum of large cells with various degrees of nuclear lobulation Progressive increase in overall size, variable cytoplasmic granules
Platelet-shedding megakaryocyte	Large multilobulated megakaryocytes with highly condensed nuclear chromatin reside adjacent to bone marrow sinuses Voluminous amounts of cytoplasm with abundant cytoplasmic granules




Megakaryoblast


Promegakaryocyte


Megakaryocyte without thrombocytes

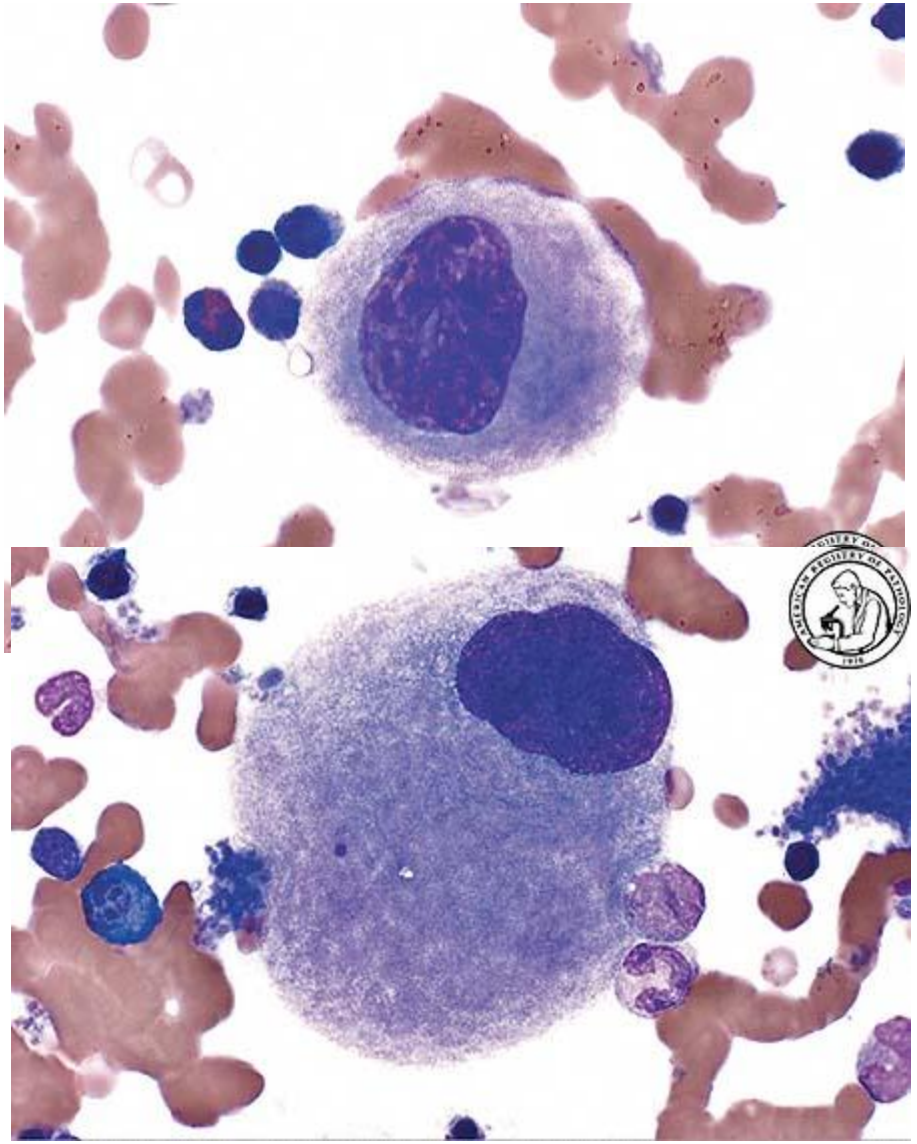

Metamegakaryocyte


Thrombocytes

Stage of Maturation	Cytochemical/Immunophenotypic Properties
Megakaryoblast	Platelet peroxidase evident by immuno-electron microscopic techniques Variable CD34 expression Expression of lineage-specific antigens such as CD41, CD42b, CD61, and Mpl1
Promegakaryocyte	Loss of CD34 but retention of the full complement of megakaryocyte-associated antigens
Platelet-shedding megakaryocyte	Expression of some megakaryocyte-associated antigens such as CD31, CD41, vWF increases with maturation

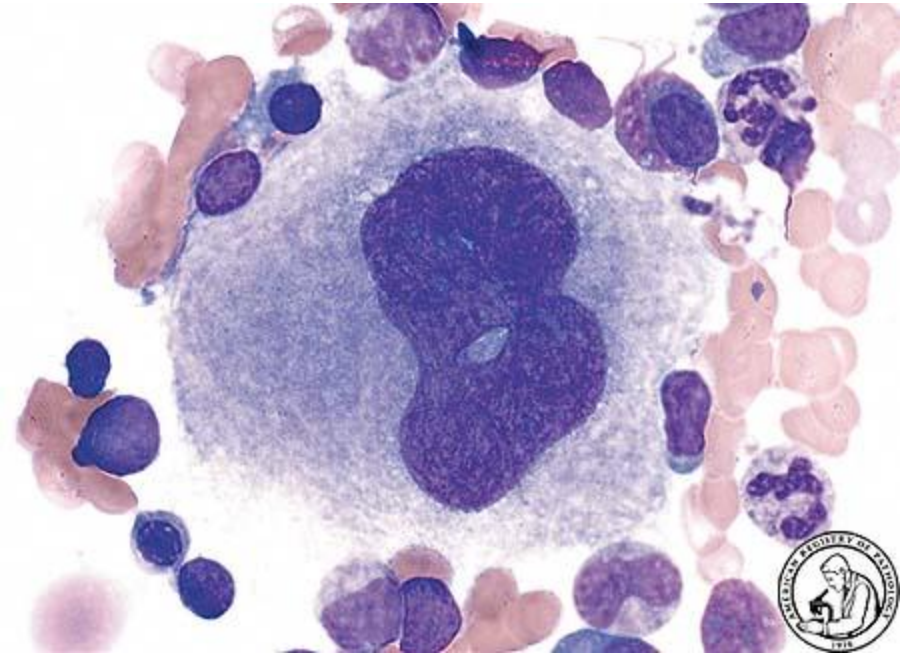
Megakarycytes

- Early to middle maturation stage megakaryocyte with round nucleus and abundant cytoplasm



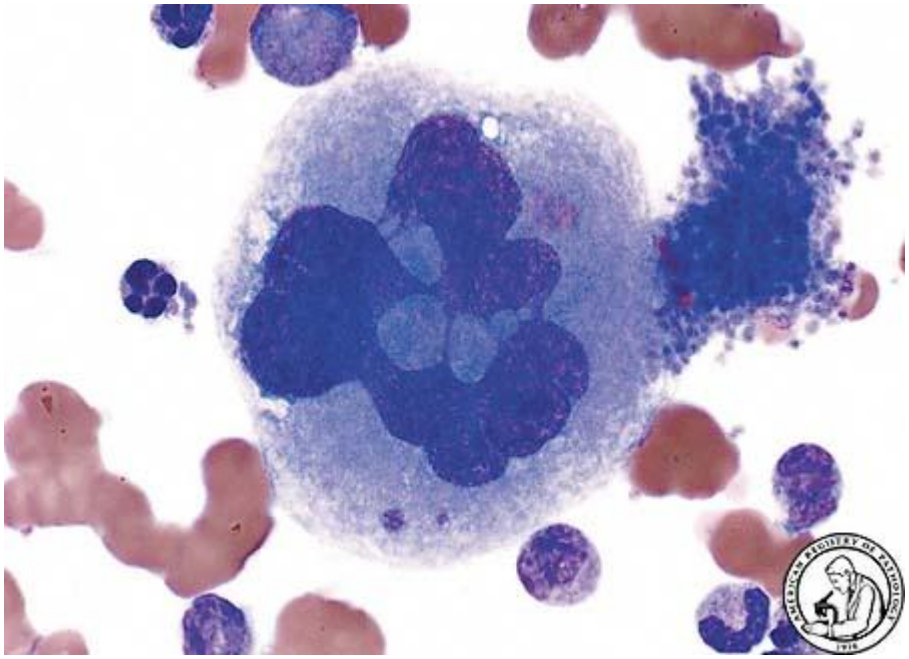
Megakarycytes

- Megakaryocyte maturation is characterized by the progressive doubling of nuclear material, with the multilobulation of a single nucleus, termed **endomitosis**



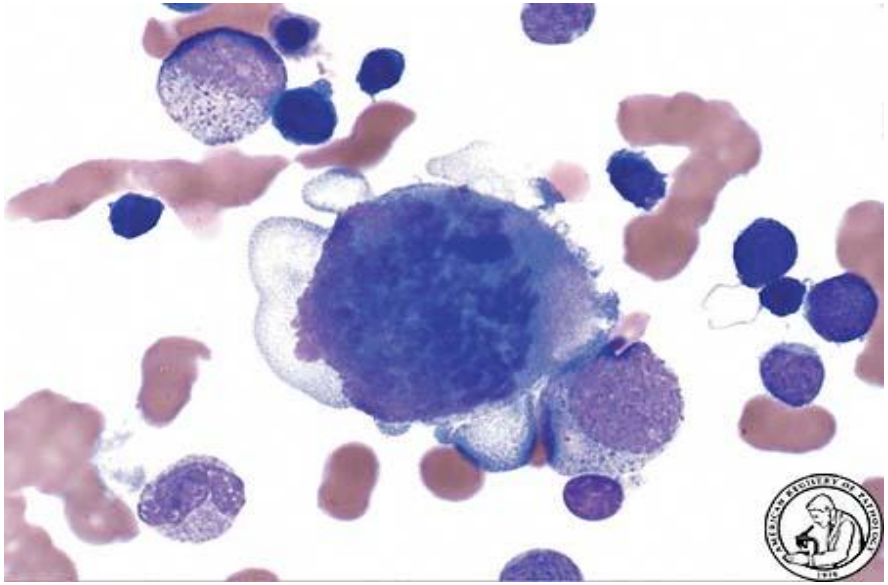
Megakarycytes

- Prominent nuclear lobulation with interconnected lobules is evident in this mature megakaryocyte with an adherent large platelet clump

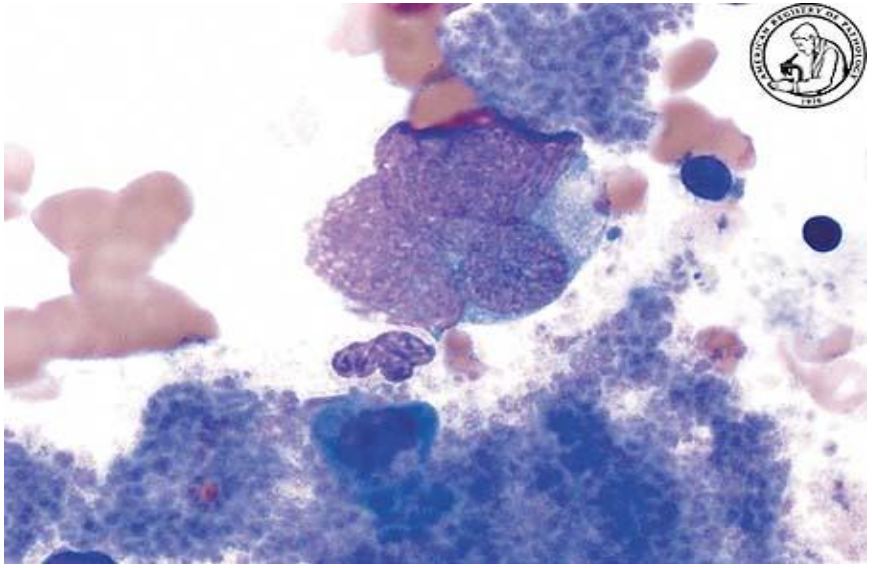


Megakaryocytes

- Thrombopoietin (produced in the liver) is an obligatory lineage specific growth factor
- Binding of thrombopoietin to its ligand (c-Mpl) activates *JAK2* → promotes meg differentiation and proliferation
- Platelets have thrombopoietin receptors

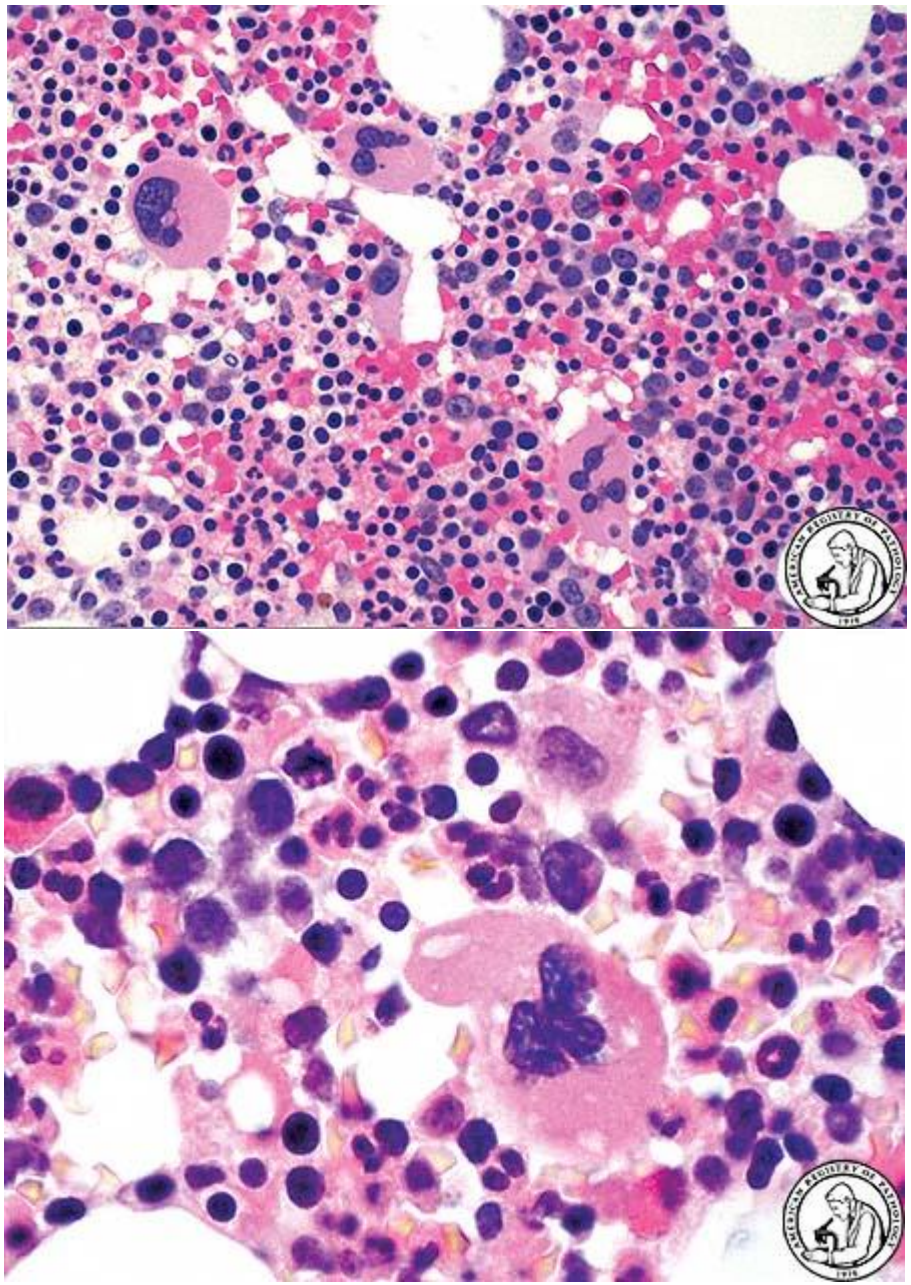


Immature megakaryocytes with prominent cytoplasmic blebbing



Megakarycytes

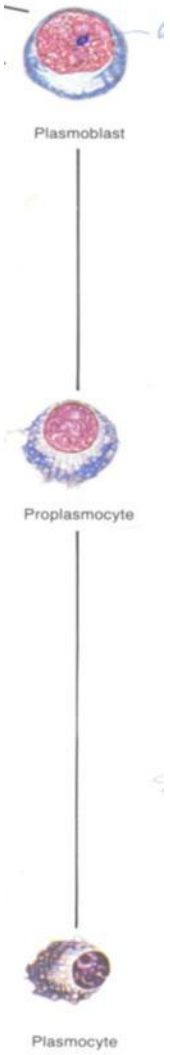
- Perisinusoidal and intrasinusoidal localization of megakaryocytes is evident in this bone marrow biopsy from an adult



Lymphoid and Natural Killer Cells

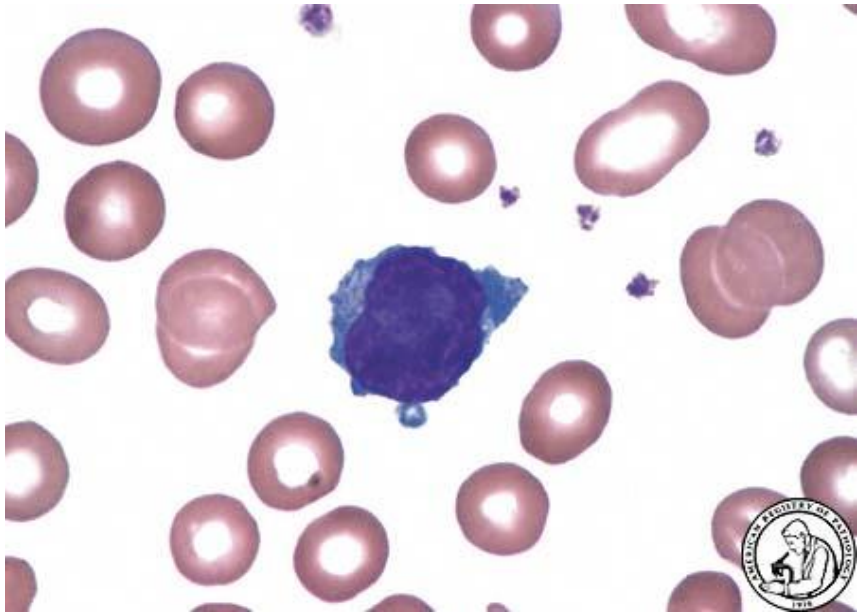
- Origin of B, T, and natural killer (NK) precursor cells from stem cells that give rise to hematopoietic and lymphoid lineages

- Immature T cells migrate to the thymus
- The bone marrow is the site of B-cell development



Lymphoid and NK Cells

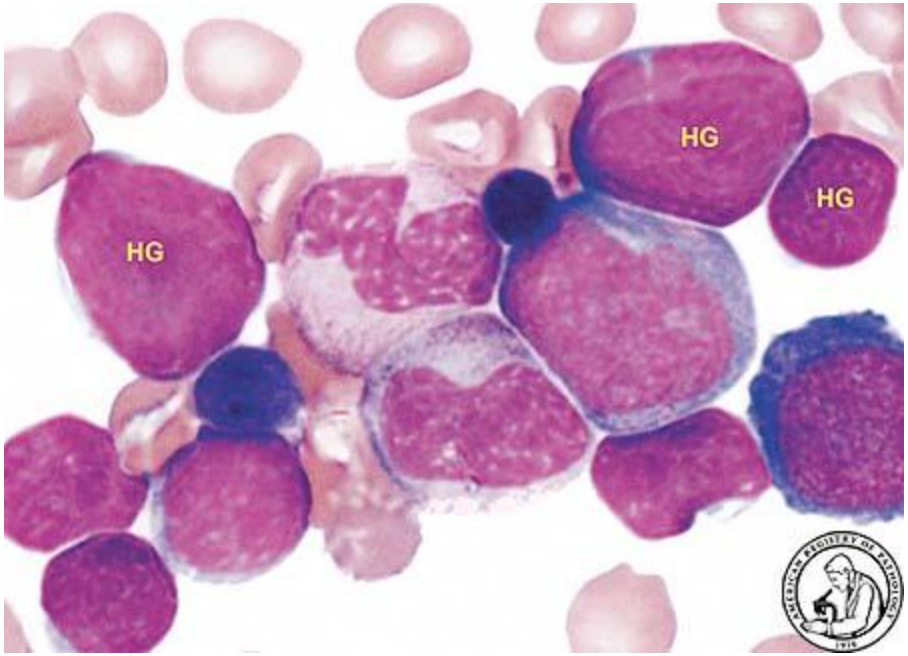
- **Hematogones** (immature benign B lymphoid cells), may be abundant in specimens from pediatric patients

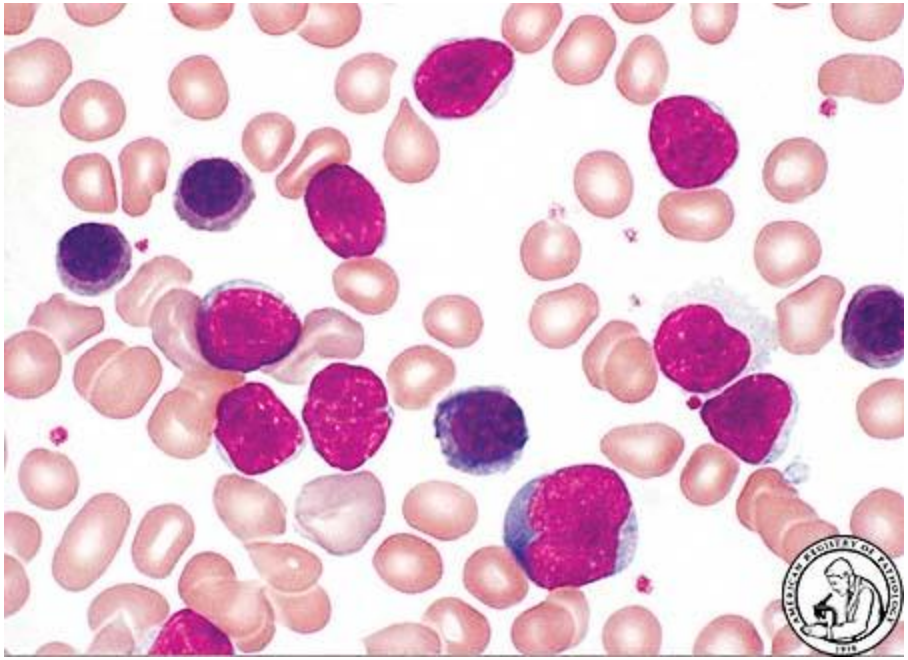


- An immature lymphoid cell with dispersed chromatin and an irregular nuclear configuration is evident in the peripheral blood of a normal infant

Lymphoid and NK Cells

- BM Hematogones
  - Nuclear chromatin is highly condensed

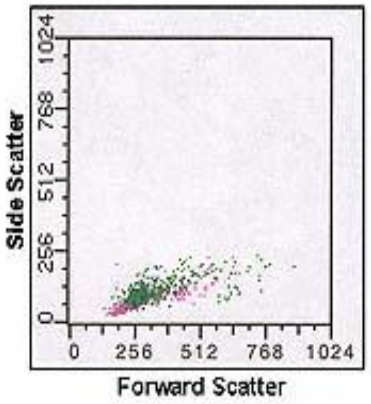


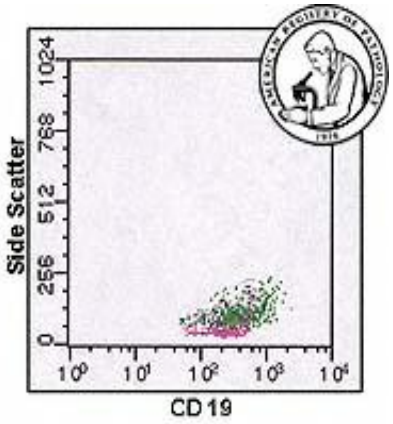


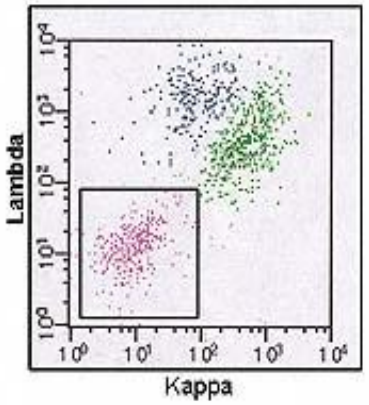
Lymphoid and NK Cells

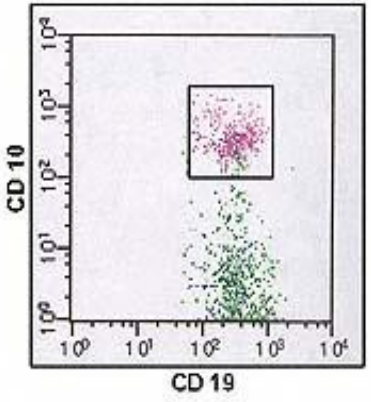
Hematogones

- By gating the lymphocyte population by forward and side scatter properties, an admixture of mature polyclonal B cells and surface immunoglobulin-negative, CD10-positive hematogones is evident in a normal bone marrow aspirate



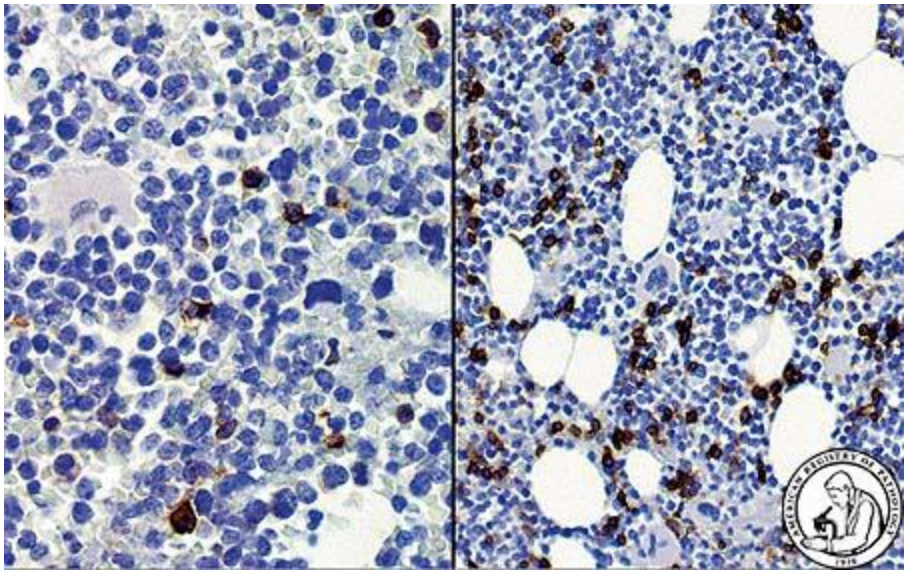






Lymphoid and NK Cells

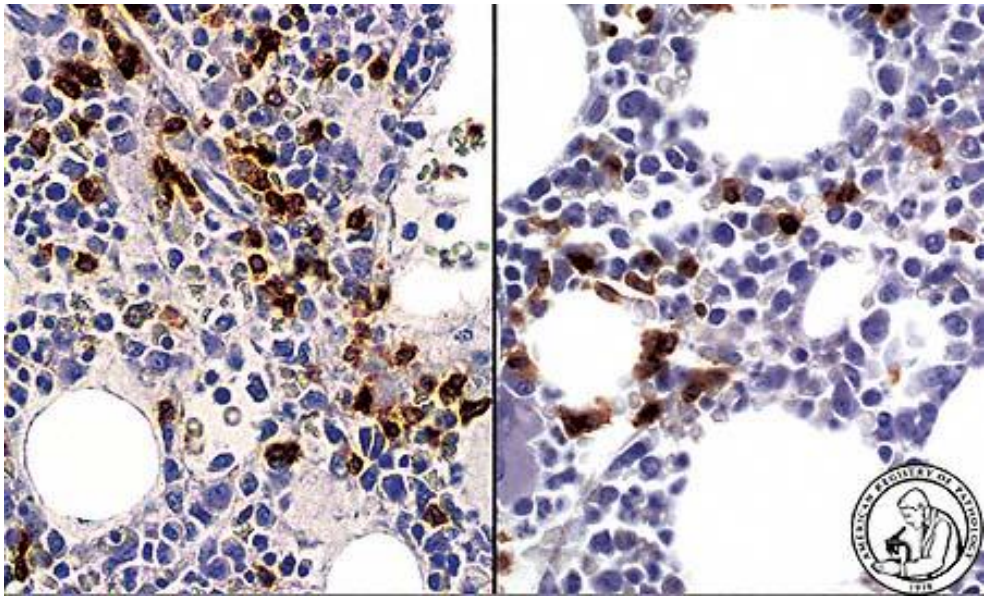
- bone marrow clot section from a premature infant with increased hematogones shows a side-by-side comparison of the number of CD3-positive T cells (left) and the number of CD20-positive B cells (right). Because of the increased numbers of hematogones, B cells are more numerous than T cells



Lymphoid and NK Cells

- Except in specimens with abundant hematogones, **T cells predominate** in normal bone marrow and exhibit a patchy, partially perivascular distribution

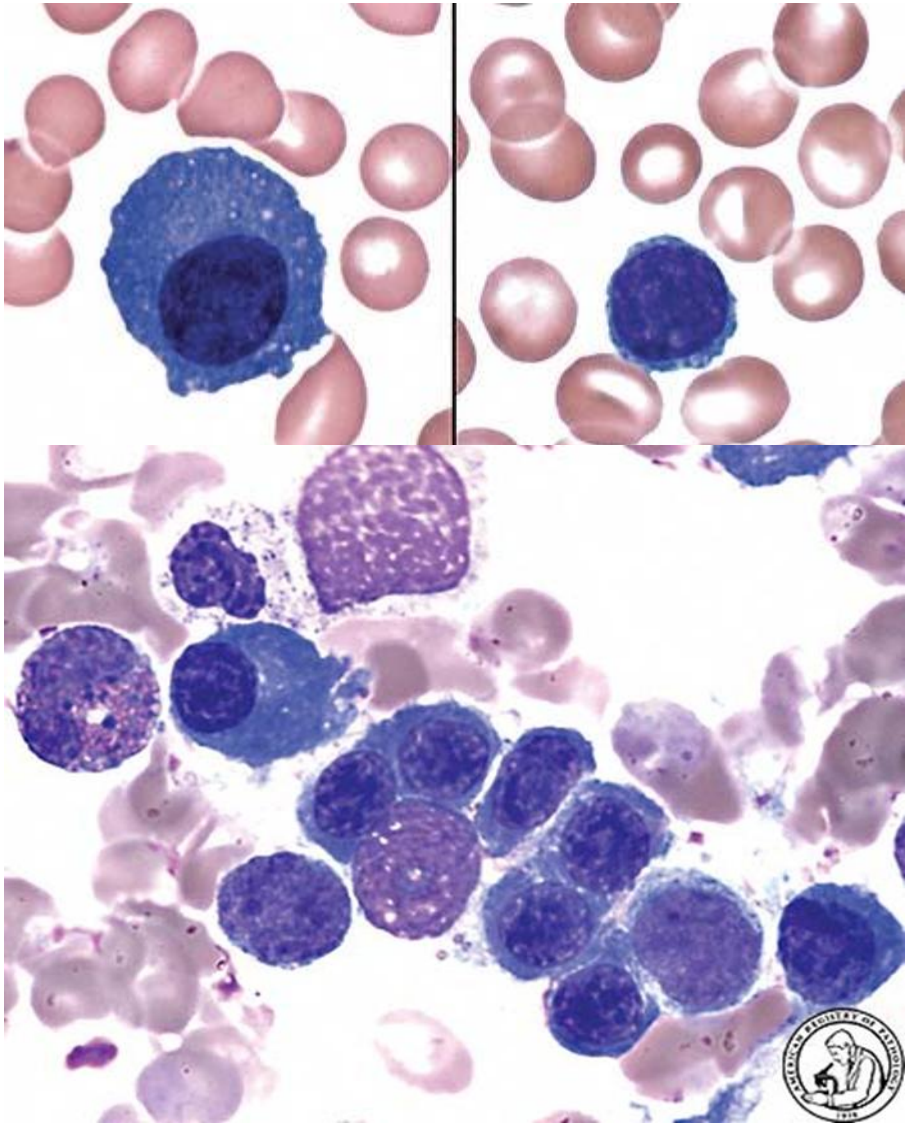
- B cells are less common and are randomly distributed and dispersed, individually or in small clusters



- The perivascular (left) and patchy interstitial (right) distribution of T cells is evident on the bone marrow core biopsy from an adult.

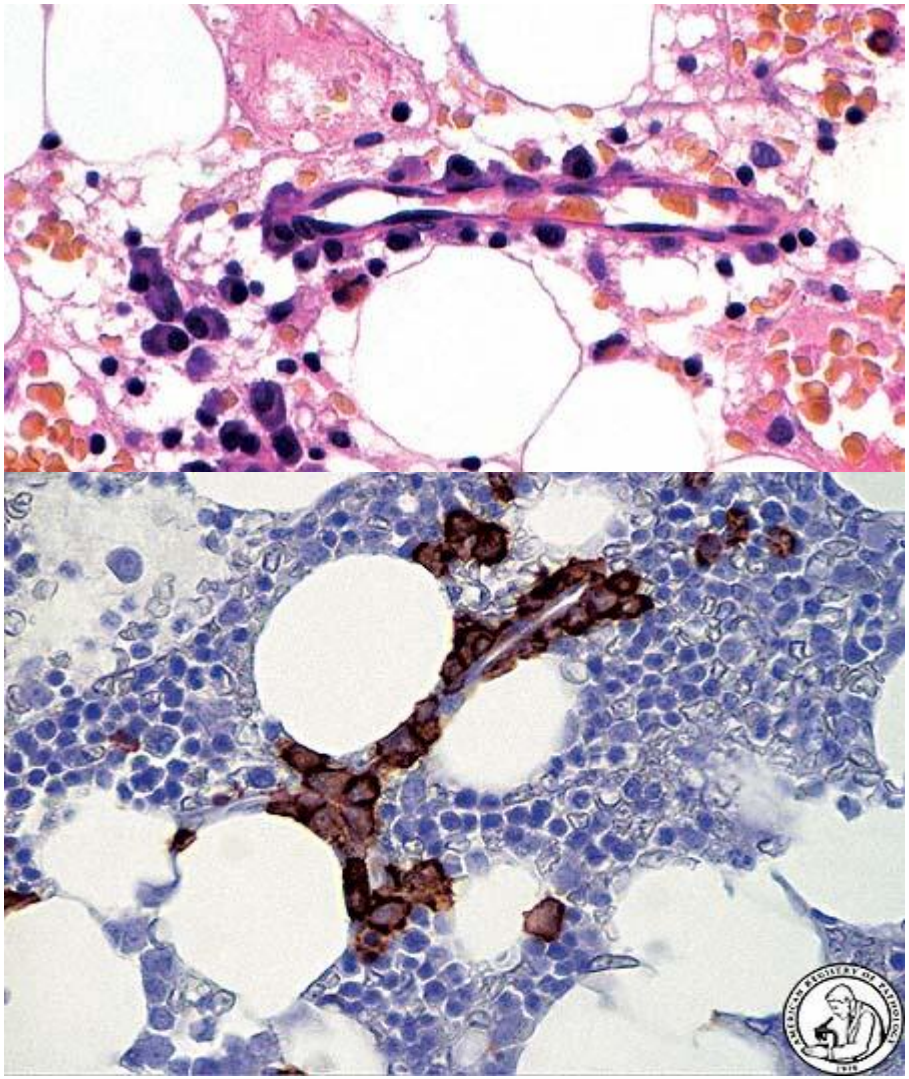
Lymphoid and NK Cells

- Mature plasma cells
  - Nuclei: eccentric with a "clockface" chromatin pattern
  - Cytoplasm: prominent paranuclear hof, and abundant basophilic cytoplasm that may contain immunoglobulin vacuoles



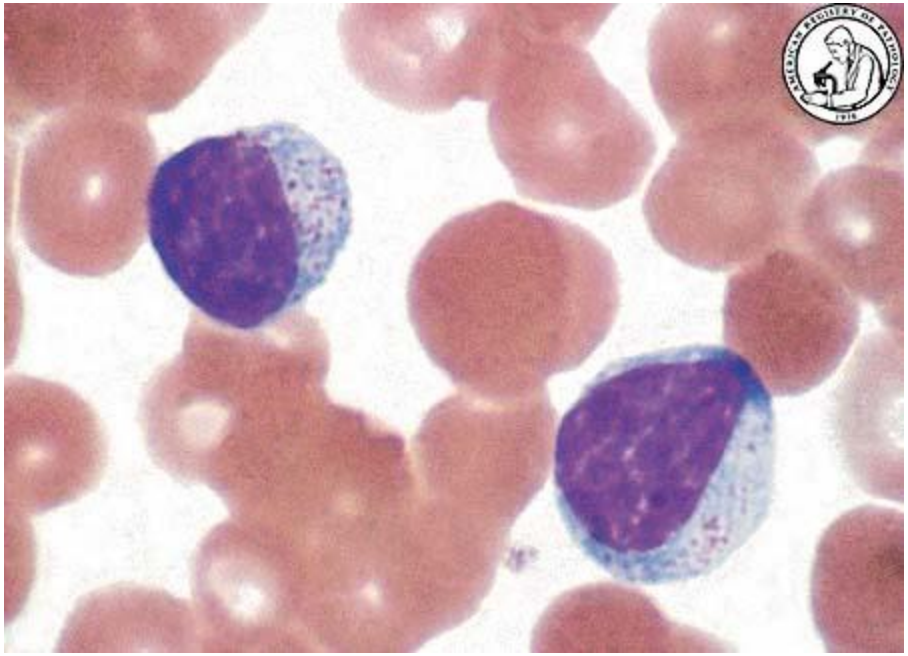
Lymphoid and NK Cells

- Plasma cells may have perivascular distribution
- CD138 highlights perivascular plasma cells
- Constitute up to 5% of plasma cells
- Benign plasma cells have a polytypic kappa to lambda light chain ratio



NK Cells

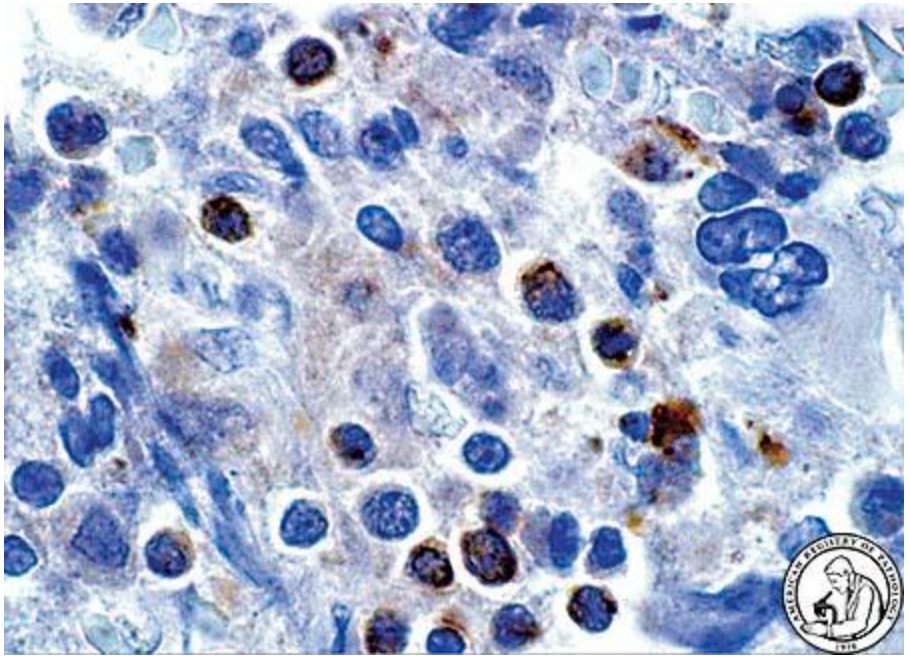
- NK cells are surface CD3-negative, CD56-positive, CD16-positive
- Produce immunoregulatory cytokines
- Also mediate cytotoxicity against target cells that lack matching major histocompatibility complex (MHC) ligands



- NK cells have features of large granular lymphocytes, although cytotoxic T cells (surface CD3 and CD8 positive) also share this morphology
- The granules contain perforin and granzymes,
- **IHC: granzyme and T-cell intracellular antigen 1 (TIA-1)**

Lymphoid and NK Cells

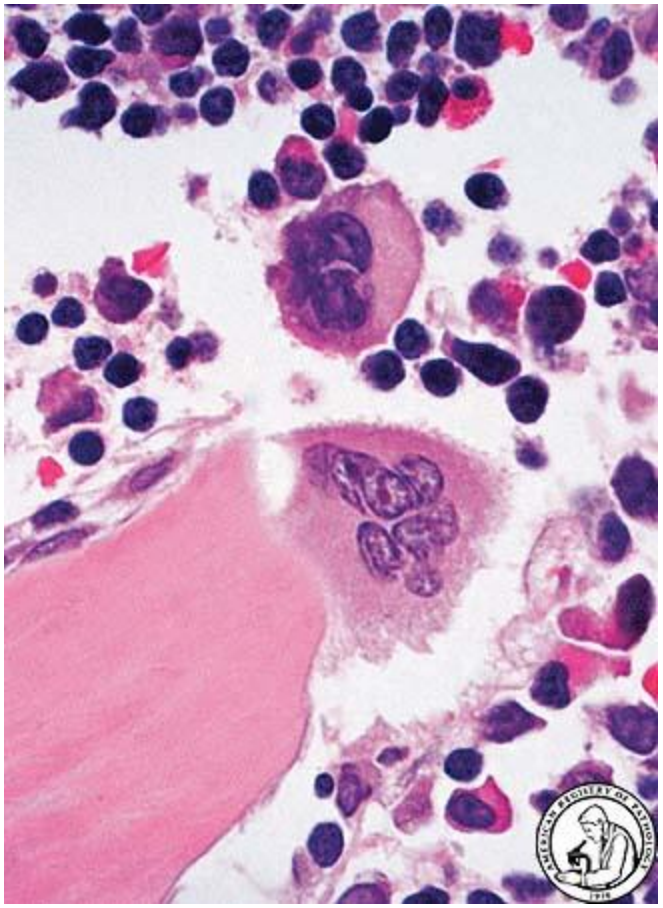
- TIA-1 IHC stain highlights cytoplasmic granules in a bone marrow core biopsy with increased large granular lymphocytes.



Bone Elements

3 elements:

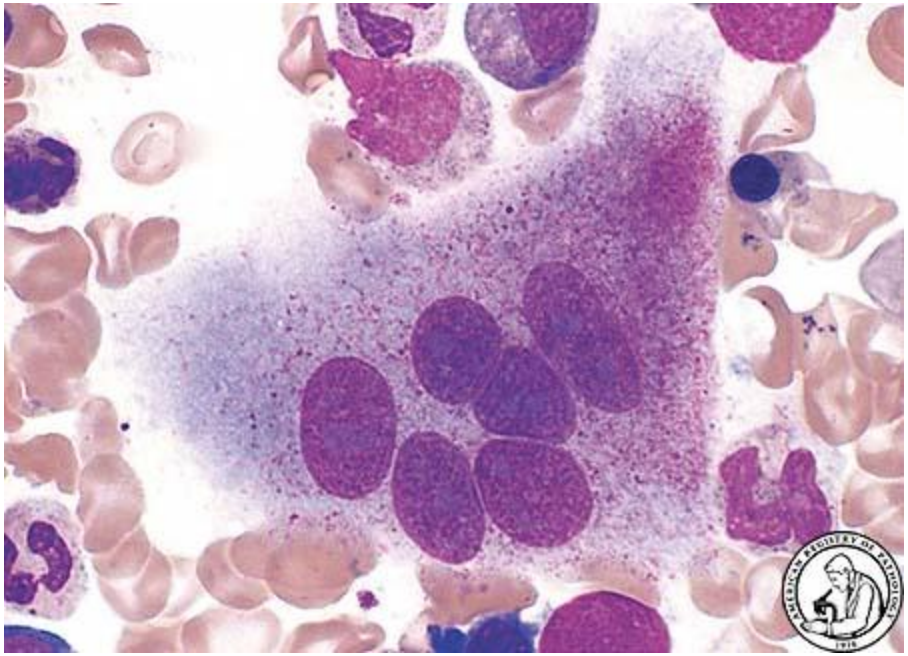
- Osteoblasts
- Osteoclasts
- Osteocytes
  - Osteoblasts assemble to form new bone in the lacunar spaces vacated by osteoclasts, a process called coupling



Bone Elements

Osteoclast

- Osteoclasts resorb bone and are derived from a common monocytic/ macrophage/ dendritic progenitor cell
- Monocytic cells differentiate into osteoclasts in the presence of M-CSF and RANKL

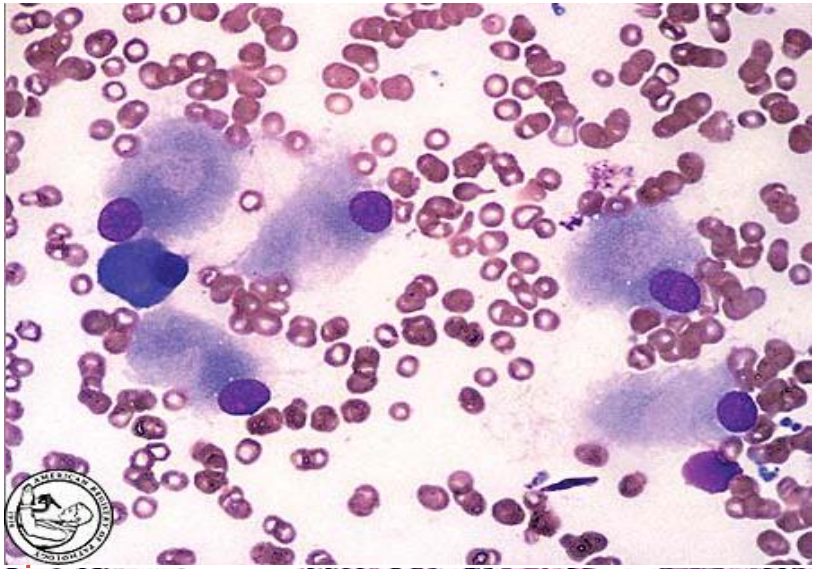


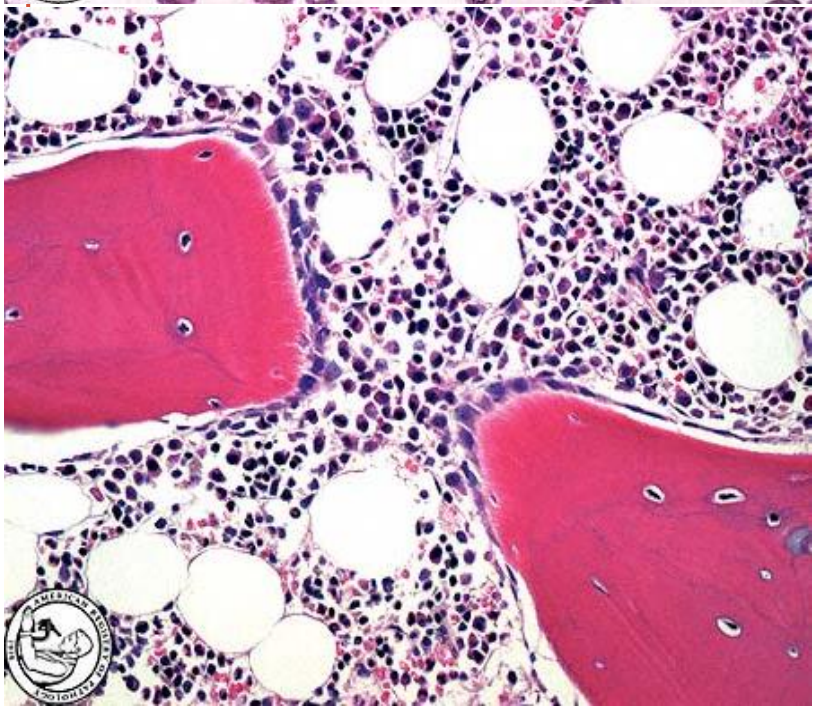
- Discrete nuclei, coarse cytoplasmic "bone sand" in mature cells, and paratrabecular localization often within scalloped spaces

Bone Elements

Osteoblast

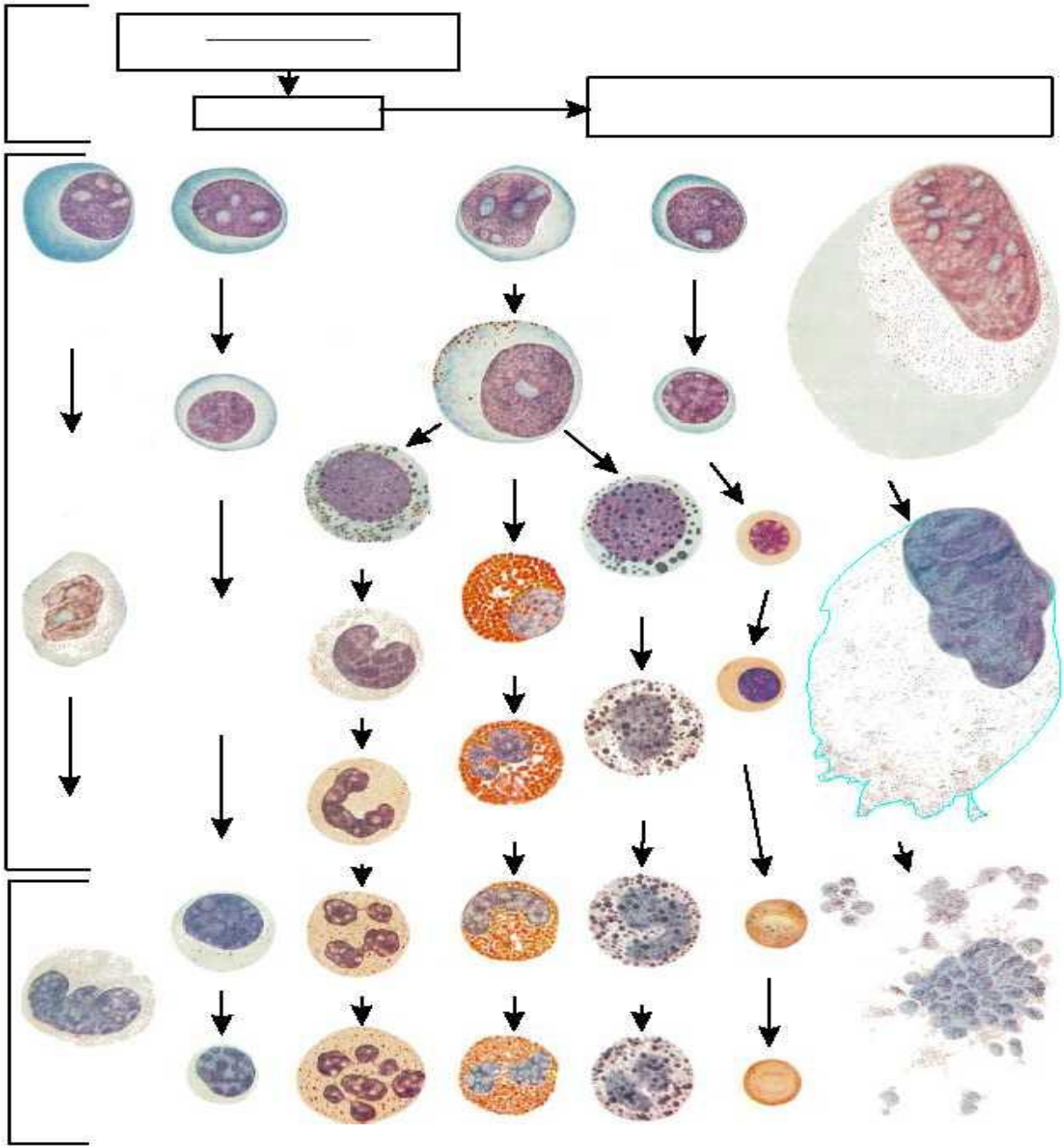
- Stromal-derived cells which produce the bony substrate
- Resemble enlarged plasma cells
- Osteoblasts also contain a cytoplasmic pale area (hof) which, unlike plasma cells, is separated from the nucleus
- If active bone remodeling is in progress, osteoblasts rim bony trabeculae in a single file





Age Related Changes

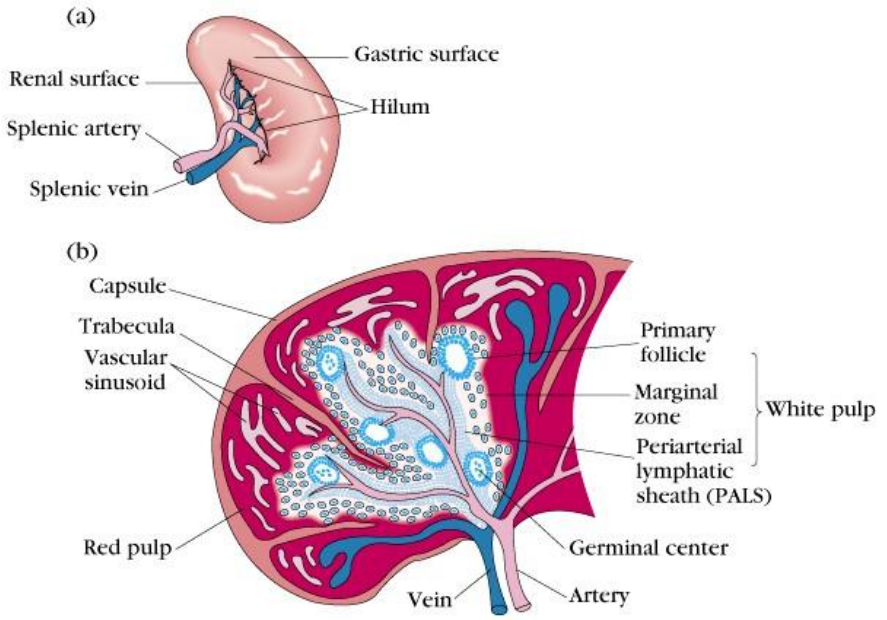
Patient Age	Cellularity	Bony Trabeculae	Cellular Composition
Newborn	Up to 100%, but may be lower	Very active bone remodeling and incomplete ossification of cortical bone	Blasts up to 5% Predominance of myeloid cells except in cases in which hematogones are numerous; myeloid to erythroid ratio of ~ 4:1 Lymphoid cells, notably hematogones, may be numerous
Infant	Variable, up to 100%, but may be lower	Very active bone remodeling and incomplete ossification of cortical bone	Blasts up to 5% Predominance of myeloid elements; myeloid to erythroid ratio: ~ 5-10:1 Erythroid elements markedly reduced during physiologic nadir Lymphoid cells, notably hematogones, may be abundant (up to 50% of cells)
Child	60-80%	Active bone remodeling	Blasts up to 5 percent, but usually lower Myeloid elements predominate; myeloid to erythroid ratio: ~ 3:1 Lymphocytes, notably hematogones, may be abundant
Young adult	50-70%	Bone remodeling may be evident, especially in young males	Blasts generally <5% Myeloid elements predominate; myeloid to erythroid ratio: ~ 3:1 Lymphocytes generally inconspicuous, but may range up to 20%
Adult	40-60%	Bone remodeling absent Osteoclasts and osteoblasts inconspicuous Bony trabeculae may be thinned (osteopenic), especially in females	Blasts generally <3% Myeloid elements predominate; myeloid to erythroid ratio: ~ 3-4:1 Lymphocytes usually inconspicuous, but may range up to 20%
Elderly	25-40%	Bone remodeling absent Osteoblasts and osteoclasts inconspicuous Bony trabeculae may be thinned (osteopenic), especially in females	Blasts <3% Myeloid elements predominate; myeloid to erythroid ratio: ~ 3-4:1 Mild dysplastic features may be noted Lymphocytes are generally inconspicuous, but may range up to 20%, especially if hematopoiesis is reduced. Lipogranulomas and lymphoid aggregates may be present



HISTOLOGY OF THE SPLEEN

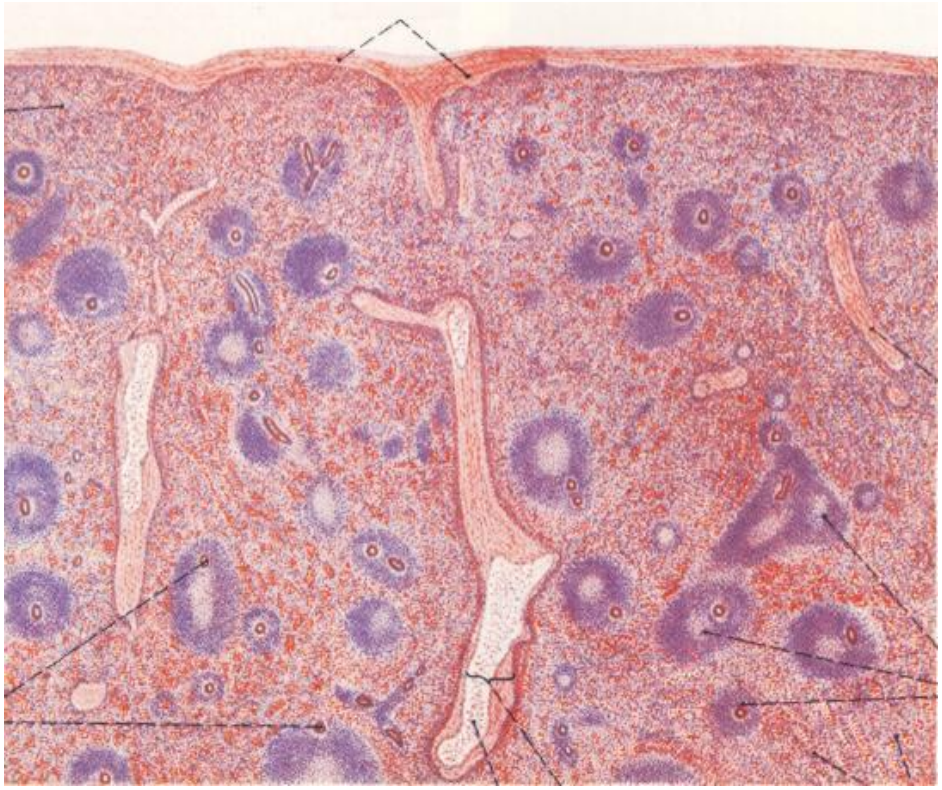
Gross Anatomy

- Normal weight 150 g
  - SD 25 g
- Hilus, where it is penetrated by vessels and nerves which follow the extensive branching network of fibrous trabeculae.
- Accessory spleens occur in about 10 percent of individuals
- Following traumatic rupture, small nodules of splenic tissue may grow on the peritoneal surface as implants (splenosis)



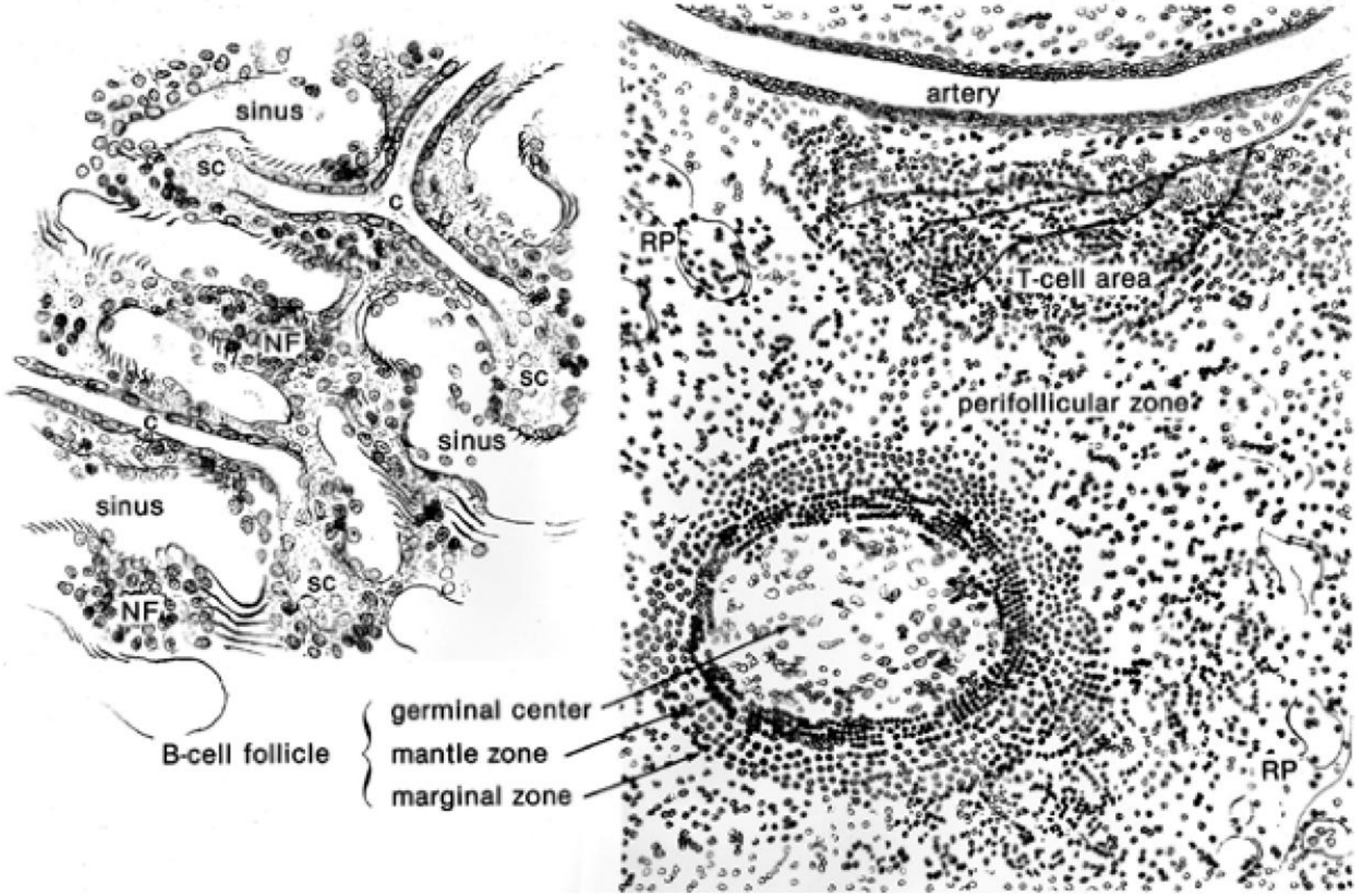
White Pulp

- Comprises the lymphoid compartment of the spleen and consists of both **follicular B-cell-rich areas** as well as **T-cell-rich periarteriolar** lymphoid sheaths.



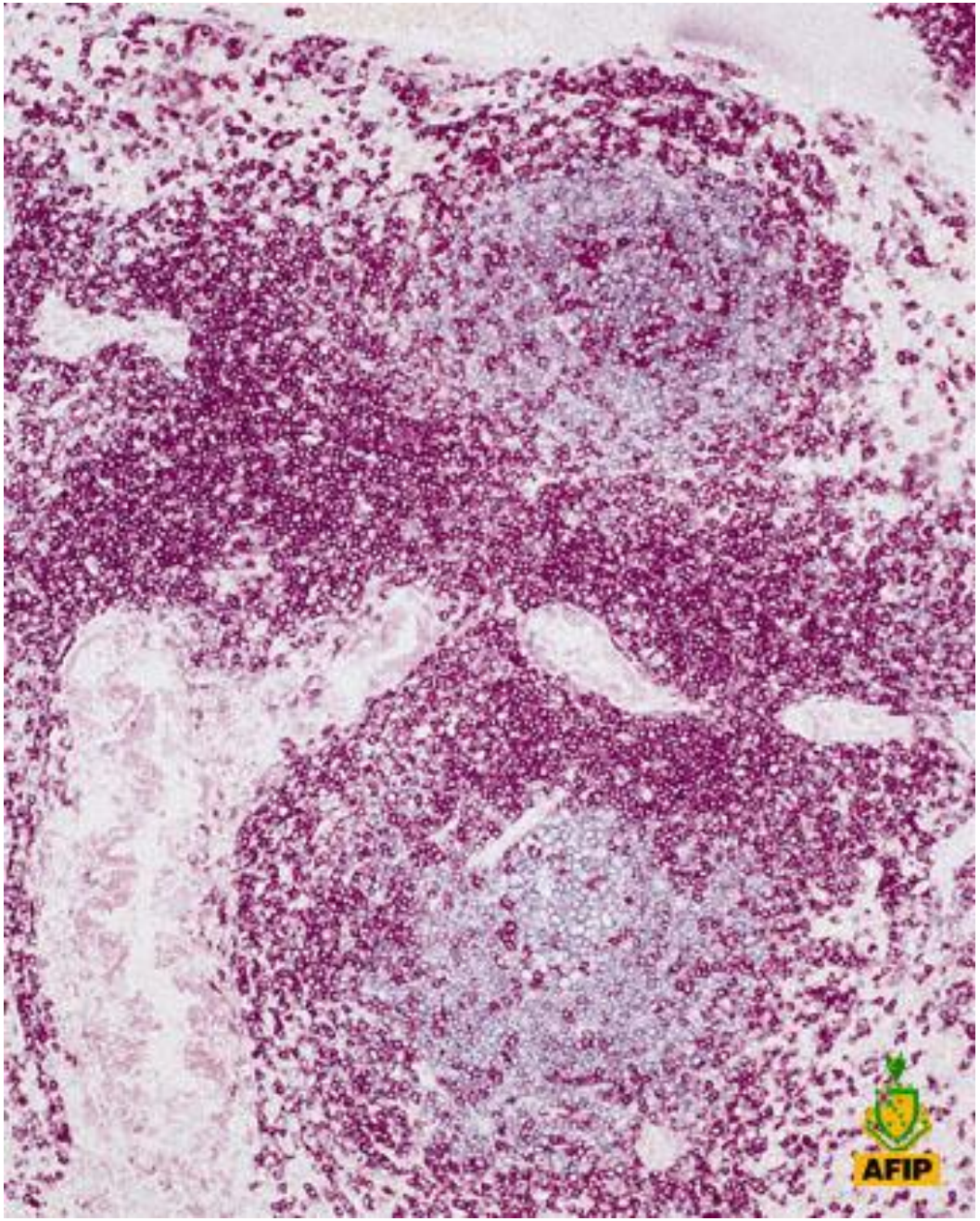
Periarteriolar T-Cell-Rich Lymphoid Sheaths

- Counterpart to the paracortical region of lymph nodes
- Lymphoid sheath, which surrounds splenic arteries as they exit the fibrous trabeculae


sinus
sc
c
NF
sc
sinus
c
sinus
sc
NF
B-cell follicle
germinal center
mantle zone
marginal zone
artery
RP
T-cell area
perifollicular zone
RP

Spleen: Periarteriolar area

The T cells predominate in the periarteriolar lymphoid sheath (labeled red with Leu-22/ CD43). The follicles, which tend to occur at arterial branch points, are labeled blue (L26/CD20).



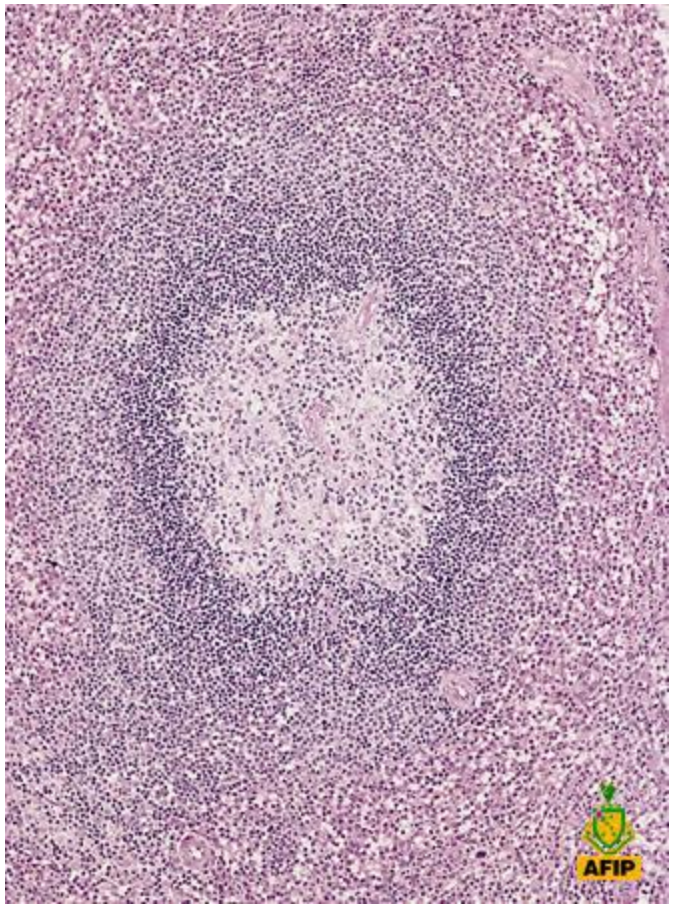
Primary and Secondary B-Cell Follicles

- Located at the periphery of the T zone and have the identical histologic and phenotypic features of primary and secondary follicles of lymph nodes

Primary and Secondary B-Cell Follicles

MARGINAL ZONE

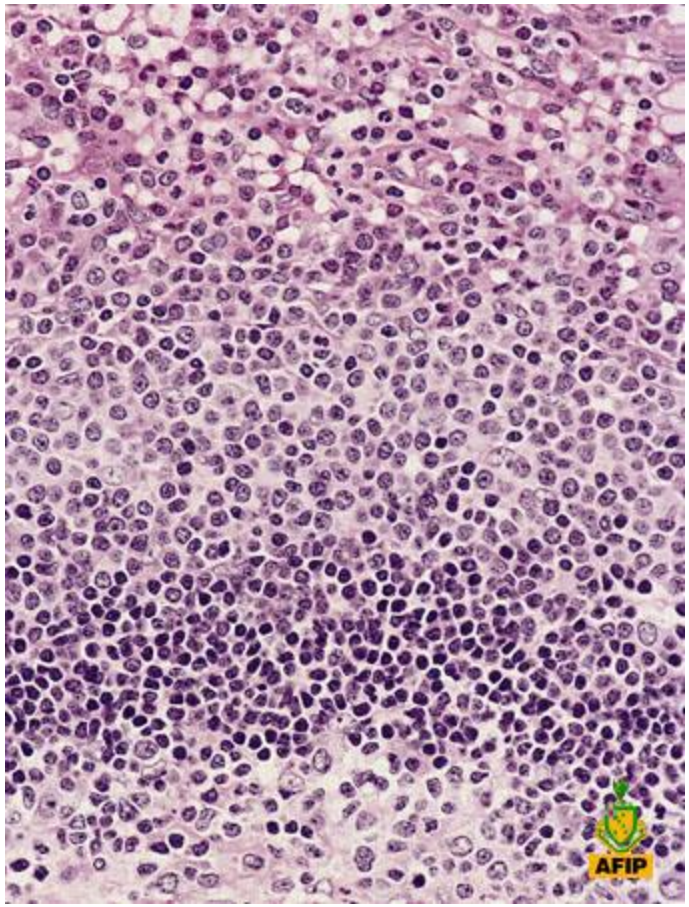
- Surrounds the primary follicle and the mantle zone of secondary follicles
- Consists of a corona of medium-sized lymphoid cells with prominent pale cytoplasm



Primary and Secondary B-Cell Follicles

MARGINAL ZONE

- The nuclear chromatin of the intermediate-sized marginal zone cells is somewhat less condensed than that of small lymphocytes
- Admixed with a variable number of plasma cells, T cells, and macrophages



Red Pulp

- 4 vascular structures:
  - Slender and nonanastomosing **arterial vessels**
  - Reticular meshwork of thin plates of cellular tissue lying between the sinusoids comprising **splenic cords**
  - Large, thin-walled venous vessels called **sinusoids**
  -
  - **Pulp veins** which drain the sinusoids

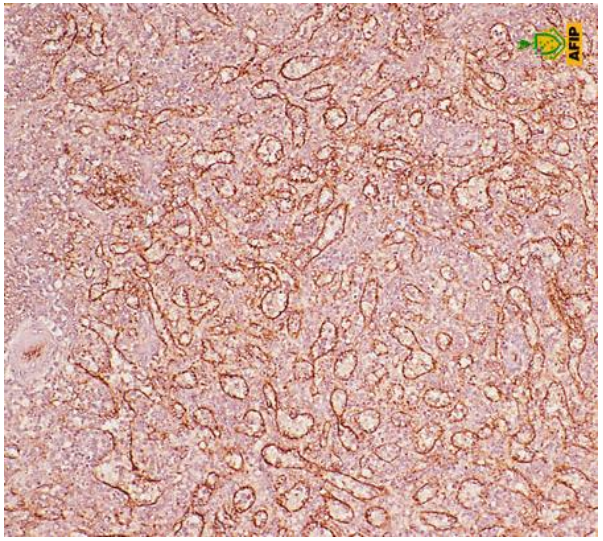
Cords

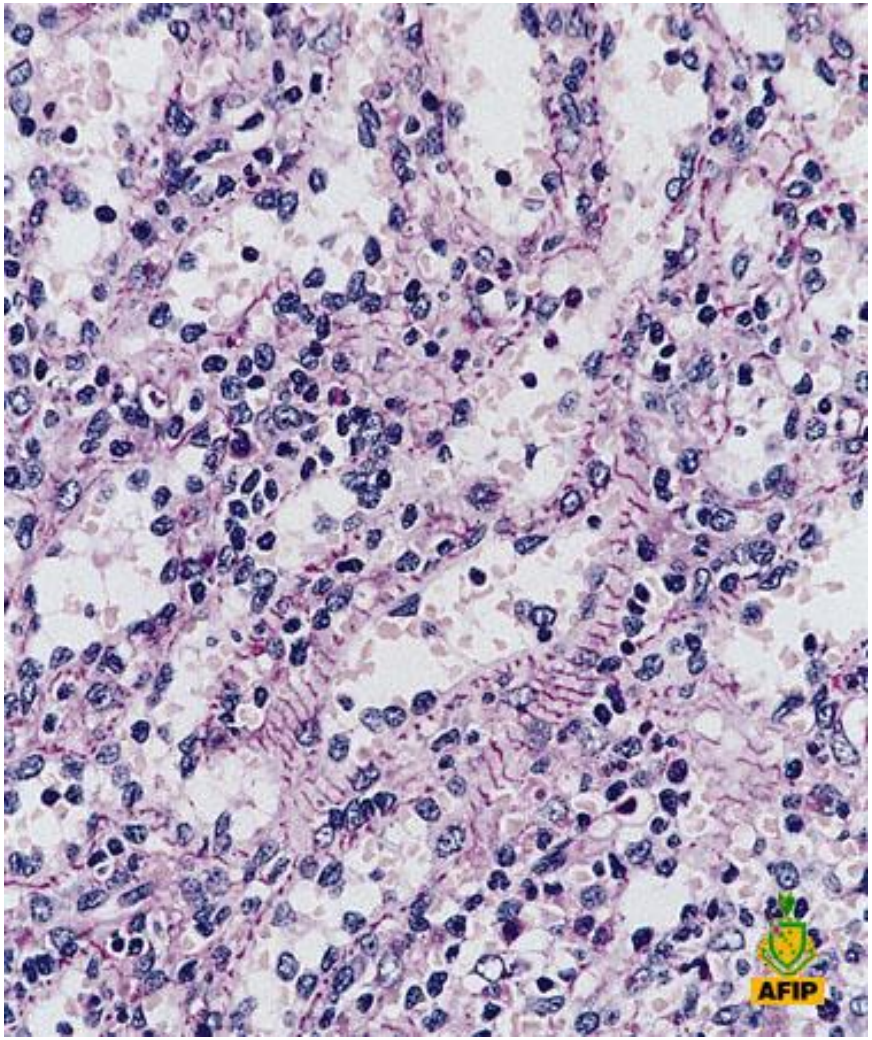
- Reticular meshwork consists of a branching system of cords lying between the sinuses
- Includes the reticular meshwork and may run through a sheath of macrophages with may run through a sheath of macrophages
- Clearance functions are also handled by marginal zone and red pulp macrophages

Sinusoids

- Are lined by tapered endothelial cells separated by slit-like spaces and surrounded by distinctive ring fibers and bridging fibers

- **Stain endothelial markers (FVIII) and CD8**

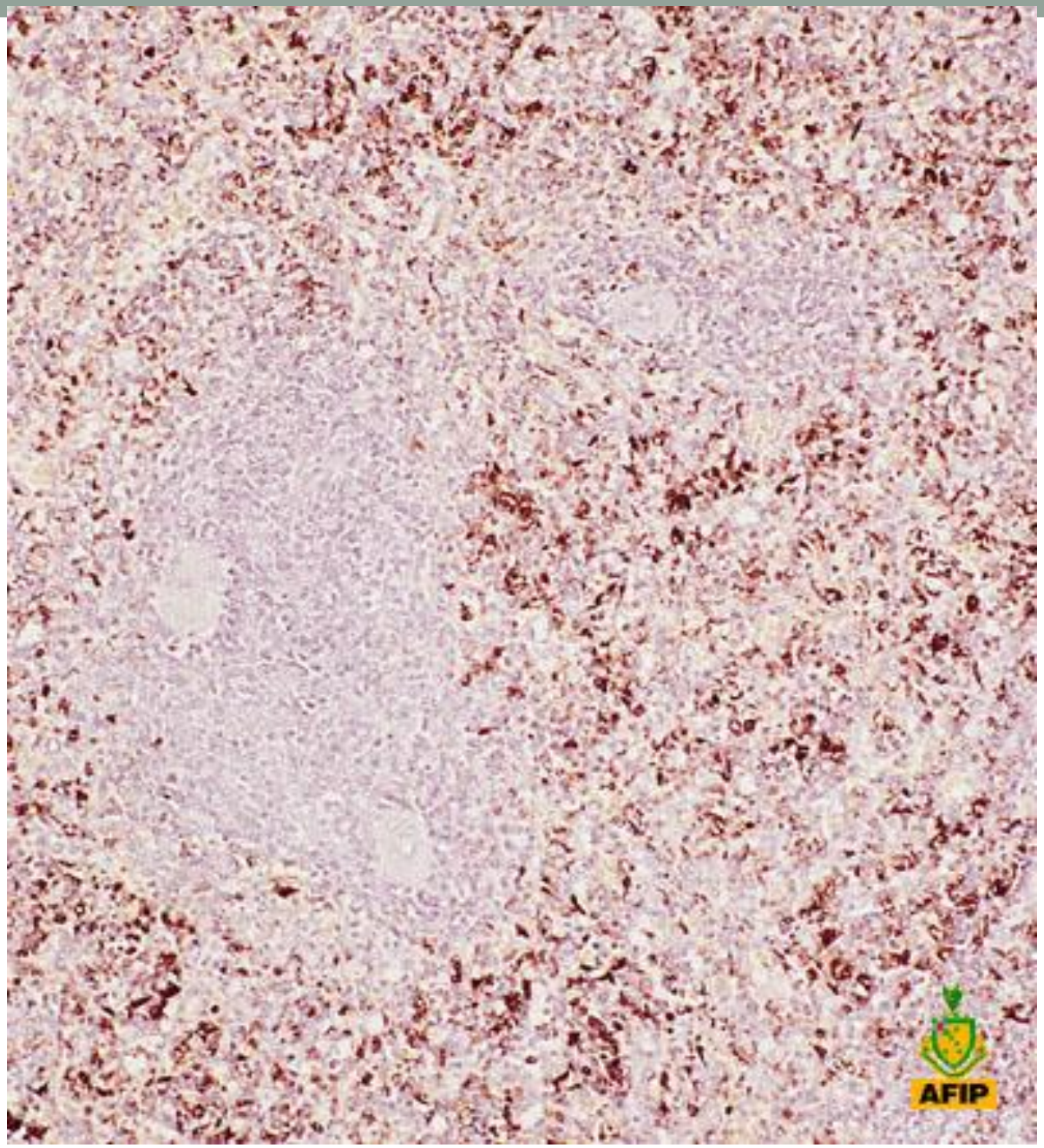




- PAS stain highlights the distinctive ring fibers and bridging fibers

Splenic macrophages

Macrophages are preferentially located in the marginal zone and red pulp cords of the spleen (labeled brown with KP-1/CD68).



Spleen, physiology

- Differentiation of reticulocytes, platelets, and monocytes
- Removal of abnormal erythrocytes
- Major site of antibody production, particularly in response to blood-borne antigens.

Useful References

Leukemias

- WHO 2008
- For a single author perspective:
  - Bone marrow -> Dr. Foucar or Dr. Bain books
  - Online free AFIP book:
  - https://www1.askafip.org/portal/page?_pageid=33,319226&_dad=portal&_schema=PORTAL&pAction=PREVIEW_PAGE&pBook=3F14&pPage=8

Lymphomas

- WHO 2008
- For an algorithmic approach → http://www.uscap.org/newindex.htm?98th/education.htm
- For benign lymph node →Ioachim's Lymph node pathology

Based on…

- AFIP Atlas of non-tumor pathology – Bone marrow by Dr. Foucar.