CLINICAL DIAGNOSIS AND MANAGEMENT BY LABORATORY METHODS

THE BONE MARROW EXAMINATION

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Bone marrow architecture

- The marrow is filled with a network of thin-walled sinusoids lined by a single layer of endothelial cells, underlain by a discontinuous basement membrane and adventitial cells.
- Within the interstitium lie clusters of hematopoietic cells and fat cells.
- Differentiated blood cells enter the circulation by transcellular migration through the endothelial cells.
- Normal megakaryocytes lie next to sinusoids and extend cytoplasmic processes that bud off into the bloodstream to produce platelets.

Bone marrow architecture

- Red cell precursors often surround macrophages (so-called nurse cells) that provide some of the iron needed for the synthesis of hemoglobin.
- Processes that distort the architecture may lead to the abnormal release of immature cells into the peripheral blood (leukoerythroblastosis).
- Carcinoma
- Granulomatous disease

Maturation of blood cells

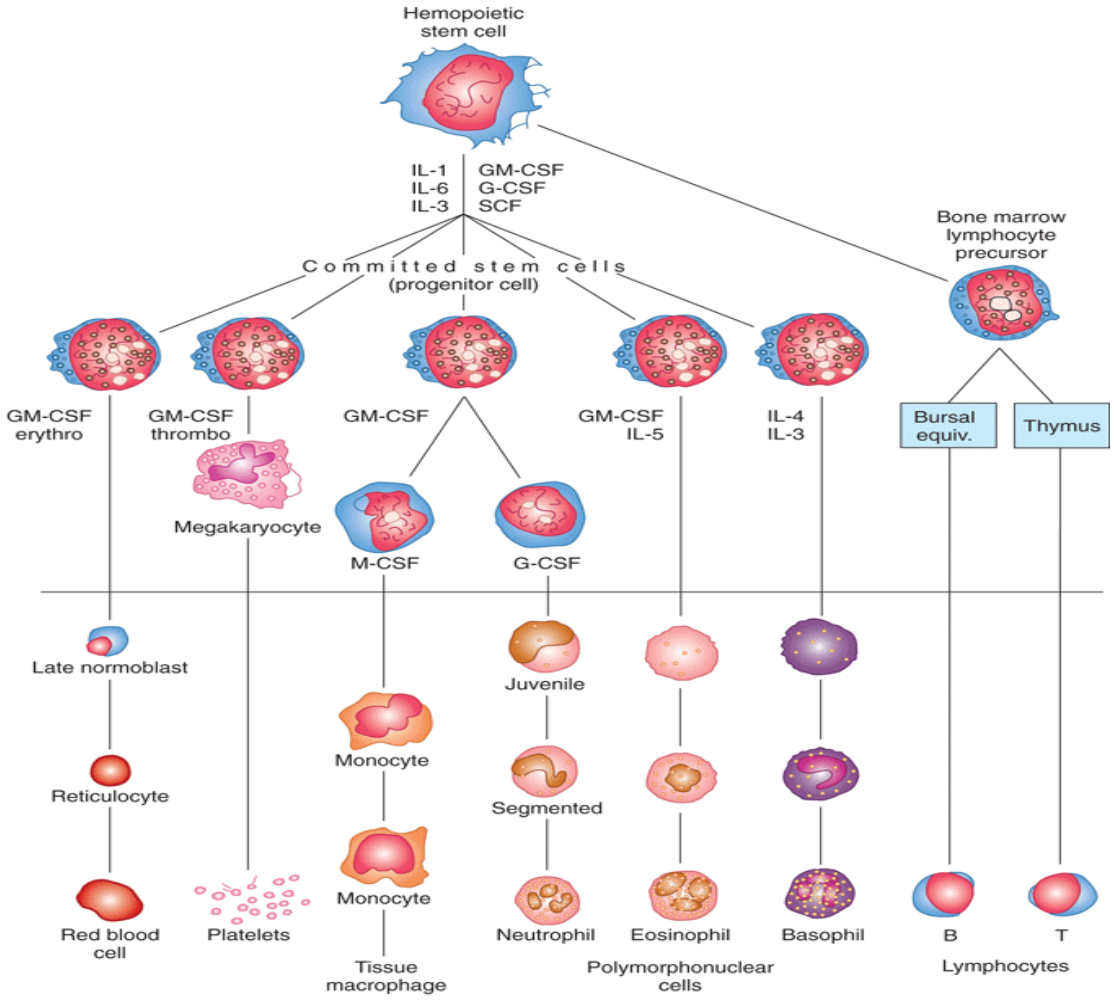
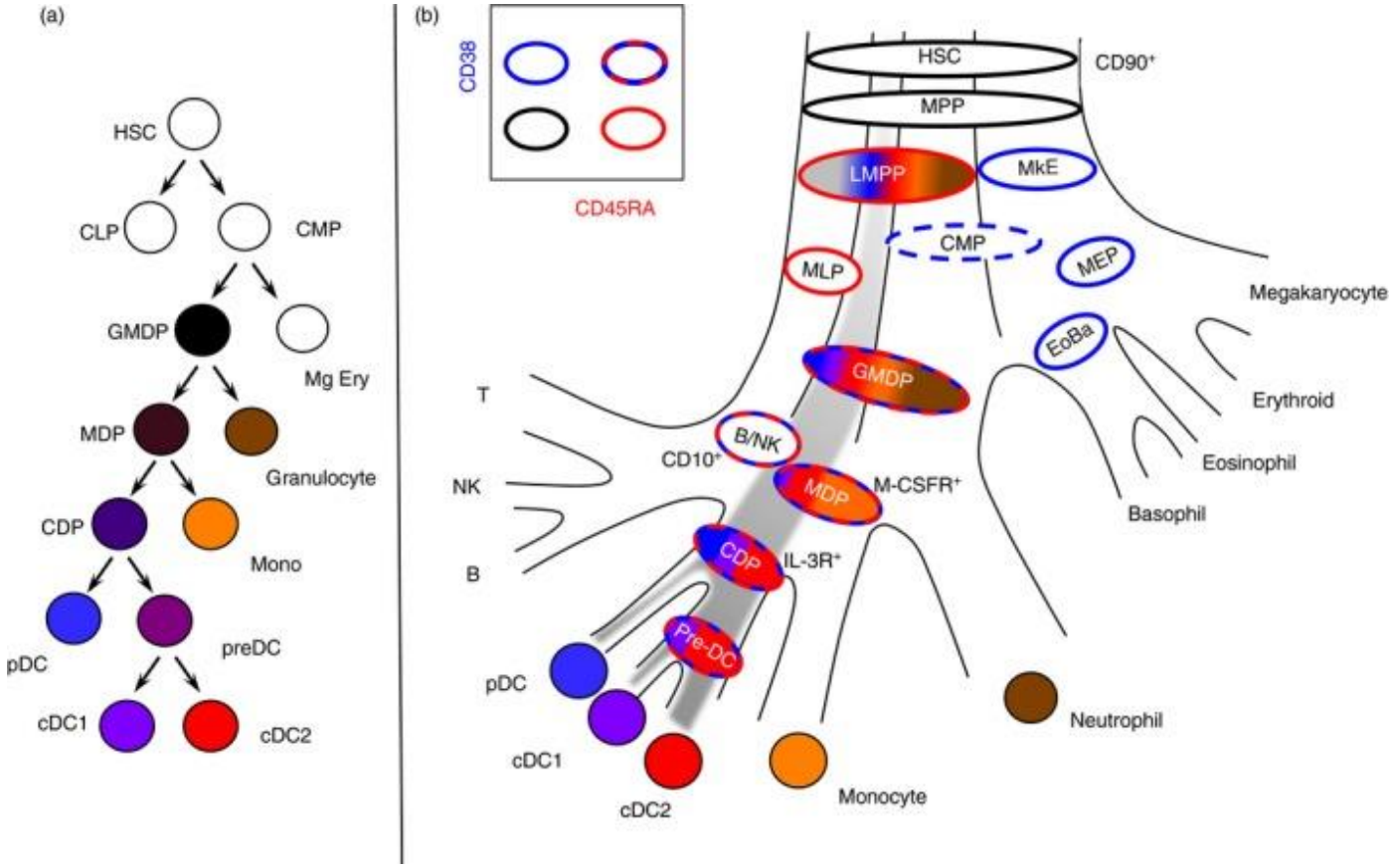


Fig. 32-3 Accessed 02/01/2010

Classic and revised models of hematopoiesis



Common myeloid progenitors are mixtures of mega-erythroid and myeloid precursors and the most significant early partitioning of cell fate occurs when megakaryocyte and erythroid potentials separate from lympho-myeloid potentials.

Doi: 10.1111/imm.12888

Bone marrow

- Erythroblasts are the very dark nuclei with scant cytoplasm.
- The paler staining larger cells with nuclei are developing neutrophils.
- Scattered cells with intensely reddish-orange cytoplasm are eosinophils.
- Lymphocytes and plasma cells are present in small numbers.

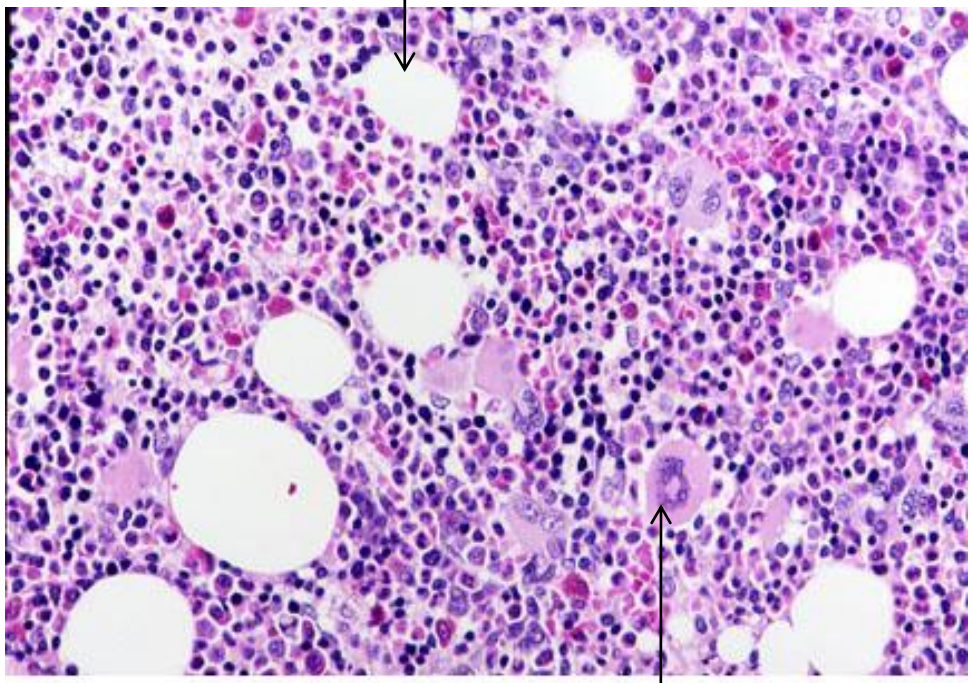


Fig. V.A.1 Accessed 02/01/2010

Clues from examination of the bone marrow

- Bone marrow cytology allows a quantitative assessment only in relative terms.
- In adults, normal marrow cellularity is 35–40%.
- The important ratio of red precursor cells to white cells is 1 : 2 for men and 1 : 3 for women.

Erythropoiesis

- Erythroblasts are the least mature cells.
- They are about three times the size of a mature red cell.
- The nuclear structure is dense. The cytoplasm is deep blue and homogeneous.
- The maturation of cells in the erythrocyte series is closely linked to the activity of macrophages which phagocytize nuclei expelled from normoblasts and iron from senescent erythrocytes, and pass these cell components on to developing erythrocytes.

Erythropoiesis

- The results of mitosis of erythroblasts are called normoblasts.
- There are two cell types with relatively dense round nuclei and grayish stained cytoplasm.
- (1) The polychromatic normoblasts are immature cells with a blue-gray cytoplasm.
- They are still able to divide.

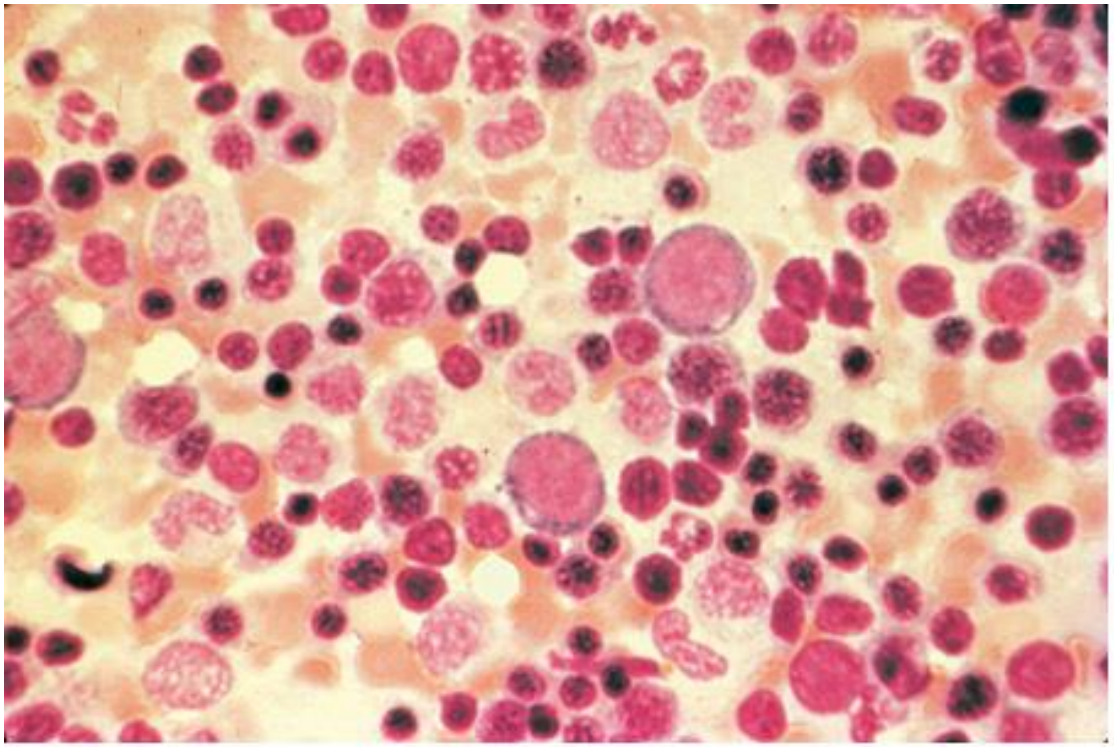
Erythropoiesis

- (2) The orthochromatic erythroblasts are immature cells with a pink cytoplasm.
- They contain hemoglobin.
- They are no longer able to divide.
- The nuclei of these gradually condense and are expelled from the cells.
- Reticulocytes are the resulting erythrocytes that possess no nucleus but contain copious ribosomes in a pink cytoplasm that precipitate into reticular [net-like] structures identified with supravital stains.

Erythropoiesis

- RBC progenitors express receptors for inflammatory cytokines which are necessary for normal differentiation to polychromatophilic normoblast
- Fas/TRAILR/TNFR, IFN-γ
- Fas/FasL/TRAIL/TNF-α are expressed during maturation from orthochromic normoblast.
- In the absence of erythopoietin, these cytokines promote erythroid cell death.
- HIF-1α, the transcription factor responsible for EPO (erythropoietin) gene transcription, is stimulated by iron deficiency and hypoxemia.

Erythroid hyperplasia



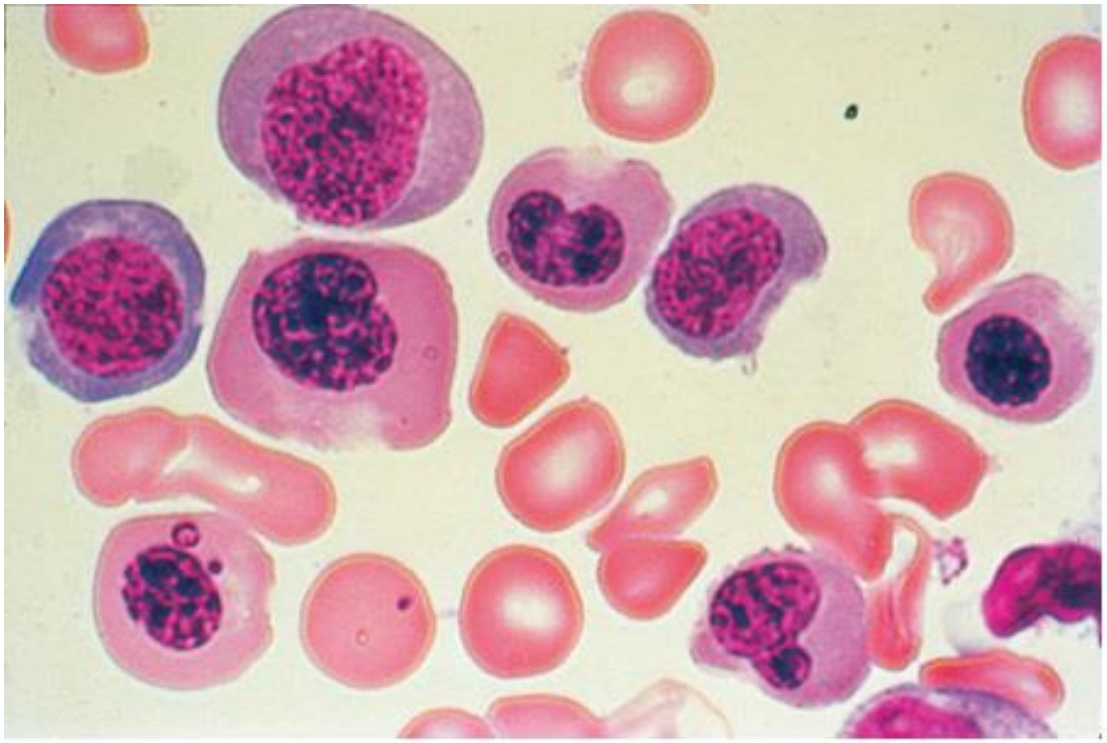
The myeloid/erythroid ratio (M/E ratio) of 1:1–2 is typical for a patient with an hemolytic anemia or recovering from blood loss.

Figs. E11-35 Accessed 02/01/2010

Clues from examination of the bone marrow

- Shifts towards erythropoiesis are seen in all regenerative anemias.
- A left shift in the erythroid series is seen in regenerative anemias except hemolysis.
- A right shift in the erythroid series is seen in hemolytic conditions
- Nests of normoblasts in marrow
- Atypical proerythroblasts predominate in megaloblastic anemia and erythremia.

Megaloblastic erythropoiesis



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Fig. e11-37 Accessed 02/01/2010

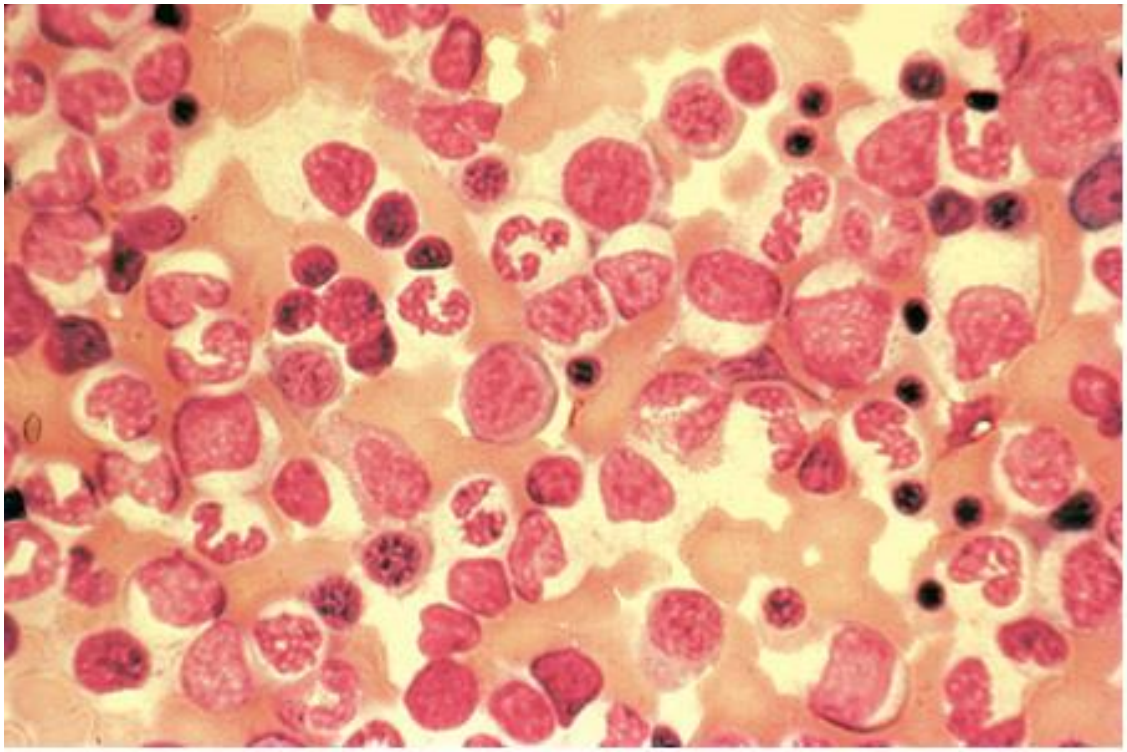
Megaloblastic red blood cell precursors from a patient with a macrocytic anemia.

Maturation is delayed with late normoblasts showing a more immature appearing nucleus with a lattice-like pattern but with normal cytoplasmic maturation.

Clues from examination of the bone marrow

- Shifts toward granulopoiesis are seen in all reactive processes and in all malignant processes of the white cell series.
- A left shift in the granulocyte series is observed in all reactive processes and at the start of neoplastic transformation.
- In acute leukemias, undifferentiated and partially matured blasts may predominate.
- In agranulocytosis, promyelocytes are most abundant.
- A right shift is diagnostically irrelevant.

Myeloid hyperplasia



The myeloid/ erythroid ratio of 3:1 suggests either a loss of red blood cell precursors or an expansion of myeloid elements.

Fig. e11-36 Accessed 02/01/2010

Myeloblasts

- Myeloblasts are the least mature cells in the granulocyte lineage.
- Mononuclear, round-to-ovoid cells
- Distinguished from proerythroblasts by the finer, “grainy” reticular structure of their nuclei and the faintly basophilic cytoplasm.

Promyelocytes

- Promyelocytes usually grow larger than their progenitor cells, the myeloblasts.
- Nuclei show an increasingly coarse chromatin structure. The nucleus is eccentric, with a the lighter zone over its indentation that corresponds to the Golgi apparatus.
- The basophilic cytoplasm contains many large azurophilic granules containing peroxidases, hydrolases, and other enzymes.
- Stain blue-red.

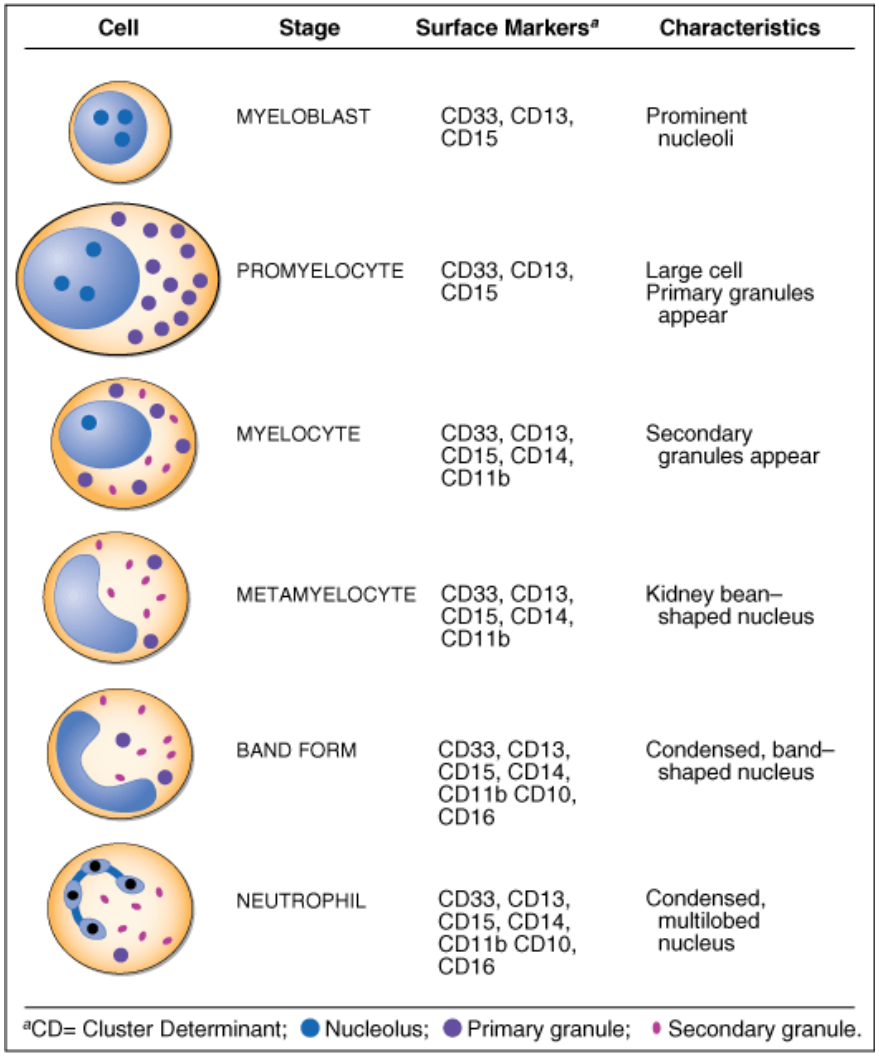
Myelocytes

- Myelocytes are always clearly smaller than their progenitors, the promyelocytes.
- Ovoid nuclei have a banded structure.
- Light blue cytoplasm which may acquire a pink tinge.
- Secondary granules appear and are evenly distributed throughout the cytoplasm.
- They do not stain red.
- They are peroxidase negative.

Metamyelocytes

- Are the product of the final myelocyte division.
- Unable to divide.
- Further nuclear maturation occurs with nuclear contraction and segmentation.
- The shape of the nuclei resemble that of a kidney bean.

Myelocyte maturation



Cell	Stage	Surface Markers[a]	Characteristics
	MYELOBLAST	CD33, CD13, CD15	Prominent nucleoli
	PROMYELOCYTE	CD33, CD13, CD15	Large cell Primary granules appear
	MYELOCYTE	CD33, CD13, CD15, CD14, CD11b	Secondary granules appear
	METAMYELOCYTE	CD33, CD13, CD15, CD14, CD11b	Kidney bean–shaped nucleus
	BAND FORM	CD33, CD13, CD15, CD14, CD11b CD10, CD16	Condensed, band–shaped nucleus
	NEUTROPHIL	CD33, CD13, CD15, CD14, CD11b CD10, CD16	Condensed, multilobed nucleus

[a]CD= Cluster Determinant; Nucleolus; Primary granule; Secondary granule.

Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Fig. 61-2
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Eosinophils

- Eosinophils arise from the same stem cell population as neutrophils and mature in parallel with them.
- The promyelocyte stage is the earliest time at which eosinophils can be morphologically defined in the bone marrow.
- Contain large granules that stain blue–red.
- At the metamyelocyte stage these granules begin to fill the cytoplasm.
- They are round, golden-red granules.
- The nuclei of mature eosinophils usually have only two segments.

Basophils

- Basophils mature in parallel with cells of the neutrophil lineage.
- The earliest stage at which they can be identified is the promyelocyte stage
- Large, black–violet stained granules are visible.
- In mature basophils, which are relatively small, these granules often overlie the two compact nuclear segments.
- Tissue basophils have a round nucleus underneath large basophilic granules.
- They are not found in blood.

Monocytes

- Arise early in the myeloid series.
- Do not demonstrate specific precursors that can be identified morphologically without special staining techniques.
- Irregular, ovoid nucleus, with invaginations and often pseudopodia-like cytoplasmic processes. The nuclear chromatin is fine.
- The gray cytoplasm varies in width, and contains a scattered population of very fine reddish granules.

Clues from examination of the bone marrow

- Plasma cell counts are also slightly elevated in reactive processes and very elevated in plasmacytoma.
- Reactive increase of lymphocytes and plasma cells with concomitant low counts in the other series is often an indication of panmyelopathy [aplastic anemia].
- Raised eosinophil counts in bone marrow have the same diagnostic significance as in blood: hypersensitivity, helminthic disease.
- Monocytes comprising more than 7% of the marrow points to myelomonocytic leukemia.

Plasma cell

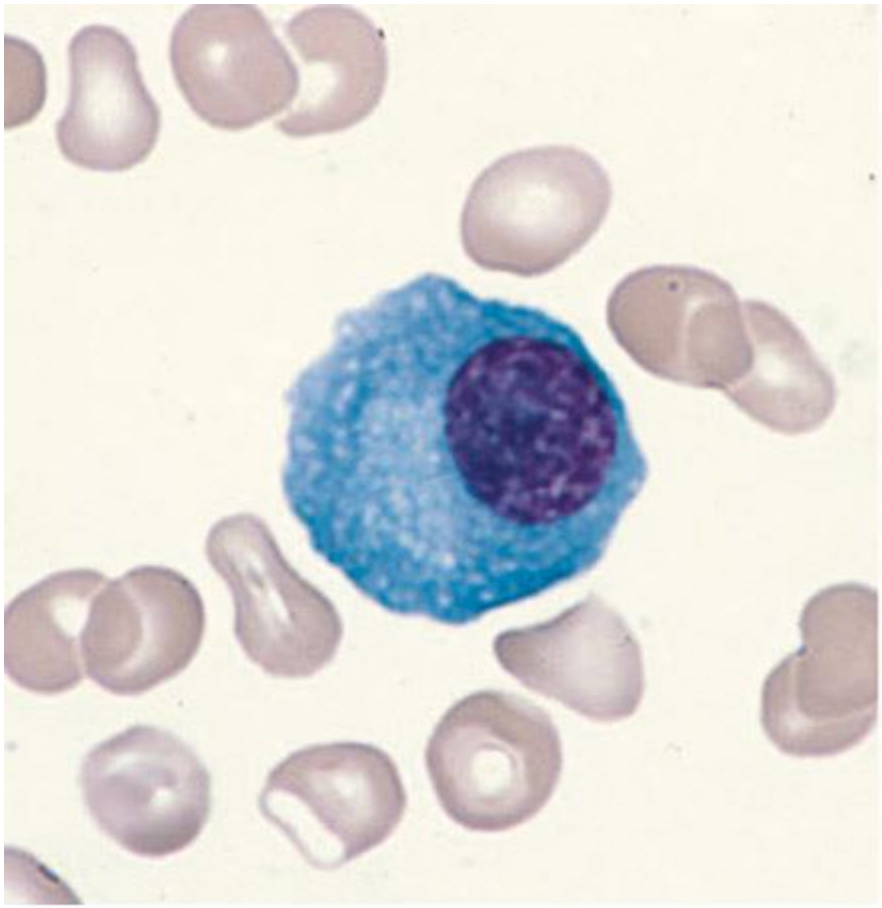


Fig. e11-55 Accessed 02/01/2010

Clues from examination of the bone marrow

- Megakaryocyte counts are reduced under the effects of all toxic stimuli on bone marrow.
- Counts increase in iron deficiency anemia, parathyroid disease, after bleeding, in essential thrombocytopenia, and in myeloproliferative disease.

Megakaryocytes

- Megakaryocytes have giant, extremely hyperploid nuclei
- 16 times the normal number of chromosome sets on average
- Built up by endomitosis.
- Thrombopoietin regulates the increase of megakaryocytes and the release of thrombocytes.
- Cytoplasm with granules is pinched off from megakaryocytes to form thrombocytes.
- The residual naked megakaryocyte nuclei are phagocytosed.

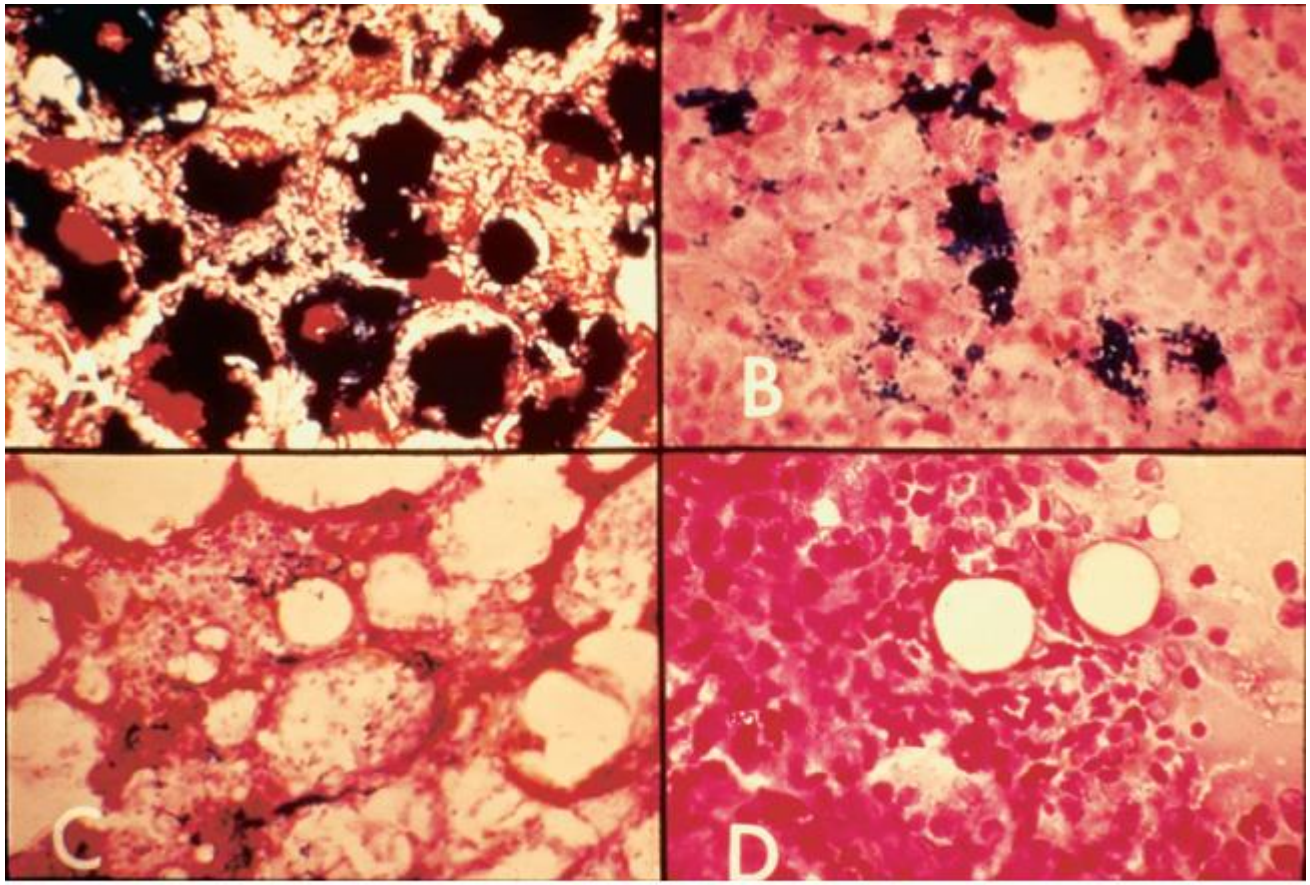
Megakaryoctyes

- Only mature thrombocytes occur in blood.
- Thrombocytes are about 1–4 μm in size and do not have a nucleus.
- Their light blue stained cytoplasm and its processes give them a star-like appearance, with fine reddish blue granules near the center.
- Abnormalities present before anemia in megaloblastic disorders.

Clues from examination of the bone marrow

- Under exogenous iron deficiency conditions the proportion of sideroblasts and iron-storing macrophages is reduced.
- However, if the shift in iron utilization is due to infectious and/or toxic conditions, the iron content in normoblasts is reduced while the macrophages are loaded with iron to the point of saturation.
- In hemolytic conditions, the iron content of normoblasts is normal
- It is elevated only in essential or symptomatic refractory anemia, including megaloblastic anemia.

Iron stores



Iron stores can be graded on a scale of 0 to 4+.
A: a marrow with excess iron stores (>4+)
B: normal stores (2–3+)
C: minimal stores (1+)
D: absent iron stores (0).

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Iron stores

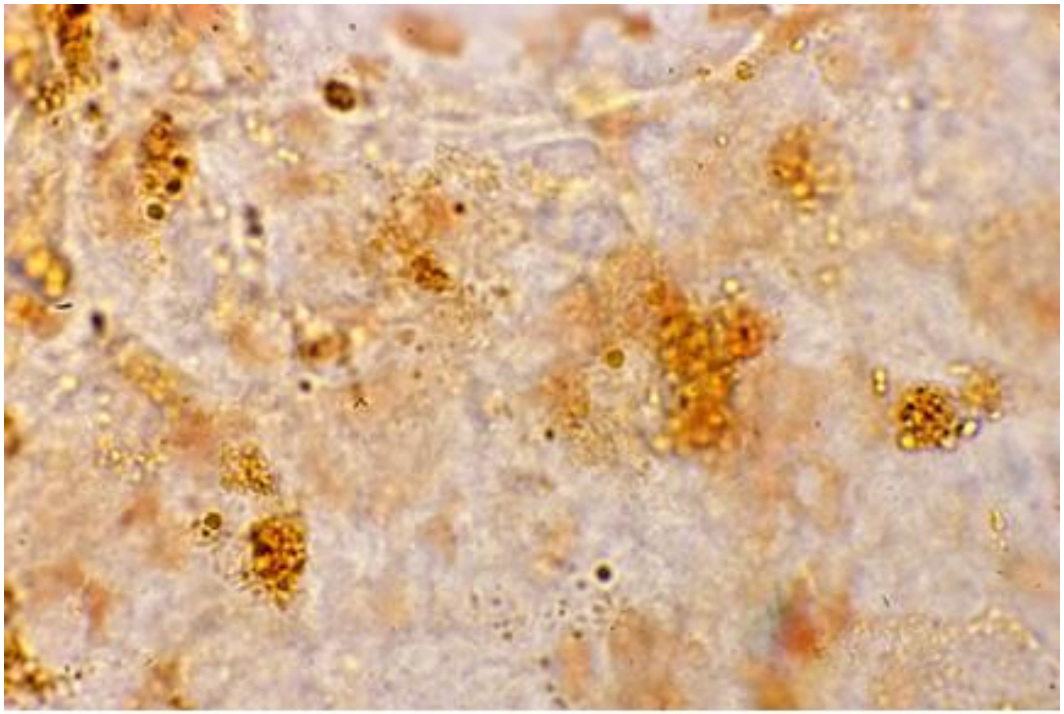

Fig. V.B.3 Accessed 02/01/2010

Hemosiderin containing macrophages