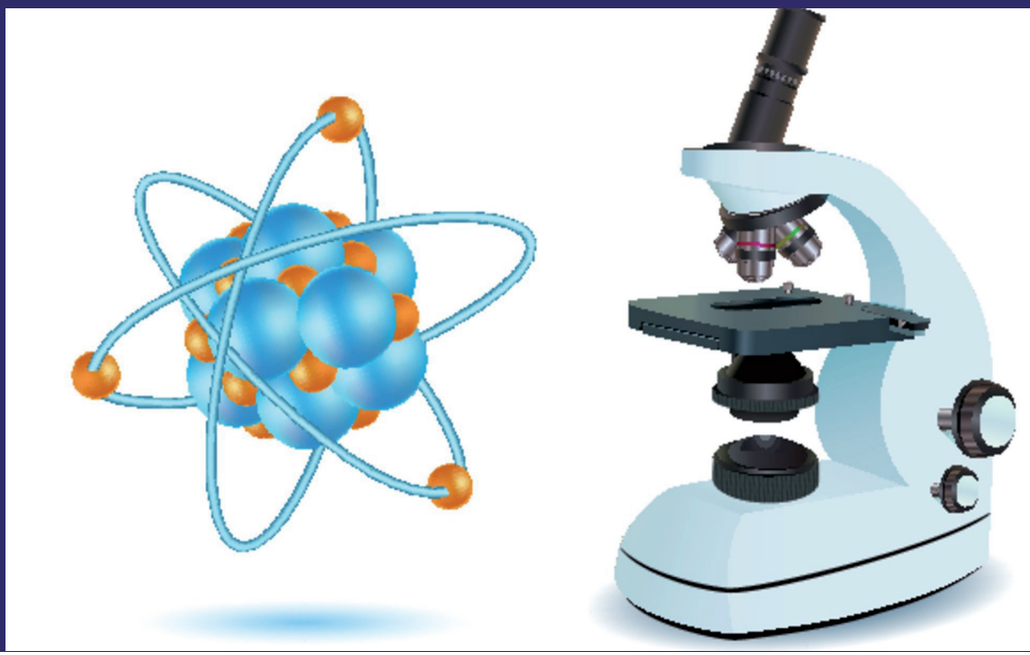
White blood cells (WBCs, leukocytes) are nucleated cells produced from bone marrow; they have a role in body immunity. They form the first line of defense of the body against invading microorganisms. White blood cells are classified either as polymorphonuclear leucocytes (or granulocytes) or as mononuclear cells. Granulocytes are further divided into three subtypes, Neutrophils, eosinophils, and basophils. Mononuclear cells indicate both lymphocytes and monocytes. The Book reviews the production, morphology, distribution and kinetics, functions, non-neoplastic disorders, and inherited and acquired abnormalities of the white blood cells. An attempt is made to provide the reader with a working knowledge of the white blood cells in contemporary hematology. This book is designed to generate an overall knowledge with the WBCs for hematologists, practitioners, educators and students to accomplish an excellent overview of the topic.



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Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes

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Characteristics of White blood cells (leucocytes)

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خصائص كريات الدم البيضاء

الدكتور محمد وائل دعبول

أخصائي في التشخيص المخبري


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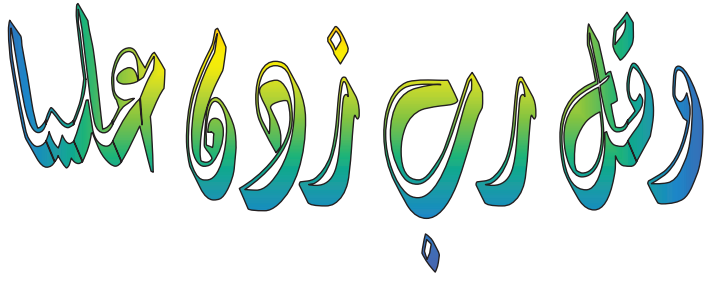
وقل رب زدني علما

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Characteristics of White blood cells (leucocytes)

INTRODUCTION:

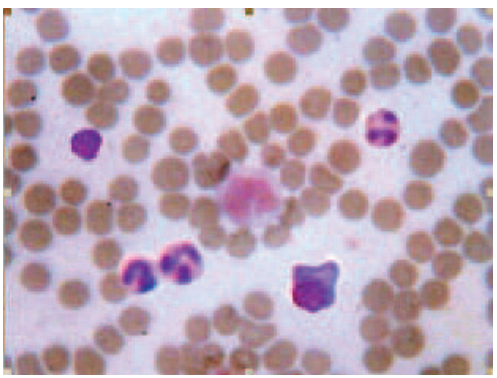
The white blood cells, or leucocytes, constitute only 1% of the total blood volume. They originate in the bone marrow and circulate throughout the lymphoid tissues of the body. There they function in the inflammatory and immune processes. Normal peripheral blood leucocytes are classified either as polymorphonuclear leucocytes or as mononuclear cells, the latter term indicating lymphocytes and monocytes. Polymorphonuclear leucocytes are also referred to as polymorphonucleargranulocytes, polymorphs or granulocytes (1). This Book reviews the production, morphology, distribution and kinetics, functions, non-neoplastic disorders, and inherited and acquired abnormalities of white blood cells. The Book will also be supplemented with Blood films in healthy and non-healthy subjects. A practical and applied approach to the subject matter, with illustrations, is incorporated in an attempt to provide the reader with a working knowledge of the white blood cells in contemporary hematology. A discussion of normal white cell physiology is introduced in this Book, which defines qualitative and quantitative white blood cell disorders and other hematologic diseases leading to abnormal white cell morphology. In conclusion, this book is designed to generate an overall knowledge with the WBCs for hematologists, practitioners, educators and students to accomplish an excellent overview of the topic.

White blood cells:

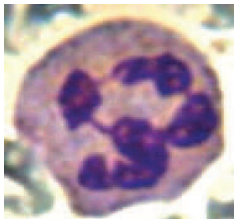
Called as that because it is after centrifugation present in buffy coat, which have a white color. White blood cells (WBCs, leukocytes) are nucleated cells produced from bone marrow; they have a role in body immunity. They form the first line of defense of the body against invading microorganisms (2)

(figure.1). Normal white cells readily identified in peripheral blood smears, are divided into mononuclear cells and polymorphonuclear leucocytes (or granulocytes). Granulocytes are further divided into three subtypes, Neutrophils (figure. 2), eosinophils (figure. 3), and basophils (figure. 4). All cells of this group (neutrophils, eosinophils and basophils) play an essential role in inflammation. They are primarily phagocytes and, with lymphocytes, antibodies and complement, are responsible for the defense against microorganisms. Neutrophils and monocytes (figure. 5) respond by phagocytosis; lymphocytes (figure. 6) and plasma cells primarily produce antibodies.

In addition to a nonspecific response to bacterial or viral infection, there are alterations in the normal WBCs blood picture (figure. 7) that may provide diagnostic clues to specific diseases, both benign and malignant. Non-neoplastic WBCs alterations may be quantitative, qualitative, or both; qualitatively, WBCs may demonstrate an increased degree of immaturity, morphologic alteration in cellular structure, or the increased production of less common types of WBCs.

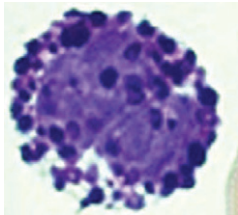


(Figure.1) Cells of a peripheral blood smear stained with Wright stain. Note the neutrophils with different lobes, the monocyte with hazy cytoplasm, the lymphocyte with high cytoplasmic/nuclear ratio and the reactive lymphocyte down right. (Mag. X 400)



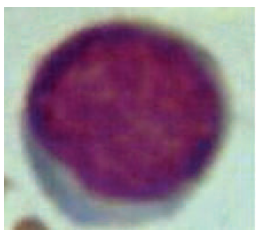
(Figure. 2) Segmented neutrophil with five lobes nucleus and a faint pink cytoplasm. (Wright stain) (Mag. X 1000)

(Figure.3) Eosinophil with a couple of lobes. Note the spherical eosinophil granules. (Wright stain) (Mag. X 1000)



(Figure. 4) Basophil. Note the large dark basophilic granules. (Wright stain) (Mag. X 1000)

(Figure. 5) Monocyte. Note the opaque grayish-blue cytoplasm and the nuclear indentation. (Wright stain) (Mag. X 1000)



(Figure. 6) Lymphocyte. Note the dense nuclear chromatin with a large nuclear / cytoplasmic ratio. (Wright stain) (Mag. X 1000)

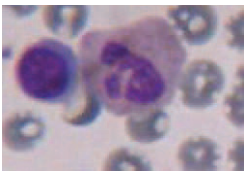
Type	*Count in adults* Average Range /mm3	Normal /mm3	*Percentage*	*Physiologic role*
Segmented granulocytes (segmented neutrophils)	3500	2000-7250	50-70	Migrate into tissue for the purpose of Foreign microorganisms Phagocytosis
Eosinophils	175	0-400	1-4	Immune defense against parasites,

				immune regulation
Basophils	50	0- 90	0-1	Regulation of the response to local inflammatory processes
Monocytes	500	100-900	2-8	Phagocytosis of bacteria, protozoa, fungi, foreign bodies. Transformation in target tissue
Lymphocytes T- and B-lymphocytes	2500	1500-3500	20-50	T-lymphocytes (70%): cytotoxic defense against viruses, foreign antigens, and tumors. B-lymphocytes (20%) antibody production.

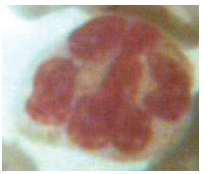
Table**1** peripheral blood cells count, percentage and physiological role

Differential cell counting is the enumeration and classification of the leukocytes seen on the blood smear. The usual procedure is to count at least 100 consecutive leukocytes in an area of good cell distribution. A uniformly thin smear of blood on a cover glass is the best preparation for such examination. As the total number of cells enumerated increases, the accuracy of the differential count is greater. Most changes in the white blood cell count are due to an increase or decrease of cells of the myeloid series. The term shift to the left in neutrophilic white blood cells, indicates an increase in the proportion of cells with only one or few lobes, whereas shift to the right represents an increase in the proportion of multisegmented forms. A right shift is said to be present if the

average lobe count is increased or if there is an increased percentage of neutrophils with five or six lobes (3) (figure 8). Normal values for absolute leukocyte concentrations are obtained by using a Coulter or similar automated counters (Table 1).



(Figure. 7) A normal WBC blood picture. Note the neutrophil accompanied with a Lymphocyte. (Wright stain) (Mag. X 1000)



(Figure. 8) A hypersegmented neutrophil with six nuclear lobes. (Wright stain) (Mag. X 1000)

A great deal of information is achieved if the blood is carefully mixed and a thin layer is spread on a glass slide to form a blood film. The blood cells are then preserved by exposure to the alcohol methanol, a process known as fixation. The fixed film of blood is stained with a mixture of several dyes so that the individual cells can be recognized when they are examined with a microscope. Two of the commonest mixtures of dyes used to stain the peripheral blood smear in daily ordinary hematologic work are the May–Grünwald–Giemsa (MGG) stain, named after its inventors, and the Wright stain. After staining, the colour of red cells is enhanced and the white cells and platelets, which would otherwise be transparent and colourless, have acquired a variety of colours, which allow their detailed structure to be recognized.

The procedure itself gives a vast amount of information. It allows visual estimation of the overall size, shape, and structure of individual RBCs, WBCs and platelets, which may have diagnostic significance in certain diseases. Pathologic early forms of the blood cells are also visible. All the figures in this chapter are of MGG or Wright-stained blood films.

Production of White Blood cells

Pluripotent cells of the early embryo originate all types of somatic cells and germ cells of adult organism. Despite different origin, all pluripotent stem cell lines demonstrate considerable similarity of the major biological properties: similar gene expression profiles, unlimited self-renewal and differentiation into various somatic and germ cells in vitro and in vivo, and similar cell cycle structure. These cells continue to proliferate in epiblast and then differentiate into multipotent precursor cells of different somatic lineages, which will give rise to terminally differentiated specialized cells. The nature of those pluripotent stem cells makes them an ideal source of cell-based products for regenerative medicine (4). Having had the unique and defining
characteristics of self- renewal and of differentiation into multiple cell types, Stem cells Poses in each cell division, an inherent asymmetry that is generally not found with other cell types. Multipotent stem cells, such as the hematopoietic stem cells (HSCs) of the bone marrow, are capable of giving rise to multiple mature cell types, but only those of a particular tissue, such as blood (5). Blood cell generation, or hematopoiesis, is one of the most well - studied physiological processes and serves as a paradigm for stem cell biology and the regulation of self – renewing tissues. During normal hematopoiesis in humans, approximately 250 billion new blood cells are produced each day to replace those lost through natural aging processes. The capacity for this remarkable job while maintaining the appropriate balance between the various types of specialized blood cells resides in a complex hierarchy of cellular elements found mainly in the bone marrow. At the apex of this hierarchy lies a relatively small population of around 50 million of those HSCs. Upon execution of a

series of asymmetric cell divisions and fate decisions; HSCs give rise to a heterogeneous pool of differentially committed progenitor cells.

Normal WBC maturation sequence begins with the blast form, derived from multipotent hematopoietic stem cells.

Neither multipotent hematopoietic stem cells nor more committed progenitors are readily morphologically identified by traditional methods, and reliable electron microscopy (EM) criteria for distinguishing myeloblasts from monoblasts or lymphoblasts are also lacking (6, 7), although pronormoblasts often can be differentiated by the presence of ferritin on the cell surface or in coated vesicles (8). Only the more mature forms of each hematopoietic cell series can be reliably distinguished from one another. Neutrophilic, eosinophilic, and basophilic granulocytes are thought to follow similar patterns of proliferation, differentiation, maturation, and storage in the bone marrow and delivery to the blood. The details of these processes are best documented for neutrophils. In the first three morphologic stages, the myeloblast, promyelocyte, and myelocyte cells are capable of replication, as shown by their uptake of tritiated thymidine (3H-TdR) and the presence of mitoses; in later stages, cells cannot divide but continue to differentiate. The morphologic boundaries of each cell compartment were defined many years ago and were based on criteria such as cell size, ratio of size of nucleus to cytoplasm, fineness of nuclear chromatin, nuclear shape, the presence or absence of nucleoli, the presence and type of cytoplasmic granules, and the cytoplasmic color of stained cells gradually during the stages of cell development. These morphologic definitions are necessarily arbitrary and do not always conform to significant biochemical or physiologic changes. Classifying a cell in one category or another often is difficult because it is actually in transition between the two. Nevertheless, it is useful to separate the cell lines into morphologic compartments and to define

normal limits of cell distribution therein because gross changes from these patterns indicate disease (9). The formation of blood cells (hemopoiesis) is determined by the interaction of multiple genes and involves cytokines and other protein factors. Regulation of blood cells production is tightly mediated by a complex network of biological humoral regulators, variously called hematopoietic growth factors (HGFs), cytokines, which usually act in synergy with each other. They are glycoproteins produced by stromal cells, T lymphocytes, liver and for erythropoietin, the kidney. Some growth factors act on receptors on the surface of primitive cells, others act on later cells already committed to a particular lineage. Growth factors inhibit apoptosis of target cells.

They also affect the function of mature cells. Growth factors in clinical use include Granulocyte/Macrophage colony - stimulating factor (GM – CSF), Granulocyte colony - stimulating factor (G – CSF), Macrophage colony - stimulating factor (M – CSF), stem cell factor (SCF), erythropoietin (EPO), and the cytokines including IL –1, IL - 3, IL –4, IL - 5, IL - 6, IL – 7, IL –8 and IL –9 (10).

BONE MARROW EXAMINATION

Bone marrow aspirates provide films on which the cytological details of developing cells can be examined. The proportions of different cells are assessed, appearances of the individual cells noted, and a search is made for the presence of cells foreign to the normal marrow, such as metastatic deposits from carcinoma. Iron stores may also be assessed (Table 2).

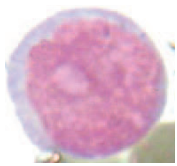
Type of cells	*Percentage*
Myloblasts	0.1- 3.5
Promylocytes	0.5 – 5.0
Mylocytes neutrophil Metamylocytes and bands Segmented neutrophils	5 – 20 10-30 7-25
Mylocytes Eosinophil Segmented Eosinophils	0.1-3.0 0.2-3.0
Mylocytes basophil Segmented basophils	0.0-0.5 0.0-0.5
Monocytes	0.0-2.0
Lymphocytes Plasma cells	5-20 0-3.5

(Table 2) Normal Mylogram

GRANULOPIOSIS:

Myelocytic (granulocytic or neutrophilic) series:

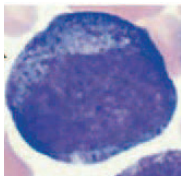
(Figure. 9 A) Myeloblast. Note the grainy reticular structure of the nucleus with the high nucleocytoplasmic ratio. (Wright stain) (Mag. X 1000)



(Figure. 9 B) Myeloblast. See the nucleoli "the lighter spot within the nucleus" and the faintly basophilic cytoplasm. (Giemsa stain) (Mag. X 1000)

Myeloblasts (figure. 9) are the least mature cells in the granulocyte lineage measure 12–20 µm and have a high nucleocytoplasmic ratio. They originate from a precursor pool of stem cells. Mononuclear, round-to-ovoid cells characterized by a large nucleus, they may be distinguished from proerythroblasts by the finer "grainy" reticular structure of their nuclei and the faintly basophilic cytoplasm (11) (figure. 9A). On first impression, they may look like large or even small lymphocytes (micromyeloblasts), but the delicate

structure of their nuclei always gives them away as myeloblasts. In some areas, condensed chromatin may start to look like nucleoli, with one to five (most often two or three) nucleoli present (12) (figure. 9B). Typically, a blast has relatively scanty basophilic cytoplasm without granules, although ultrastructural examination and cytochemistry show that granules are actually present. The French-American-British (FAB) group and subsequently supported by the WHO, describe a category of blasts sporadically the cytoplasm contains azurophilic granules (13). Although a myeloblast does have characteristic cytological features it is not always possible to make the distinction between a myeloblast and a lymphoblast on a traditional-stained film.



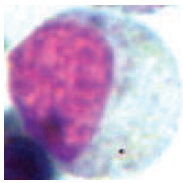
(Figure. 10 A) Progranulocytes. Note the large azurophilic cytoplasmic granules. (Giemsa stain) (Mag. X 1000)

(Figure. 10 B) Progranulocytes. See the eccentric nucleus with lower nucleocytoplasmic ratio. (Wright stain) (Mag. X 1000)

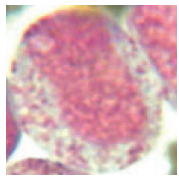
Progranulocytes (promyelocytes) (figure. 10) are next in sequence. They are the product of myeloblast division, and usually grow larger than their progenitor cells with a diameter of 15–25 µm. They are similar to the blast but have a variable number of cytoplasmic granules (14). In comparison with the myeloblast, the nucleocytoplasmic ratio is lower and the cytoplasm is more basophilic. During maturation, their nuclei show an increasingly coarse chromatin structure. The nucleus is eccentric (figure. 10 B), the lighter zone over its bay-like indentation corresponds to the Golgi apparatus. The wide layer of basophilic cytoplasm contains copious large azurophilic granules containing peroxidases, hydrolases, and other enzymes. Those azurophils or primary granules (dark granules) are produced only during the promyelocyte stage; they

also exist scattered all around the nucleus (figure. 10 A). The granules contain many antimicrobial compounds beside (MPO), a protein that catalyzes the production of hypochlorite (OCl -) from chloride and hydrogen peroxide produced by the oxidative burst. MPO constitutes approximately 5% of the dry weight of the neutrophil (15) and imparts the greenish coloration to pus. It is an enzyme whose activity is a classic marker of myeloid differentiation. Other components of azurophilic granules include lysozyme, which degrades bacterial peptidoglycans, bactericidal permeability-increasing protein, which has antibacterial activity against certain gram-negative bacteria, azurocidin, which has antibacterial as well as antifungal activity against Candida albicans, the serine proteinases elastase, cathepsin G, proteinase 3, esterase N, and others (16, 17). These azurophilic granules fuse with phagocytic vesicles resulting in the delivery of their contents to the ingested organism (5). Azurophil granule production ceases at the end of the promyelocyte stage, coincident with the loss of peroxidase activity from the rough endoplasmic reticulum.

Ordinarily, both previous cell types are encountered only in the bone marrow, where they are the most actively dividing cells and main progenitors of granulocytes. In times of increased granulocyte production, promyelocytes and (in rare cases) myeloblasts may be released into the blood stream (pathological left shift). Under strong regeneration pressure from the erythrocyte series during the compensation phase following various anemias, immature white cell precursors like the red cell precursors, may be swept into the peripheral blood. Bone marrow involvement by tumor metastases also increases the permeability of the blood–bone marrow barrier for immature white cell precursors (2).



(Figure. 11A) Myelocyte. Note the condensed nuclear chromatin and round nucleus. (Wright stain) (Mag. X 1000)

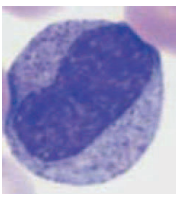


(Figure. 11B) Myelocyte. Note the cytoplasmic secondary granules. (Wright stain) (Mag. X 1000)

Myelocytes (figure. 11) are the product of promyelocyte division. They are smaller than their progenitors measuring 10–20 µm in diameter. In this stage a differentiation of the myelocytes into the neutrophil, eosinophil, and basophil series can be recognized (13). The cells can be identified as belonging to their lineage by the presence of specific or secondary granules with the staining characteristics of these cell lines. The nuclear chromatin in myelocytic stage is more condensed and the nucleus itself is round or oval, sometimes with a slight flattening along one side (figure. 11A). The ovoid nuclei have a banded structure. Often there is a small, localized, pale or clear area next to the flattened portion (if present) of the nucleus, called the myeloid spot. The cytoplasm is mildly basophilic and granular to varying degrees (figure. 11B), although sometimes granules are absent. Cytoplasm becomes lighter with maturation and in some cases acquires a pink tinge. Special types of granules, which no longer stain red like the granules in promyelocytes ("specific granules" peroxidase-negative) also called secondary granules, are evenly distributed in the cytoplasm. They fuse with phagocytic vesicles. It is believed that these granules are largely for release into the extracellular space. The known contents of these granules include apolactoferrin (18), the major specific granule protein, vitamin B 12-binding protein (19), plasminogen activator and collagenase. Lysozyme and some gelatinase are also present in specific granules. Release of specific granule contents may modify the inflammatory

process (20). Secondary granules also contain a number of membrane-bound molecules that are expressed on the cell surface. This includes CD11, CD18, CD66a, CD66b, NB-1, f-met-leu-phe (FMLP) receptors, C5a receptors, and cytochrome b 558. When cells are stimulated, the surface expression of many of these membrane proteins is increased and some of the up-regulated molecules may be derived from those secondary granules. Patients who lack those granules; are susceptible to repeated skin and respiratory infections and have defective neutrophil chemotaxis and adhesion. In these patients, the importance of the secondary granules in neutrophil function could be revealed.

Myelocyte morphology is wide-ranging because myelocytes actually cover three different varieties of dividing cells.

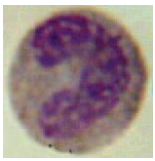


(Figure. 12 A) Metamyelocyte. Note the smaller indented nucleus. (Giemsa stain) (Mag. X 1000)

(Figure. 12 B) Metamyelocyte. The cytoplasm is faint pink and less basophilic. (Wright stain) (Mag. X 1000)

Metamyelocyte is next. Metamyelocyte (figure 12) measures 10–12 μm in diameter. The boundary between the myelocyte and metamyelocyte compartments is best defined by the fact that myelocytes divide and are actively involved in protein synthesis, as evidenced by the presence of nucleoli, abundant endoplasmic reticulum, and polysomes. Differentiation between myelocytes and metamyelocytes before was defined mainly in terms of nuclear shape. This characteristic is recognized as a poor criterion because it has been shown in studies of human neutrophils that myelocyte nuclei may become a markedly indented in shape (figure. 12 A) and subsequently may revert to an

oval configuration and enter mitosis. Consequently, in classifying cells at this stage the observer should pay particular attention to evidence in the nucleus and cytoplasm that protein synthesis has decreased or stopped. This determination is made on the basis of the fact that the nuclear chromatin is coarse and clumped and that the cytoplasm is faint pink and is essentially the color of the mature cell in stained preparations (figure. 12 B). Overall, in metamyelocyte the nucleus begins to indent; when it does, the cell is called a (juvenile). As it continues to mature, the nucleus becomes more and more indented and is characterized by a clearly horseshoe-shaped nucleus without nucleoli (even by EM examination) (21). The nuclear chromatin becomes more and more condensed, clumped with considerable clumping evident along the nuclear membrane and the cytoplasm becomes progressively less basophilic filled with primary, secondary, and tertiary granules, but the secondary granules predominate (22). The entire cell size becomes somewhat smaller with the nucleus taking up increasingly less space. The endoplasmic reticulum is sparse as are polysomes, thus signifying the virtual completion of protein synthesis. From this stage on, only further maturation of the nucleus occurs by contraction and the distinctions (between metamyelocytes, band neutrophils and segmented neutrophils) are merely conventional (23). During increased cell production in response to stress or triggers especially infections, myelocytes and metamyelocytes normally appear in the peripheral blood. Under these conditions they are however, more abundant than myeloblasts or promyelocytes.



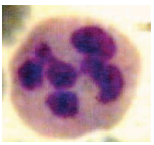
(Figure. 13 A) Band cell. Note the

(Figure. 13 B) Band cell. The

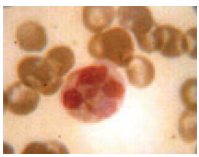
nuclear chromatin condensation.
. (Wright stain) (Mag. X 1000)

cytoplasm is very slightly eosinophilic in color
(Wright stain) (Mag. X 1000)

The band (stab) neutrophil stage. The band stage (figure 13) is characterized by further condensation of nuclear chromatin. There is some disagreement as to what constitutes a band as opposed to a metamyelocyte or band as opposed to an early mature polymorphonuclear leukocyte (24). Basically, a band is distinguished from a late metamyelocyte when the nucleus has indented more than one half its diameter and has formed a curved rod structure that is roughly the same thickness throughout (figure. 13 A). As the band matures, nuclear indentation continues and may also occur in other areas of the nucleus. When at least one area of nuclear constriction becomes a thin wire, the cell has reached the final stage of maturity called the polymorphonuclear (poly) or segmented neutrophil (25). Before classifying a cell as a polymorphonuclear form, anything less clear-cut, whether because of overlapping of nuclear lobes or incomplete constriction is classified as a band form. The nuclear chromatin at this stage becomes more dense and clumped. The cytoplasm is a very slightly eosinophilic in color or at least there is no basophilia. There usually are small irregular granules which often are indistinct. Banded neutrophilic granulocytes may occur in small numbers (up to 2%) in a normal blood count. This is of no diagnostic significance.

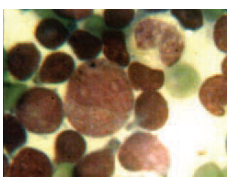


(Figure. 14 A) Segmented neutrophil. Note the nuclear segments with denser chromatin appearance. (Wright stain) (Mag. X 1000)

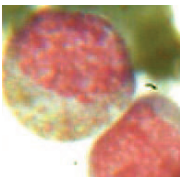


(Figure. 14 B) Segmented neutrophil. The cytoplasm has a faint pink appearance. (Wright stain) (Mag. X 400)

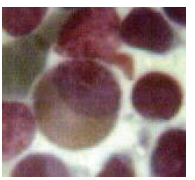
Segmented neutrophils (figure. 14) represent the final stage in the lineage forming gradually without any clear transition or further cell divisions, by increasing contraction of their nuclei. Finally, the nuclear segments are connected only by narrow chromatin bridges which should be no thicker than one-third of the average diameter of the nucleus. The chromatin in each segment forms coarse bands or patches and is denser than the chromatin in band neutrophils (figure. 14 A). The cytoplasm is faint pink and contains fine specific granules that sometimes give only a ground-glass appearance (figure. 14 B). The abundant azurophilic or primary granules have usually lost their dark-staining characteristics by this stage and are often barely visible dots in light microscope, but can be seen with EM. The number of segments increases with the age of the cells. The mechanism and purpose of nuclear segmentation remains unclear.



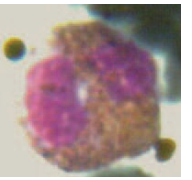
(Figure. 15-1) Note the eosinophilic Promyelocytes with a couple of nucleoli inside the nucleus. (Wright stain) (Mag. X 400)



(Figure. 15-2) Myelocytic eosinophil with round nucleus and secondary granules. (Wright stain) (Mag. X 1000)



(Figure. 15-3). Metamyelocyte stage with golden-red granules filling the cytoplasm. (Wright stain) (Mag. X 400)



(Figure. 15-4) Mature eosinophil. Note the Nucleus with two Lobes. (Wright stain) (Mag. X 1000)

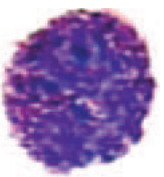
(Figure. 15) Eosinophilic series

Eosinophilic Granulocytes (Eosinophils) production (figure 15)*:*

Eosinophils arise from the same stem cell population as neutrophils and exhibit the same maturation phenomena; with the exception that only one type of granules is recognized. The earliest point at which eosinophils can be

morphologically defined in the bone marrow is at the promyelocyte stage. Promyelocytes contain large granules that stain blue–red (figure. 15-1), not until they reach the metamyelocyte stage do these become a dense population of increasingly round, golden-red granules filling the cytoplasm (figure. 15-3). In humans, these homogeneous granules appear to be formed throughout all the subsequent stages of maturation. The Charlot–Leyden crystals found between groups of eosinophils in exudates and secretions have the same chemical composition as the eosinophil granules. The nuclei of mature eosinophils usually have only two segments and the lobes are larger than those seen in neutrophils (figure. 15-3). The presence of eosinophils with more than two nuclear lobes suggests cell activation as occurs in parasitic diseases.

(Figure. 16-1) Promyelocytic stage with large stained granules. (Giemsa stain) (Mag. X 1000)



(Figure. 16-2) Basophil at a later stage of maturation. (Giemsa stain) (Mag. X 1000)

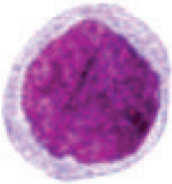
(Figure. 16-3) Mature basophil with few basophilic granules. (Giemsa stain) (Mag. X 1000)

(Figure. 16) Basophilic series

Basophilic Granulocytes (Basophils) production (figure 16):

Basophils (basophilic granulocytes) like eosinophils exhibit the same maturation phenomena of neutrophil lineage. The earliest stage at which they can be identified is the promyelocyte stage, at which, large black–violet stained granules are visible (figure. 16-1). In mature basophils, which are relatively small, these granules often obscure the two compact nuclear segments (figure. 16-2). The granules are water-soluble and thus may be dissolved in the process of staining and washing; the cells may then appear vacuolated with only a few

or no basophilic granules remaining. Similar but somewhat larger are tissue basophils or tissue mast cells— normally do not circulate in the blood. Tissue basophils have a round nucleus underneath large basophilic granules.



(Figure. 17-1) Monoblast (Giemsa stain) (Mag. X 1000)

(Figure. 17-2) Promonocyte (Giemsa stain) (Mag. X 1000)

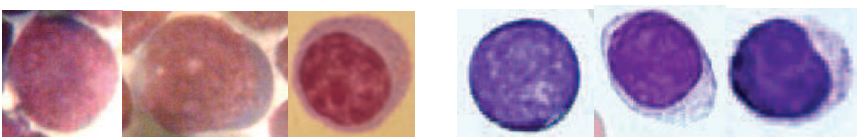
(Figure. 17-3) Mature monocyte (Giemsa stain) (Mag. X 1000)

(Figure. 17) Monocytic series

MONOCYTES PRODUCTION:

Monocytes (figure. 17) are produced in the bone marrow; their line of development arises in the bone marrow from progenitors committed to mononuclear phagocyte production and branches off at a very early stage from that of the granulocytic series, but does not contain any distinct specific precursors that can be securely identified with diagnostic significance in everyday morphological studies. Initiating in bone marrow with monoblast then heading to promonocyte, later the monocytes are released into the blood. Differentiation occurs rapidly with a maturation time of 50 to 60 hours associated with two rounds of replication and morphologic maturation marked by progressive lobulation of the nucleus. After a short time in the circulation, monocytes migrate into different tissues either randomly or specifically in response to chemotactic stimuli. Owing to their great motility and adhesiveness, mature monocytes are morphologically probably the most diversified of all cells. Measuring anywhere between 20 and 40 µm in size, their constant characteristic is an ovoid nucleus usually irregular in outline with invaginations and often pseudopodia-like cytoplasmic processes. The fine “busy” structure of

their nuclear chromatin allows them to be distinguished from myelocytes, whose chromatin has a patchy streaky structure and also from lymphocytes, which have dense homogeneous nuclei. Monocytes contain in their cytoplasm both primary (peroxidase-positive) and secondary (peroxidase-negative) granules. The primary granules of monocytes like those of neutrophils contain myeloperoxidase. Secondary granule fusion with the membrane on stimulation of monocytes results in up-regulation of Mac1 and p150,95 and is thought to play a role in adhesion and diapedesis of stimulated monocytes (26). The basophilic cytoplasmic layer varies in width, stains a grayish color, and contains a scattered population of very fine reddish granules that are at the very limit of the light microscope resolution. These characteristics vary greatly with the size of the monocyte, which in turn is dependent on the thickness of the smear. Where the smear is thin, especially at the feathered end, monocytes are abundant, relatively large and loosely structured and their cytoplasm stains light gray–blue. In thick dense parts of the smear some monocytes look more like lymphocytes: only a certain nuclear indentation and the gray–blue staining of the cytoplasm may still mark them out.



(Figure. 18-1) Lymphoblast (Wright stain) (Figure. 18-2) Prolymphocyte (Mag. X 1000) (Figure. 18-3) Lymphocyte (Figure. 18 A) Lymphoblast (Giemsa stain) (Figure. 18 B) Prolymphocyte (Figure. 18 C) Lymphocyte (Mag. X 1000)

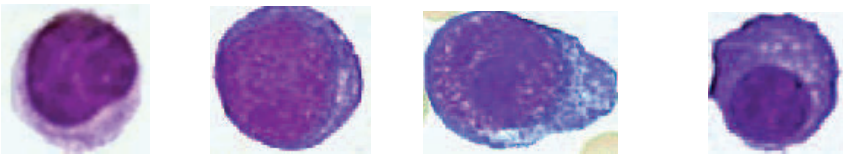
(Figure. 18) Lymphocytic series

LYMPHOPOIESIS

Lymphocytes (figure. 18) and Plasma Cells production (figure. 19):

Lymphocytes originate from a lymphoid stem cell committed to lymphopiosis. The earliest lymphocytes are identified as lymphoblasts and prolymphocytes. Lymphoblasts contain a large round nucleus with a small or moderate amount of basophilic cytoplasm. The nuclear chromatin strands in lymphoblasts are thin evenly stained and not clumped. One or several nucleoli are usually demonstrable (figures. 18-1, A). Prolymphocytes have an intermediated chromatin pattern that has begun to clump in some areas of the nucleus but does not appear as clumped as in mature lymphocytes. Nucleoli are less distinct than in lymphoblast (figures. 18-2, B). In case of doubt, the cell should be called a lymphocyte. Although the marrow remains the principal site of 'virgin' lymphocyte formation, the majority of circulating lymphoid cells (mature T and B cells) are produced in peripheral lymphoid tissue: lymph nodes, spleen, thymus and the lymphatic islands of the intestinal mucosa and respiratory tracts. Lymphocytes (figures. 18-3, C) usually comprise less than 10 percent of the normal myelogram, and the progenitor lymphoblasts are difficult to differentiate from other blast cells. Isolated plasma cells are generally not difficult to find in marrow cell trails. They comprise up to 4 percent of the normal marrow cell population. Note that lymphoid stem cells are not able to initiate immune responses until they have been processed either in the thymus or the bursa-equivalent organs. A small fraction of the lymphocytes are known as NK cells (natural killer cells). Immature precursor of lymphocytes, are practically never released into the blood and are therefore of no practical diagnostic significance. The cells encountered in circulating blood are mostly "small" lymphocytes with oval or round nuclei 6–9 μm in diameter. Their chromatin may be described as dense and coarse. Detailed analysis under the microscope reveals not the patch-like or banded structure of myeloblast

chromatin, or the “busy” structure of monocyte chromatin, but slate-like formations with homogeneous chromatin and intermittent narrow, lighter layers that resemble geological break lines. Nucleoli are rarely seen. The cytoplasm wraps quite closely around the nucleus and is slightly basophilic. Only few lymphocytes display the violet stained stippling of granules, about 5% of small lymphocytes and about 3% of large ones. The family of large lymphocytes with granulation consists mostly of NK cells. An important point is that small lymphocytes—which cannot be identified as T- or B-lymphocytes on the basis of morphology—are not functional end forms, but undergo transformation in response to specific immunological stimuli. The final stage of B lymphocyte maturation (in bone marrow and lymph nodes) is plasma cells, whose nuclei often show radial bars and whose basophilic cytoplasm layer is always wide. Intermediate forms (“plasmacytoid” lymphocytes) also exist.



B-lymphocyte | Plasmacytoid lymphocyte | Plasmacytoid lymphocyte with wide cytoplasmic layer | Plasma cell

(Figure. 19) Plasma Cells production
(Giemsa stain) (Mag. X 1000)

White Blood cells morphology, circulating kinetics, function, and activity

PERIPHERAL WHITE BLOOD CELLS:

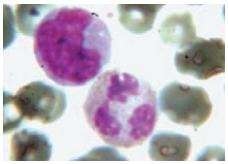
The usual initial diagnostic approach to blood disorders is blood counting and blood film examination. Various parameters make up the normal blood count (Table. 3). Blood films on glass slides are usually stained with one of the Romanowsky stains (for example, Giemsa or Wright's). During blood film examination, the white cell numbers and morphology are assessed and a differential count is performed, if this has not been provided by an electronic laboratory blood counter. Representative examples of white cells found in normal blood are shown in Fig. 20, 21, 22.

Very occasionally, either epithelial or endothelial cells are seen during blood film examination. It is likely that these have been aspirated from the skin or the blood vessel wall during vein puncture. Other rare appearances include neutrophil-platelet rosetting and neutrophil aggregation, neither of which is usually of clinical significance.

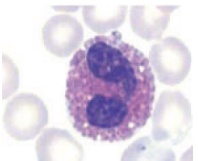
	Total Leukocytes		**Neutrophils**	
	Mean	**Normal Range**	**Mean**	**Normal Range**
Birth	22.0	9.0-30.0	13.2	3.6-24.0
1-4 wk	12.0	5.0-21.0	4.8	2.0-8.4
6 mo	11.9	6.0-17.0	3.8	1.0-8.5
1 yr	11.4	6.0-17.0	3.8	1.5-8.5
2 yr	10.6	6.0-17.0	3.5	1.5-8.5
4 yr	9.1	5.5-15.5	3.8	1.5-8.5
6 yr	8.5	5.0-14.5	4.3	1.5-8.0
8 yr	8.3	4.5-13.5	4.4	1.5-8.0
10 yr	8.1	4.5-13.5	4.4	1.8-8.0
16 yr	7.8	4.5-13.0	4.4	1.8-8.0
21 yr	7.4	4.5-11.0	4.4	1.8-7.7

NOTE: Expressed as cells ×10 3/mm 3

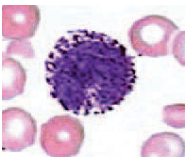
Table 3



(Fig. 20)
Large lymphocyte in upper left.
Mature neutrophil in bottom right.
(Wright stain) (Mag. X 400)



(Fig. 21) Eosinophil
Note the bilobed nucleus and
the spherical eosinophil granules.
(Wright stain) (Mag. X 1000)



(Fig. 22) Basophil
See how the purple-black granules
are obscuring the nucleus.
(Giemsa stain) (Mag. X 1000)

Neutrophils (polymorphs):

Neutrophils (Fig. 20) are so named because of their neutral staining with Wright stain. The mature neutrophil measures 12–15 µm in diameter. The cytoplasm is acidophilic with many fine granules. The azurophilic or primary granules have usually lost their dark-staining characteristics by this stage but can be seen with EM. The nucleus has clumped chromatin and is divided into two to five distinct lobes by filaments which are narrow strands of dense heterochromatin bordered by nuclear membrane (27). The nucleus tends to follow an approximately circular form since in the living cell the nuclear lobes are arranged in a circle around the centrosome. In normal neutrophil there may be some non-granular cytoplasm protruding at one margin of the cell. This may represent the advancing edge of a cell in active locomotion. In normal females a ‘drumstick’ may be seen protruding from the nucleus of a proportion of cells.

Neutrophil kinetics is the processes of cell multiplication, maturation, storage, and delivery to the tissues and sites of infection or cell damage. Production and differentiation of neutrophil in the bone marrow takes from 6 to 10 days. Large numbers of band and segmented neutrophils are held in the marrow, liver and spleen as a reserve pool, which, in the normal state, contains 10–15 times the number of neutrophils in the peripheral blood. From the maturation

compartment cells flow into the blood and are distributed in two sites: the circulating bloods granulocyte pool (CGP) and the marginal granulocyte pool (MGP). Cells in these two pools are in constant equilibrium. After being released from the marrow, they spend 6–12 hours in the circulation before migrating into tissues where they perform their phagocytic function. Within minutes, adherence of neutrophils to the endothelial cells of the vessel wall and their subsequent migration into the tissues can be seen at a local site of tissue damage or infection. After initial adherence, neutrophils project microscopically visible pseudopods between the endothelial cells and force a passage between them. This directed movement, chemotaxis, is induced by the binding of a variety of chemoattractant molecules such as n-formyl peptides, leukotriene B 4, and platelet-activating factor to specific membrane receptors. Further migration is then delayed by the basement membrane and periendothelial cells until a passage into the surrounding connective tissue is found. Once neutrophils leave the blood, they do not return in significant numbers. The sites into which neutrophils normally disappear are poorly understood. They survive two to four days in the tissues before being destroyed during defensive action or as a result of senescence. Neutrophils play a critical role in host defense by phagocytizing and digesting microorganisms. Inappropriate activation of neutrophils may result in damage to normal host tissues. In the resting uninfected host, the production and elimination of neutrophils are balanced resulting in a fairly constant concentration of neutrophils in peripheral blood. When an infection occurs, chemotactic agents are generated that result in migration of neutrophils to the site of the infection and activation of neutrophil defensive functions. This effect is often associated with an increased release of neutrophils from the bone marrow after production through a regulating mechanism with the aid of a large number of growth

factors or colony-stimulating factors such as G-CSF and granulocyte-macrophage colony-stimulating factor GM-CSF. When the neutrophil reaches the infecting organism it has to destroy it. This destruction is generally accomplished by phagocytosis of the agent, which occurs when a neutrophil meets a particle it envelops it with pseudopodia which fuse around it forming a phagosome. The process of Endocytosis is by which the material is taken into a cell, enclosed within pieces of plasma membrane and therefore, never occurs free within the cytoplasm of the cell. It is followed by rapidly release and fuses with azurophilic and specific granules into the phagocytic vesicle, terminating with killing and digesting of the organism. Neutrophils are best known for their function of phagocytosing invading pathogens such as bacteria or other foreign material (e.g., a uric acid crystal found in a gouty knee joint). Once pathogenic material has been phagocytosed, it is degraded in lysosomal granules within the neutrophil via lysosomal enzymes such as lysozyme and myeloperoxidase. Energy derived from glycolysis and the pentose monophosphate pathway within the neutrophils is required for phagocytosis and lysosomal fusion. NADP (nicotinamide adenine dinucleotide phosphate) formed in the pentose pathway is regenerated by NADPH oxidase enzyme producing NAD, the oxidized form of the coenzyme and hydrogen peroxide (H202). Thus, the energy burst in neutrophils derived significantly from the pentose monophosphate pathway results in the production of H202, which reacts with myeloperoxidase released from the lysosome and in the presence of halides such as chloride or iodide, effects the killing of bacteria. Myeloperoxidase (MPO) which is present in the primary granules in the neutrophils, with chloride presence, converts H202 to toxic hypochlorous acid (HoC1-) or bleach, which in turn is converted to chlorine. Lactate produced during glycolysis results in the lowering of pH, thus assisting further the destruction of the intruding pathogen by lysosomal

constituents, which is optimal at an acid pH. H202 can also be generated by other reactions such as the action of superoxide dismutase on superoxide anion, which itself is formed when NADPH oxidase catalyzes the monovalent reduction of oxygen. The regeneration of NADP can also be achieved when H202 reacts with reduced glutathione and the enzyme glutathione peroxidase to produce oxidized glutathione. The latter can react with an NADPH-linked glutathione reductase to generate NADR Thus, the continual supply of NADP for the pentose monophosphate pathway, and hence the maintenance of phagocytic efficiency of the neutrophil, is achieved by several enzyme systems within the neutrophil.

Neutrophils possess a cell-surface molecule called L-selectin, which recognizes carbohydrate components such as sialyl-Lewis x, an adhesion molecule on the vascular endothelium. In response to acute reaction, neutrophils marginate along the capillary and venule walls in the vicinity of target tissue. As the neutrophil rolls along the vascular endothelium, the binding of L-selectin on the neutrophil surface to sialyt-Lewis X on the vascular endothelium arrests its movement. The activated neutrophil sheds L-selectin from its surface and replaces it with other cell adhesion molecules such as integrins. During the acute phase response, inflammatory mediators such as bacterial lipopolysaccharide and cytokines, interleukin-1, and tumor necrosis factor induce the expression of E-selectin on the blood vessel wall. Integrins on the surface of activated neutrophils bind E-selectin on the blood vessel wall, facilitating the entry of the neutrophil into tissue spaces via a process referred to as diapedesis. A variety of inflammatory mediators such as complement components, leukotrienes, histamine, prostaglandins, and particularly chemokines (a group of chemoattractant cytokines) serve to recruit the activated neutrophil through the vessel wall to the tissue site of infection (28). The

phagocytosis of bacteria at the infected site by activated neutrophils is facilitated by its recognition of complement (C3b) coated bacteria. In neutrophils, several monoclonal antibodies were described that recognize subpopulations of those neutrophils; the clinical significance of neutrophil subpopulations is unclear.

Eosinophils:

The eosinophil (Fig. 21) is slightly larger than the neutrophil with a diameter of 12–17 μm. The nucleus is usually bilobed but occasional nuclei are trilobed. The cytoplasm of eosinophils is weakly basophilic. Eosinophil granules are spherical and considerably larger than those of neutrophils; they pack the cytoplasm and stain reddish-orange (27).

Although less is known about the kinetics of eosinophil production, differentiation, circulation and migration, it is likely that the mechanism is similar to that for neutrophils. Eosinophils make up approximately 3% of the bone marrow from healthy individuals, of which 37% are fully differentiated, and the remainders are promyelocytes/myelocytes and metamyelocytes. The appearance of newly matured cells in the blood occurs approximately 2.5 days from the time of the last mitotic division. Three cytokines, interleukin (IL)-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) that are produced by CD4 and CD8 T lymphocytes from peripheral blood as well as inflamed tissues are critical for stimulation of bone marrow production of eosinophils. Three different populations of eosinophils normodense, hypodense, and primed eosinophils are present. They can be characterized based on their intrinsic buoyant density and responsiveness to stimuli. Of the mature granulocytes, eosinophils display the most intense staining with peroxidase. The intensity of staining appears to be related to their basic protein content. Eosinophils like neutrophils are capable of phagocytosis. Human eosinophil

granules contain eosinophil major basic protein (MBP). MBP constitutes a major proportion of total granule protein and damages several parasites such as schistosomes, Trichinella spiralis larvae, and trypanosomes as well as many types of mammalian cells including respiratory epithelium (29). The granules are membrane-bound organelles with a crystalloid core. Eosinophils are particularly important in allergic and parasitic diseases. Following appropriate stimulation, the granule contents may be released outside the cell at large targets such as helminth parasites. Eosinophils release histaminase and aryl sulphatase, which inactivate histamine and a slow-reacting substance of anaphylaxis (SRS-A) released from mast cells. In some disease states, evidence for the involvement of eosinophils has been reported by the detection of MBP or erythropoietin at the site of pathology (30). Eosinophils are features of allergic and non-allergic asthma, and a correlation between the degree of bronchial hyperresponsiveness (a clinical feature of asthma) and peripheral blood eosinophilia has been observed in subjects who exhibited a dual response after allergen challenge. Based on the discovery of intensely stained deposits of eosinophil MBP in the airways of individuals who died from fatal asthma, recent studies suggest that eosinophils may have a destructive role in allergy and asthma (9). Atopic dermatitis and other cutaneous lesions such as urticaria, are often associated with deposition of eosinophil products such as the eosinophil granule MBP.

Basophils And Mast Cells:

The basophil (Fig. 22) is of similar size to the neutrophil (10–14 μm in diameter). The nucleus is usually obscured by purple-black granules, which are intermediate in size between those of the neutrophils and those of the eosinophil (31).

Although both basophils and tissue mast cells are of bone marrow origin, their relationship is not entirely clear. Both cells are round and have irregular short surface projections and cytoplasmic secretory granules containing crystalline structures. The cytoplasm of both cells is generally pink, the nucleus is purplish or blue and the cytoplasmic granules are dark blue to purple or even blackish (32). Basophils do have aggregates of cytoplasmic glycogen while mast cells have little. The nucleus of mast cells can be round or lobed, whereas that of the basophil is generally multilobed. Basophils have an abundance of condensed chromatin positioned at the periphery of the nucleus, whereas mast cells have little condensed chromatin. Mature basophils and mast cells differ markedly in their surface phenotype, stored mediators and in the synthesis of new mediators after activation. One prominent difference in surface phenotype is that mature mast cells express CD117 or c-kit, the receptor for stem cell factor (SCF), whereas mature basophils do not. Mediators that are produced by the two types of basophilic leucocytes are another obvious source of their distinctions. A major distinction between the two cell types in stored mediators lies in the proteinases that are abundant in mast cells. The cytokine and proteinase profiles of the two cell types are also distinct. In a model of allergic asthma, an inhibitor of mast cell–specific tryptase has promising bronchodilatory and anti-inflammatory effects. Such drugs perhaps, will help distinguish between the roles of mast cells and basophils in allergic inflammation.

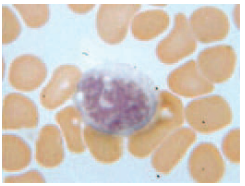
Mast cells and basophils are prominent players in allergic inflammation and other immune and inflammatory events. The granules of both cells contain heparin and pharmacological mediators such as SRS-A and histamine (33, 34). Both cells express high-affinity to IgE. Release of SRS-A and histamine follows an allergen–IgE complex binding to the cell surface via Fc receptors for

IgE. Several challenges remain including perhaps the most obvious question, what functions distinguish both basophils and mast cells?

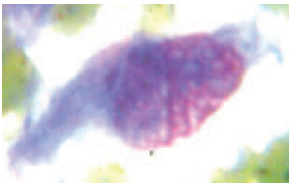
The early phase of the allergic responses appears to be critically important that there is a pool of circulating mature basophils and also a large tissue repository of mast cells. Under certain circumstances a large influx of basophils can occur into local tissues e.g., cutaneous basophilic hypersensitivity and asthma and the numbers of mast cell progenitors in the circulation can be increased. However, there is much less evidence to suggest that neurogenic inflammation is a major pathway in basophil-mediated reactions when compared to mast cells. As given the close anatomic association between mast cells and nerves and the abundant evidence for their functional interdependence, it is likely that as a component of allergic and other inflammatory reactions, mast cell–dependent neurogenic pathways are important.

Mast cells may be important in the defense against parasites and innate antimicrobial immunity and the importance of the possible role of their toll-like receptors is emphasized. A great interest in the expression and function of these receptors on mast cells and basophils has arised.

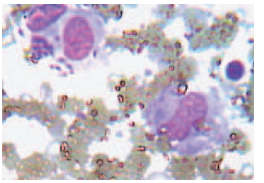
Monocytes:



(Fig. 23) Monocyte
See the lobulated nucleus and the opaque grayish-blue cytoplasm (Wright stain) (Mag. X 400)



(Fig. 24) Macrophage (Wright stain) (Mag. X 1000)



(Fig. 25) A couple of macrophages and a lymphocyte in the upper left (Wright stain) (Mag. X 400)

The monocyte (Fig. 23) is the largest normal peripheral blood cell with a diameter of about 12– 20 µm. It has an irregular, often lobulated nucleus and

opaque grayish-blue cytoplasm with fine azurophilic granules (35). The cell outline is often irregular and the cytoplasm may be vacuolated (27).

Monocytes spend only a short time in the marrow. After circulating in blood, monocytes leave the vascular system in an exponential manner with a half-life of 4.5 to 10.0 hours (mean of 8.4 hours). They enter the tissues, where they mature and carry out their principal functions. In the tissues, they differentiate in response to soluble stimuli to become tissue macrophages with characteristic morphologic and functional qualities. Activation is the term for this process of differentiation. The mononuclear phagocytes of the skin are called Langerhans cells. They make up 3 to 8% of the epidermal cells. Langerhans cells have a relatively clear cytoplasm, a lobulated nucleus without organelles characteristic of keratinocytes and melanocytes and a unique cytoplasmic granule, the Birbeck granule.

Monocytes and macrophages function can be modulated by different substances such as CK, chemokine; CSF, colony-stimulating factor; GF, growth factor; GM-CSF, granulocyte macrophage CSF, (IFN) interferon; (IL) interleukins, (LPS) lipopolysaccharide, M-CSF, macrophage CSF, (RNS) reactive nitrogen specie, (ROS) reactive oxygen species, (TGF) tumor growth factor, (TNF) tumor necrosis factor and (VD 3) vitamin D 3. Their extravascular lifespan may be as long as several months or sometimes years. These cells are divided in tissues into the phagocytic macrophages, whose role is to remove particulate antigens and the antigen-presenting cells, whose main function is to present antigens to lymphocytes.

Macrophages participate in a wide variety of important activities in the body including removal of dead, senescent, foreign, or altered cells or foreign particles, regulation of the function of other cells, processing and presentation of antigens in immune reactions, participation in various inflammatory

reactions and destruction of microbes and tumor cells. Monocytes are active phagocytes. Ingestion and adherence to microorganisms are facilitated by special surface receptors for the Fc portion of IgG and for complement (for example, C3b) with which the microorganisms may be coated. Many antigens on the surface of macrophages have been characterized. Antigens of the major histocompatibility complex are expressed by mononuclear phagocytes class I or HLA-A, B, and C as well as class II or HLA-DR. Monocytes carry other surface markers including receptors for lymphokines such as g-interferon and migration inhibition factor.

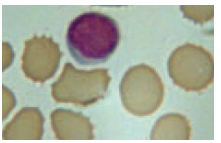
Monocytic lysosomes contain acid hydrolases and peroxidase, which are important in the intracellular destruction of microorganisms. They also produce complement components, prostaglandins, interferons, monokines, such as interleukin-1 and tumor necrosis factor and haemopoietic growth factors such as colony-stimulating factors. Mononuclear phagocytes internalize substances by either pinocytosis (the uptake of solutes) or phagocytosis (the uptake of particulates). Most of the internalized vesicles fuse with lysosomes and the contents of the vacuoles are processed in the lysosome. Particles are phagocytosed either through opsonins (e.g., Ig, C', or fibronectin) or by ill-defined, opsonin-independent mechanisms.

Reticuloendothelial system: phagocytes and antigen-presenting cells:

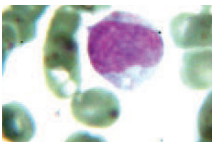
In addition to the wandering or free tissue macrophages, the monocyte-derived phagocytic cells form a network known as the reticuloendothelial system (RES) and are found in many organs (Figs. 24, 25). This system includes the Kupffer cells in the liver, alveolar macrophages in the lung, macrophages of various serosal surfaces, mesangial cells of the kidney, brain microglia and macrophages of the bone marrow, splenic sinuses and lymph nodes.

Antigen-presenting cells (APCs) are found primarily in the skin, lymph nodes, spleen and thymus. Following stimulation, the Langerhans' cells of the skin migrate via the afferent lymphatics into the paracortical areas of draining lymph nodes where they interdigitate with T cells presenting them with antigens carried from the skin (36). Other specialized APCs include the follicular dendritic cells found in the germinal centers of lymph nodes and other lymphoid tissues and the interdigitating follicular cells found in the thymus

Lymphocytes:



(Fig. 26) Normal lymphocyte (Wright stain) (Mag. X 400)



(Fig. 27) Large lymphocyte (Wright stain) (Mag. X 400)

Lymphocytes are produced in the bone marrow from pluripotent stem cells. They fall into two main groups with different functions, T cells and B cells (37). The two groups are indistinguishable by traditional Wright staining (fig. 26, 27). T cells are processed initially in the thymus. B cells differentiate in birds in an organ known as the bursa of Fabricius. Both cells assist the phagocytes in the defense of the body against infection and other foreign invasion and constitute the major cells in specific immunity (38). Lymphocyte values between 1500 and 4000 /mm 3 and about 35% reflect normal output of the lymphatic system. Elevated absolute lymphocyte counts, often along with cell transformation, are observed predominantly in viral infections or in diseases of the lymphatic system. Relative increases at the expense of other blood cell series may be a manifestation of toxic or aplastic processes. A spontaneous decrease in lymphocyte count is normally seen only in very rare congenital diseases such as agammaglobulinemia Bruton disease, DiGeorge

disease chromosome 22q11 deletion syndrome. Some systemic diseases also lead to low lymphocyte counts (Hodgkin disease, active AIDS).

T cells:

T cells, which comprise 65–80 percent of the circulating lymphocyte population are 5 to 8 µm in diameter with a high nucleocytoplasmic ratio (39). The cytoplasm forms a narrow light-blue rim around a purple nucleus with densely packed chromatin. The sequence of events in T-cell development appears to be initial expression of nuclear terminal deoxynucleotidyl transferase (TdT) and the surface antigen CD7 followed by CD2. Mature T cells carry a marker (antigen CD2), which binds sheep erythrocytes. Adherence of thymocytes to epithelial cells is mediated by CD2. CD2 and CD7 expression on the cell surface may precede arrival in the thymus (40). Those early cells are Potentially, capable to differentiate to other lineages such as natural killer (NK) cells, dendritic cells and monocytes (41). Rearrangement of the T-cell receptor genes occurs in the sequence d, g, b and finally, the CD2 antigen. CD3 antigen are expressed on the surface later, although intracytoplasmic CD3 is one of earliest markers. At this stage, the majority of the CD34 + cells are still negative for CD3, CD4 and CD8 (triple negative, or TN), but a small subpopulation expresses low levels of CD4 (42). This population may appear as an intermediate between the TN thymocytes and the main cortical population that are positive for both CD4 and CD8 (DP) (double-positive stage). In the process of differentiation, CD34 is progressively lost while the intensity of CD7 decreases. The CD4 and CD8 antigens are expressed in medullary thymocytes after the T- cell receptor gene rearrangement is complete. The DP thymocyte (the double- positive stage) (CD4/CD8) differentiates to two phenotypically and functionally distinct lineages of T cells: CD4 and CD8. The TCR (T cell receptor) of the CD4 cells interacts with peptides bound to class II MHC

molecules, whereas the TCR of the CD8 cells recognizes peptide–class I MHC complexes. The CD4 and CD8 proteins are not clonally distributed and are known as co-receptors because they co-recognize the same ligands as the TCR (9). The mechanism by which the separation of the lineages from DP thymocytes is achieved remains unresolved (43). CD8 (suppressor) cells are the major subpopulation of T cells in the marrow, while CD4 (helper) cells predominate in the peripheral blood. T cells contain a number of lysosomal acid hydrolases such as b- glucuronidase and acid phosphatase, which may be detected cytologically as discrete masses in the Golgi zone in the cytoplasm. The T-cell surface contains an antigen receptor consisting of a and b chains, each with variable and constant portions. The genes for these polypeptide chains on chromosomes 14 and 7 are rearranged in T cells in a manner similar to the rearrangement of immunoglobulin genes in B cells, resulting in a wide diversity among T lymphocytes. Close by the T-cell receptor is a complex of proteins termed the CD3 complex, which consists of g, d and e chains. This complex is responsible for transducing signals derived from interaction of antigen with the T-cell receptor to the cell interior. T4-helper/inducer and T8-suppressor lymphocytes are regulatory cells that function to regulate both humeral as well as cell-mediated immunity. A normal ratio of T4: T8 is 1.6 to 2.0. Various subpopulations of T lymphocytes are derived including the effector T lymphocytes, which include cytotoxic T lymphocytes (T-CTL) and delayed hypersensitivity (T-DH) T lymphocytes. Both types along with macrophages play major roles in cell-mediated immunity, which is important in protection against intracellular pathogens. T suppressor cells are other subpopulations. They control antibody production and inflammation produced by T cells, balance ratio of immunoglobulin classes and block activation of T-and B-cell clones reactive to self.

T lymphocytes circulate through the body surveying for abnormal or malignant cells. They are unlike other white cells, long-lived cells with a have life between 15-30 years. It is postulated that T lymphocytes are not replaced during adult life.

Lymphocyte locomotion: was described by Lewis (44), who observed that the cell first extends a pseudopod separated from the rest of the cell by a groove that encircles the cell body. Eventually, as the groove deepens, the nucleus is pushed forward through the constriction ring, giving rise to the classic shape of the motile lymphocyte, resembling a hand mirror or pear. The advancing front is occupied by the nucleus, which is separated by a deep constriction from the rest of the cytoplasm that trails behind, forming the handle of the mirror. The cytoplasmic tail is called the uropod (from Greek ura, "tail," and podi, "foot").

B cells:

This subpopulation of lymphocytes comprises 5–15 percent of the circulating lymphocyte population. B-lymphocytes and plasma cells are characterized by their ability to produce antibodies (immunogobulin). They are responsible for "humoral" immunity. The physiologic mechanisms controlling the cell growth and orderly synthesis of immunoglobulin are regulated by complex set of interrelations result in the release of specific antibodies in response to initiating antigen. This normal response under certain circumstances may become uncontrolled and the process of antibody production becomes excessive, and may lead to the production of abnormal immunoglobulin type. B-lymphocytes derive from cells expressing high levels of CD34 (45). Markers defining the first stage of development are CD19, and occasionally, some other markers such as CD2 or CD7 are detected. The immature B-cell stage is characterized by expression of only IgM without IgD. Mature B cells are defined by IgD expression and the presence of endogenously produced immunoglobulin

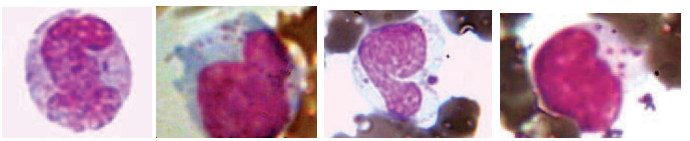
molecules inserted into the surface membrane where they act as receptors for specific antigens. Fluorescein-labelled specific antibodies may be used to demonstrate these surface-bound immunoglobulin molecules, for example, CD10, CD19, CD20 and CD22. They also express HLA-DR. B cells which have matured and secrete immunoglobulin are termed plasma cells. The surface immunoglobulins are individual to each clone of B cells and are identical to those secreted as antibodies by the B lymphocyte or plasma cell. The immunoglobulin (Ig) may be divided into one of five classes: IgG, IgA, IgM, IgD, IgE, which are further divided into subtypes. Each immunoglobulin molecule consists of light chains (k or l) and heavy chains (gamma, alpha, mu, delta, and epsilon respectively), which determine the class of immunoglobulin. Both heavy and light chains contain constant and variable regions. The heavy chain genes are arranged in the order given above on chromosome 14 and the light chain genes on chromosomes 2 for (k) and 22 for (l) (9).

The immunoglobulins are expressed in the cytoplasm in pre-B cells before they can be detected on the surface, while the nuclear enzyme TdT is expressed early in B-cell ontogeny. Diversity is produced by differences in the rearrangement of the genes of the immunoglobulins they secrete for the variable (V), diversity (D), joining (J) and constant (C) regions, and also by insertions of a variable number of bases in ‘N’ regions by TdT.

Gene rearrangement processes are mediated by a recombinase enzyme system and recombination signal sequences (RSS), which recognizes specific joining sequences consisting of a palindromic heptamer and nonamer separated by spacer regions of 12 or 23 base pairs. The sequence starts with the heptamers, which border the 3' side of each V and D segment and the 5' side of each D and J segment. The gene rearrangement first requires back-to-back fusion of the heptamer–nonamer sequences. These sequences and a circular intervening

sequence including the sequences to be deleted are excised and the ends of two gene segments joined up. Class switching is achieved by deletion of the constant region genes upstream from the gene to be expressed. The majority of B cells carry HLA-DR antigens, which are important in the regulation of the immune response. Complement receptors for C3b and C3d are also found on more mature B cells.

Natural killer (NK) cells:



(Fig. 28) Large Granular Lymphocytes. Note the azurophilic granules inside the cytoplasm. (Wright stain) (Mag. X1000)

LGLs are large cells (Fig 28) with pale blue cytoplasm and a high cytoplasmic to nuclear ratio. Their main histologic feature is the presence of azurophilic granules (46). These cells constitute 2 to 6% of the peripheral white cells and approximately 10 to 15% of the peripheral blood lymphocytes. They are larger than the typical lymphocytes (10 to 12 µm) with a larger amount of cytoplasm that contains peroxidase-negative granules. There is a minor population of 'lymphocytic' cells which do not carry markers of either T or B cells and which are known as 'non-T, non-B' cells or 'third population' cells. The sequence of differentiation of these cells is not known. The majority of 'null 'cells appear as large granular lymphocytes in the peripheral blood. This population of cells contains the majority of natural killer (NK) cells, which were originally described on a functional basis according to their capability of killing certain tumor cells of hematopoietic origin in the absence of prior stimulation or sensitization. Natural killer (NK) cells are antibody-independent cellular cytotoxic cells (AIDCC), and do not require major histocompatibility complex (MHC) recognition. They are able to directly kill tumor and virus-infected cells

(1). They are involved in graft rejection. NK cells are a heterogeneous population with respect to phenotype and target specificity. Although the majority of the CD56 + NK cells are CD3 -, small numbers of CD45 +/CD3 + cells have been detected and large granular lymphocyte (LGL) leukemias with the same phenotype have been reported (47). Small numbers of circulating 'null' cells are also immature T or B and myeloid or erythroid progenitor cells. NK cells probably include cells of mainly T-cell (CD81), (CD161) and myeloid-cell lineages. Their proliferation is stimulated by interleukin-2, interleukin-4, interleukin-12, interleukin-13, and g-interferon (9).

Lymphocyte proliferation and differentiation:

T and B cells proliferate and develop in reactive lymphoid tissue, for example, lymph nodes and lymphoid tissues of the alimentary and respiratory tracts and spleen. Both T and B cells acquire receptors for antigens, which commit them to a single antigenic specificity and are activated when they bind their specific antigen in the presence of accessory cells.

Antigen-presenting cells (APCs) interact with T cells bearing the appropriate receptor for that particular antigen, provided there is major histocompatibility complex (MHC) recognition (class I for CD8 and class II for CD4 cells) (48). Adhesion molecules are involved in the cell-cell binding. Differentiated cytokine-secreting effector cells is then distinguished into two major categories, Th1 cells produce primarily IFN, IL-2, and TNF, whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13. Both types of cells produce IL-3, TNF-a, and granulocyte-macrophage colony-stimulating factor. B cells with the appropriate surface receptor (immunoglobulin) for the antigen are also stimulated.

Subsequently, these stimulated T and B cells proliferate and differentiate under the stimulus of factors released from antigen-presenting cells (IL-l, IL-6 and IL-7) and activated T-helper cells (IL-2, IL-4 and IL-9). The T-helper lymphocytes

also secrete cytokines especially IL-2 and IL-4, which cause B cells to multiply to produce antibodies and to stop replication. Clones of both effector and memory B cells are produced. When the memory cells are stimulated at a later date by their specific antigen, they are able to proliferate again in an accelerated fashion due to the secondary response (49). Activated T cells become responsible for cell-mediated immunity and secrete lymphokines. Some lymphokines activate killer T cells, enabling them to attack an invading organism or cell, and induce macrophages to stay at the site of infection and help to digest the cells they have phagocytosed. Other lymphokines may also have a direct action on organisms by inhibiting proliferation or activating apoptosis.

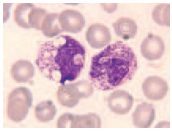
T-helper cells are important in the initiation of a B-cell response to antigens. T-suppressor cells reduce the B-lymphocytic response, and T-cytotoxic cells are capable of directly damaging cells recognized as foreign or virus-infected.

Activated B cells are responsible for humoral immunity. The B cell goes through several stages of development, beginning with the Pro-B cell, followed by the Pre-B cell, which in turn matures into a B cell bearing a mature immunoglobulin-M (IgM) B cell receptor on the cell surface. The population of these early B cells expresses an adhesion and signaling cell-surface molecule called CD5. As these "CD5" lymphocytes mature, and before they encounter a foreign antigen, they shed the CD5 marker and become B2 cells, which co-express IgM and IgD molecules on there cell surface. As B2 cells encounter a foreign antigen, they further differentiate in the presence of co-stimulatory signals either into plasma cells that produce class-specific antibodies (IgG, IgA, IgM, IgD or IgE) of one specificity or into a memory cell (50).

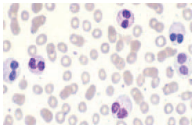
Lymphocyte circulation:

Lymphocytes from the primary lymphoid organs of the marrow and thymus migrate via the blood through postcapillary venules into the substance of lymph nodes and into unencapsulated lymphoid collections of the body and into the spleen. T cells home to the paracortical areas of these nodes and to the periarteriolar sheaths of the spleen. B cells accumulate selectively in germinal follicles of lymphoid tissue, in the subcapsular periphery of the cortex and in the medullary cords of the lymph nodes. Lymphocytes return to the peripheral blood by the efferent lymphatic system and the thoracic duct. The median duration of a complete circulation is about 10 hours. The majority of recirculating cells are T cells. B cells are mainly sessile and spend long periods in lymphoid tissue and the spleen. Many lymphocytes have long life spans and may survive as memory cells for several years (51).

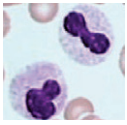
White Blood cells abnormalities



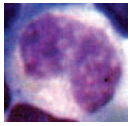
(Fig. 29) Toxic granulation with reddish violet granules. (Wright stain) (Mag. X400)



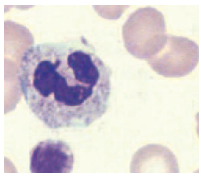
(Fig. 30) Pelger- Huët anomaly. Note the bisegmented nuclei. (Wright stain) (Mag. X100)



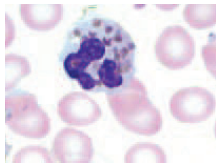
(Fig. 31) Pelger-Huët anomaly. See the band-like forms nuclei. (Giemsa stain) (Mag. X400)



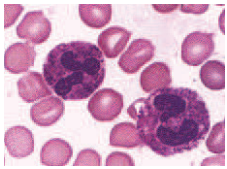
(Fig. 32) Pelger-Huët anomaly with chromatin condensation. (Wright stain) (Mag. X1000)



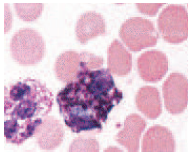
(Fig. 33)May–Hegglin anomaly. Note the giant platelet down-left. (Giemsa stain) (Mag. X1000)



(Fig. 34) ChŽdiak–Higashi syndrome. See the azurophilic granules. (Giemsa stain) (Mag. X1000)



(Fig. 35) Alder–Reilly anomaly. See the deep purple granules. (Giemsa stain) (Mag. X1000)



(Fig. 36) Mucopolysaccharidosis (Giemsa stain) (Mag. X1000)

TOXIC GRANULATION:

This term is used when the normally faint stippled granules in segmented neutrophils stain an intense reddish violet, usually against a background of slightly basophilic cytoplasm; unlike the normal granules, they stain particularly well in an acidic pH (5.4). In toxic granulation the primary granules remain strongly azurophilic; this may be related to a higher concentration of acid mucosubstances than in normal neutrophils (52) (Fig 29). It is an accentuation of normal neutrophilic cytoplasm granules, which become enlarged or appear as short, rod-shaped structures of irregular width, either dark blue-black, or the same color as the nucleus. This phenomenon is a consequence of activity against bacteria or proteins and is non-specific, observed in serious infections, toxic or drug effects, tissue destruction, burns, or autoimmune processes (e.g., chronic polyarthritis), myelodysplasia, and leukemia (32), and occurs with cytokine therapy (G-CSF and GM-CSF).

HERDITARY VARIATION IN WHITE CELL MORPHOLOGY:

Pelger– Huët anomaly:

The most important of various hereditary abnormalities of WBC morphology, is the Pelger-Huët nuclear anomaly (53) (Fig. 30,31, 32). This condition, is a hereditary segmentation anomaly of granulocytes that results in round, rod-

shaped, or bisegmented nuclei. There is also an increase in band-like forms and forms with round or oval nuclei resembling myelocytes. The inheritance is autosomal dominant. It results from a defect in the LBR gene encoding the lamin B receptor (27, 54). The condition appears to be of no clinical significance and the affected cells have not been shown to be functionally abnormal. Occasional unsegmented neutrophils with round nuclei are also seen, particularly during infection or toxic stress response as a nonhereditary condition (pseudo-Pelger formation). Those unsegmented neutrophils are distinguished from myelocytes by a lower nucleocytoplasmic ratio and by the condensation of nuclear chromatin and the maturity of the cytoplasm. 'Pseudo-Pelger' cells occur in acute myeloid leukaemia, myelodysplastic syndromes, agranulocytosis, and in some patients with metastatic tumor to bone marrow, or under conditions of drug toxicity (2).

May–Hegglin anomaly:

In this rare condition, which has a dominant inheritance pattern, abnormal condensations of RNA appear as mildly basophilic inclusions in the neutrophil cytoplasm (Fig.33). The majority of affected individuals has thrombocytopenia and also has giant platelets. Although most have no clinical abnormality, in some patients there are hemorrhagic manifestations (55). Similar cytoplasmic inclusions, which are termed Dhole bodies, may be seen in neutrophils during severe infections and occasionally in normal pregnancy (27) (see Fig. 33).

ChŽdiak–Higashi syndrome

Death usually occurs in this rare disorder, in infancy or childhood as a result of recurrent bacterial infections or hemorrhage. This severe anomaly is associated with giant neutrophil granules (56). The neutrophils of patients with this syndrome display an abnormal fusion of azurophilic primary granules or specific secondary granules with phagocytosing vacuoles (Fig. 34). A similar

granular abnormality is seen in granulopoietic cells in the marrow and in eosinophils, monocytes and lymphocytes and in all cells containing lysosomes. Bacterial killing is compromised since the fusion of the giant lysosomal granules with the phagocytosed bacteria is delayed. The inheritance is autosomal recessive in the gene LYST that codes for a cytoplasmic protein regulating vacuole formation and function (57). Chemotaxis of neutrophils is also affected, apparently due to a defect in the microtubules. Affected children usually have neutropenia and thrombocytopenia, and suffer from recurrent severe infections, especially with Staphylococcus aureus and beta-hemolytic streptococci. Clinical examination frequently reveals partial albinism and marked hepatosplenomegaly.

Alder's (Alder–Reilly) anomaly

This anomaly gives rise to deep purple granules in neutrophils (Fig. 35). Similar abnormal granules are found in other granulocytes, monocytes and lymphocytes. The inheritance is autosomal recessive and the majority of affected individuals have no clinical problems. Similar leucocyte abnormalities are seen in patients with mucopolysaccharide storage disorders, such as Hurler's and Maroteaux–Lamy syndromes, and occasionally in amaurotic family idiocy.

Mucopolysaccharidoses VI and VII

Abnormal granulation of blood granulocytes and monocytes, together with lymphocyte vacuolation, is found in the Maroteaux–Lamy syndrome –, which is also known as mucopolysaccharidosis VI. The white cell abnormality may also be seen in patients with mucopolysaccharidosis VIII (Fig. 36). These lysosomal storage disorders are caused by inherited deficiency of enzymes concerned in the breakdown of acid mucopolysaccharides. Storage-related abnormalities of connective tissue, the heart, the bony skeleton and the central nervous system

produce clinical disabilities similar to, but milder than, those found in classic Hurler's syndrome mucopolysaccharidosis I–H.

Other causes of lymphocyte vacuolation

Similar lymphocyte vacuoles may be found in rare patients with inherited defects of enzymes, which are involved in the catabolism of oligosaccharide components of glycoproteins, for example, mannosidosis, and in the rare Spielmeyer–Vogt syndrome.

Chronic Granulomatous Disease (CGD)

In this disease there is there is an X-linked defect in the gene which code for gp91 ph°x. At a lesser frequency, a defect in the gene for the cytoplasmic p47 ph°x, which is autosomal recessive, is seen. The disease is characterized by a defect in any one of the four subunits of neutrophil NADPH oxidase, which catalyzes the monovalent reduction of oxygen to superoxide anion, which in turn serves as a substrate for H202. Hence, in CGD, the neutrophils are deficient in H202 production, thus they are defective in destroying invading pathogens (58). Normal neutrophils with efficient phagocytic function in contrast with neutrophils in CGD, exhibit dark-blue cytoplasmic staining by classical nitroblue tetrazolium staining test. Patients with CGD are susceptible to recurrent infections by catalase-positive microorganisms such as Staphylococcus aureus, Serratia marcescens, Aspergillus and Nocardia species, and Burkholderia cepacia. These catalase-positive organisms can inactivate their endogenous H202, and thus are resistant to the CGD H2O2-deficient neutrophil attack. In contrast, CGD patients rarely have infections with catalase-negative organisms. Mortality rate is higher in the X-linked form of CGD.

LEUKOCYTOSIS:

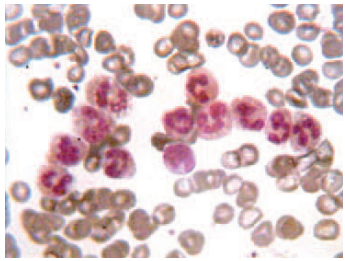
The term leucocytosis refers to an increase in white blood cells (usually to above 10 ×10 3/mm 3) (13). Leucocytes comprised a number of different types of cells, which are not necessarily related to each other biologically, and increases in their numbers generally have different causes. The most frequent cause is an increase in blood neutrophils. Most cases of infectious leukocytosis are associated with a left shift and neutrophils often show a toxic granulation. Other leucocytosis involves a predominance of one of the other white cell types found in the blood. Leukocytosis with a predominance of segmented neutrophilic granulocytes without the less mature forms is called granulocytosis or neutrophilia. Causes of leucocytosis are seen in (Table 4).

Causes of Leukocytosis

Malignancies and chronic diseases	Physiological & Metabolic causes	Hematologic disorders and infectious causes	Drugs & toxic substances
Malignant neoplasms Leukemia and myeloproliferative disorders Carcinoma Lymphoma Sarcoma Connective tissue diseases Rheumatic fever Rheumatoid arthritis Inflammatory bowel disease	Ovulation, labor, pregnancy Emotional disorders; fear, agitation Newborn (maximal 38,000/mm3) Strenuous exercise Splenectomy, functional asplenia Anoxia Azotemia Acute gout Burns Seizures Acidosis Diabetic coma Thyroid storm	Hematologic diseases Hemolytic anemia Transfusion reaction Acute hemorrhage Megaloblastic anemia during therapy Bacterial, viral, fungal, protozoal, spirochetal Acute infections	Drugs Steroids Epinephrine Serotonin Histamine heparin, acetylcholine Endotoxin mercury Camphor Lithium Lead Poisoning

(Table 4)

Neutrophil leucocytosis (neutrophilia)



(Fig. 37) Neutrophilia

(Wright stain) (Mag. X400)

An increase in neutrophils in the blood of more than 7.5 ×10 3/mm 3 is one of the most frequent abnormalities found in blood counts and blood films (Fig. 37). Causes are:

-Acidosis of various etiologies, e.g., nephrogenic diabetes insipidus.

-All kinds of toxic stress, e.g. uremia, diabetic acidosis, acute gout attacks, and convulsions.

-Tissue necrosis, e.g., burns, abscess, trauma, hemorrhage, infarction, carcinomatosis, active alcoholic cirrhosis, or surgery and after myocardial or pulmonary infarction.

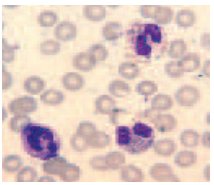
- Medications e.g., drugs, or noxious chemicals, such as Nicotine, Corticosteroids, Adrenaline, Digitalis, Allopurinol, Barbiturates, Lithium, Streptomycin and Sulfonamide.

- Some persons also have an idiopathic or familiar increase in leukocytes.

- Other etiologies, e.g., pregnancy, connective tissue diseases and cigarette smokers, heavy smokers are reported to have total WBC counts that average 1,0 ×10 3/mm 3 or even more above those for nonsmokers (2).

Clinically there is often fever due to the release of leucocyte pyrogen. In most neutrophilias there is an increase in the number of band forms; occasionally, the so-called left shift with more primitive cells such as metamyelocytes and myelocytes appear in the peripheral blood. In most causes of reactive neutrophil leucocytosis, toxic changes appear in the neutrophil cytoplasm and on occasions there are Dhole bodies. The neutrophil alkaline phosphatase score is characteristically elevated. Although neutrophilia is the characteristic response to bacterial infection, this is not invariable. Certain infections are characterized by either a normal white cell count (WBC) or even leucopenia and neutropenia (Table 4).

Eosinophil leucocytosis (eosinophilia)



(Fig. 38) Eosinophilia

(Wright stain) (Mag. X400)

Eosinophilia is the term applied to an increase in blood eosinophils above 0.4 ×10 3/mm 3 (Fig. 38), while eosinophils regulate the immune response, especially the defense against foreign proteins. Eosinophilia arises in a number of medical conditions, among which parasitic diseases involving tissue-invasive helminthes and allergic disorders, including atopy and drug reactions,

are by far the most commonly observed in developing and industrialized countries, respectively.

Eosinophils react against parasites and most often, are elevated with infestation by various flukes. With roundworms infection, such as Ascaris, Strongyloides, and Trichinella (Trichina) eosinophilia is apparent. The condition known as

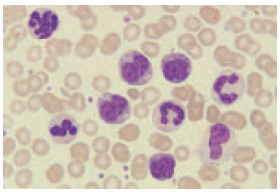
visceral larva migrans, caused by the nematode Toxocara canis (common in dogs) is sometimes seen in humans associated with high eosinophils. Further disorders with eosinophilia are IgA deficiency, Addison's disease, disorders of autoimmunity, vasculitis (especially Churg-Strauss syndrome), and eosinophilic gastroenteritis. In Certain bacterial infections, eosinophilia may occur e.g., in scarlet fever and brucellosis. An eosinophilia is a frequent sign of allergic reactions. There are numbers of pulmonary eosinophilic syndromes of varying severity, characterized by transient pulmonary infiltrates, cough, fever and peripheral eosinophilia. Steroid treatment usually results in resolution of symptoms and prompt clearing of the infiltrates. Similar pulmonary changes may occur in some parasitic infestations when migrating parasites lodge in the lungs. In miscellaneous conditions, eosinophilia is reported in 20% of polyarteritis nodosa cases and 25% of sarcoidosis patients. Eosinophilia has also been reported in up to 20% of patients with Hodgkin's disease. Eosinophils have been associated with inflammation of the gastrointestinal tract including eosinophilic esophagitis, eosinophilic gastroenteritis, and eosinophilic colitis. Eosinophilic gastroenteritis is also associated with peripheral blood eosinophilia. Eosinophilia is commonly seen in patients with acquired immunodeficiency syndrome. In those infected individuals, it may relate to intercurrent parasitic infestation. In 1989, an epidemic of eosinophilia-myalgia syndrome occurred in the United States secondary to the ingestion of L-tryptophan. The eosinophilia-myalgia syndrome is a multisystem disorder, which was found to be caused by contaminants in some L-tryptophan preparations used as nutritional supplements (13). The clinical signs of the eosinophilia-myalgia syndrome were contractures of the skeletal muscles, scleroderma-like skin lesions, and damage to the heart and lung. The pathophysiology of eosinophilia is the production of interleukin (IL)-5 mediated

by activated T-lymphocytes. Patients treated with the cytokines GM-CSF, IL-2, and IL-3 often develop eosinophilia.

Basophil leucocytosis (BASOPHILIA)

A basophil leucocytosis (>50 basophils/mm3) is seen most frequently in patients with polycythaemia Vera, chronic granulocytic leukaemia where it is a sign of acceleration or impending blast crisis. Basophils may be increased in other "myeloproliferative" diseases. Basophil leucocytosis can occur during many infections, in allergic reactions, as a sign of thyrotoxicosis, in systemic mastocytosis. Due to their role in anaphylactic reactions, elevated basophil counts are seen above all in hypersensitivity reactions of various kinds. Moderate increases in blood basophils also occur in myxoedema, chickenpox, smallpox and ulcerative colitis.

LEUKAEMOID REACTION:



(Fig. 39) Leukaemoid reaction

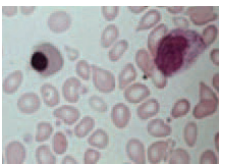
Note the immature neutrophils presence.

(Wright stain) (Mag. X400)

It is a hematological abnormality that simulates leukaemia and thus may be confused with it. The leukaemoid reaction is defined as a nonleukemic WBC count more than 50 ×10 3/mm 3 or a differential count with more than 5% metamyelocytes or earlier cells. It is a benign case but with excessive leucocytosis characterized by the presence of immature cells (blasts, promyelocytes and myelocytes) in the peripheral blood. Whereas most leukaemoid reactions involve blood granulocytes (Fig. 39), lymphocytic

reactions also occur. The majority of these reactions occur in association with severe or chronic infections, severe toxic states (burns, tissue necrosis, etc.). They sometimes manifest as a feature of widespread metastatic cancer or severe haemolysis, and juvenile rheumatoid arthritis. Leukaemoid reactions occur more frequently in children. Children with Down syndrome sometimes have a leukemoid reaction in the first few months of life, which later subsides spontaneously. The main problem is to distinguish these reactions from chronic granulocytic leukaemia. Changes such as toxic granulation, Dhole bodies and a high neutrophil alkaline phosphatase score, are characteristically found in leukaemoid reactions, while large numbers of myelocytes and the presence of the Philadelphia chromosome indicate chronic granulocytic leukaemia.

LEUCOERYTHROBLASTIC REACTION:



(Fig. 40) Leukoerythroblastosis reaction

Note the metamyelocyte in the upper right and the nucleated RBC in the left.

(Wright stain) (Mag. X400)

Can be defined as the presence of both immature WBCs (metamyelocytes or earlier cells) and nucleated RBCs in the peripheral blood smear. This reaction is most frequently found when there is a distortion of marrow architecture, due either to proliferative disorders of the marrow like in osteomyelosclerosis, or marrow infiltrations, or to the presence of extramedullary erythropoiesis. Approximately 25%-30% of patients with leukoerythroblastosis have metastatic tumor in the bone marrow, about 20% have leukemia, about 10% have myeloid metaplasia or polycythaemia vera, and about 8% have hemolytic anemia. Severe acute hemorrhage account for about 5%.

NEUTROPENIA (GRANULOCYTOPENIA):

Neutropenia is defined by a blood WBC count less than 4,0 ×10 3/mm 3 and neutrophil count of less than 2.5 ×10 3/mm 3 (13). It should be noted, however, that many African and Middle Eastern populations have normal ranges with significantly lower limits than this. Clinical problems related to recurrent infections are associated with absolute levels below 1.0 x 10 3/mm 3, and neutrophil counts of less than 0.2 ×10 3/mm 3 carry very high risks. Neutropenia may be selective or part of a general pancytopenia. . Agranulocytosis is present if granulocytes are below 0.5 ×10 3/mm 3. Conditions associated with neutropenia include: 1- conditions associated with pancytopenia, such as megaloblastic anemia, aplastic anemia, acute or aleukemic leukemia, hypersplenism of varying etiology (e.g., cirrhosis, systemic lupus, Gaucher's disease), and paroxysmal nocturnal hemoglobinuria, 2- drug-induced neutropenia (agranulocytosis), 3- certain infections, such as typhoid, some viral infections (e.g., Epstein-Barr, in the first week of illness, and the hepatitis viruses), overwhelming bacterial infection (septicemia, miliary tuberculosis), and 4- cyclic and chronic idiopathic neutropenia. Congenital neutropenias occur in several syndromes such as reticular dysgenesis. Kostmann syndrome, which is defined as a congenital agranulocytosis. Defects in the *ELA-2* gene encoding neutrophil elastase were identified in about half of cases with severe congenital neutropenia (SCN). Myelokathexis, a rare disorder with morphological aberrations of neutrophils, also manifests as neutropenia. Alloimmune neonatal neutropenia is observed in less than 0.3% of pregnancies and caused by the transplacental transfer of immunoglobulin (Ig) G antibodies to neutrophils. It resolves by 6–8 wk after delivery.

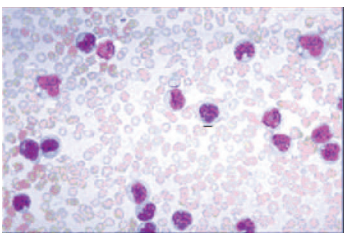
The majority of neutropenias are caused by reduced granulopoiesis; but, in some patients, the reduced neutrophil counts are caused by increased removal

of neutrophils by the reticuloendothelial system or by shift of neutrophils from the circulating population to the other tissues. Bone marrow examination is essential in all patients presenting with severe neutropenia.

In many, there will be evidence of leukaemia or other infiltrations. In those with selective depression of granulopoiesis there is a reduction in granulocyte precursors. In some cases there is absence of granulopoietic cells but in others promyelocytes and myelocytes are present with no evidence of mature neutrophils. Acute severe neutropenia is usually managed with antibiotics, colony-stimulating factors (primarily granulocyte colony stimulating factor [G-CSF]), or a combination of these agents. Acute febrile neutropenia is managed primarily with prompt administration of broad-spectrum antibiotics. In chronic neutropenia, no therapy may be necessary however, patients with severe chronic neutropenia and recurrent fever and infections benefit from long-term treatment with G-CSF (59).

In Felty's syndrome: About one percent of patients with rheumatoid arthritis have associated splenomegaly and neutropenia. Some of these patients also show skin ulceration over the anterior surface of the tibia. The neutropenia in Felty's syndrome is thought to result from anti-neutrophil autoantibodies; the bone marrow characteristically shows increased granulopoiesis. In patients with recurrent infections, splenectomy often results in a return to normal of blood neutrophil numbers.

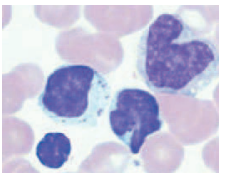
MONOCYTOSIS:



(Fig. 41) Monocytosis
(Wright stain) (Mag. X100)

Monocytosis by definition occurs when monocytes count in peripheral blood (>1.0 ×10 3/mm 3) is observed. Monocytosis is observed during some infections like tuberculosis, kala-azar, sub-acute bacterial endocarditis, disorders of autoimmunity (Fig 41). A relative monocytosis is common during recovery from cytostatic therapies and after bone marrow transplantation. As phagocytic defense cells, an elevation of the monocyte population above 7% and above 850/µl indicates an immune defense reaction. Monocytosis in cases of infection is always present at the end of acute infections (32). It becomes chronic especially in endocarditis lenta, listeriosis, brucellosis, tuberculosis, monocytosis and in cases of a non-infectious response, e.g., collagenosis, Crohn disease, ulcerative colitis. Monocytosis is also present in neoplasia, e.g., paraneoplastic in cases of disseminating tumors, bronchial carcinoma, breast carcinoma, Hodgkin disease, and myelodysplasias. Phagocytosis of erythrocyte and white blood cells (hemophagocytosis) may occur in some viral infections and autoimmune diseases. Since cells are phagocytic, they are occasionally found to have ingested red cells, cryoglobulin, microorganisms, malarial pigment, amastigotes of leishmania parasites and rarely, melanin or bilirubin (27).

LYMPHOCYTOSIS:



(Fig. 42) Lymphocytosis

Note a couple of LGL cells in the middle, one normal
lymphocyte in the bottom and a monocyte in the upper right.
(Wright stain) (Mag. X1000)

Lymphocytosis is present if the lymphocyte count in peripheral blood is increased to more than 4.0 ×10 3/mm 3 (13) (Fig 42). Lymphocytosis is most commonly associated with a normal or a decreased total WBC count. In fact, lymphocytosis seen in the majority of viral infections is actually a relative type due to a decrease in granulocytes, while total (absolute) lymphocyte numbers remain constant. A real (absolute) lymphocytosis with leukocytosis occurs in Infants with pertussis and in children with acute infectious lymphocytosis, (an unusual viral disease), in lymphocytic leukemia, and in some infants with adenovirus infection. They may all have very high lymphocyte counts. Lymphocytosis also is observed during many bacterial infections: for example, rickettsiosis, brucellosis, tuberculosis and shigellosis. Patients who are treated with the cytokine interleukin (IL)-2 develop an increase in the number of lymphocytes as a result of the proliferation of CD16+ natural killer cells. Lymphocytoses with large numbers of atypical or 'reactive' cells are most often seen in infectious mononucleosis and in other viral illnesses including infectious hepatitis, and in toxoplasmosis. Lymphatic cells in lymphocytosis, show a wide variability and transform easily. This is usually seen as enlarged nuclei, a moderately loose, coarse chromatin structure, and a marked widening of the basophilic cytoplasmic layer. Clinical findings, which include acute fever symptoms, enlarged lymph nodes, and sometimes exanthema, help to identify a lymphatic reactive state. Unlike the case in acute leukemias, erythrocyte and thrombocyte counts are not significantly reduced. Although the granulocyte

count is relatively reduced, its absolute value rarely falls below the lower limit of normal values.

In the following non-malignant diseases, normal Lymphocytes morphologically predominate in the blood analyses:

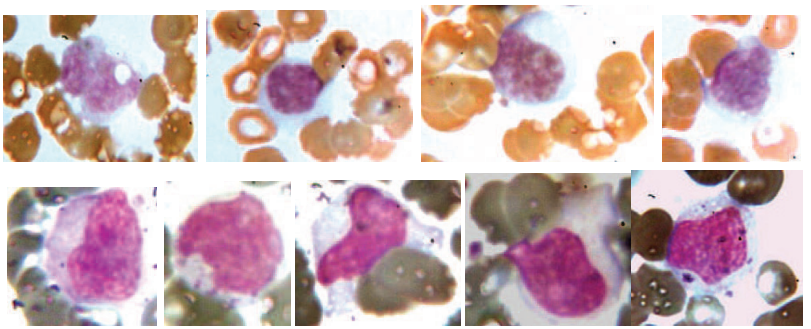
- Whooping cough (pertussis) with clear leukocytosis and total lymphocyte counts up to 20 and even 50 ×10 3/mm 3; occasionally, slightly plasmacytoid differentiation.
- Infectious lymphocytosis, a pediatric infectious disease with fever of short duration. Lymphocyte counts may increase to 50 ×10 3/mm 3.
- Chickenpox, measles, and brucellosis, in which a less well-developed relative lymphocytosis is found, and the counts remain within the normal range.
- Hyperthyroidism and Addison disease, which show relative lymphocytosis.
- Constitutional relative lymphocytosis, which can reach up to 60% and occurs without apparent reason (mostly in asthenic teenagers).
- Absolute granulocytopenias with relative lymphocytosis.

Transformed, “stimulated” lymphocytes predominate in the CBC in the following diseases with reactive symptoms:

- Lymphomatous toxoplasmosis, which does not usually involve significant leukocytosis. Slightly plasmacytoid cell forms are found.
- In rubella infections the total leukocyte count is normal or low, and the lymphocytosis is only relatively developed. The cell morphology ranges from basophilic plasmacytoid cells to typical plasma cells.
- In hepatitis the total leukocyte and lymphocyte counts are normal. However, the lymphocytes often clearly show plasmacytoid transformation.
- The most striking reactive changes in lymphocyte morphology are seen in infectious mononucleosis, an illness

caused by an acute infection by the Epstein–Barr (EB) virus (60), or cytomegalovirus [CMV] infection (13).

Infectious mononucleosis:



(Figs. 43) Infectious mononucleosis

These figures reveal the different manifestations of the reactive atypical lymphocytes seen in peripheral blood films obtained from patients infected with infectious mononucleosis.

(Wright stain) (Mag. Upper figrs. X400 -Lower figrs. X1000)

Infectious mononucleosis (glandular fever) is a disorder characterized by sore throat, fever, lymphadenopathy and atypical lymphocytes in the blood. It is predominantly a disease of adolescents and young adults. Suggested criteria alerting laboratory staff to the possibility of infectious mononucleosis is lymphocytes comprising at least 50% of peripheral blood leucocytes and atypical lymphocytes comprising at least 10% of circulating lymphocytes (27). In infectious mononucleosis, varying proportions of the mononuclear cells (more than 20%) may be extensively transformed into round cells (Pfeiffer cells, virocytes) (32). The chromatin of these stimulated lymphocytes has changed from a dense to a looser, more irregular structure, and nuclei are two- to three-fold larger than those of normal lymphocytes. The cytoplasm is vacuolated, relatively wide and basophilic. The degree of transformation and

the proportions of the different cell morphologies change almost daily. The disease is the result of infection with Epstein–Barr (EB) virus. In affected patients, heterophil antibodies against sheep red cells are found in the serum at high titers “Paul–Bunnell test”

Infectious mononucleosis is characteristically accompanied by an increase of CD8+ lymphocytes. Most patients present with lethargy, malaise and fever. On examination the majority show lymphadenopathy. There is usually generalized inflammation of the oral and pharyngeal surfaces with follicular tonsillitis, and some patients show palatal petechiae. There may be periorbital and facial oedema or a rash .Palpable splenomegaly occurs in over half the patients. Occasionally there are subcapsular haematomas of the spleen, which have a tendency to rupture. Jaundice due to liver involvement occurs in a minority of patients.

The diagnosis is suspected by finding a moderate lymphocytosis (10–20 ×10 3/mm 3) and large numbers of atypical lymphocytes in the peripheral blood film. A number of conditions, including toxoplasmosis, infectious hepatitis, HIV infection and follicular tonsillitis, acute leukaemia are likely to create initial problems of diagnosis. Absence of a quantitative reduction of hematopoiesis in all the blood cell series, however, makes leukemia unlikely, Fine-needle aspiration cytology and lymph node biopsy of affected nodes may be helpful. In infectious mononucleosis the cytology is dominated by reactive lymphocyte changes while in toxoplasmosis, characteristic small groups of histiocytes may be found.

PRIMARY IMMUNODEFICIENCY DISORDERS:

Defect in the adenosine deaminase gene (ADA deficiency) that occurs as an autosomal recessive on chromosome 20, can cause severe combined immunodeficiency (SCID), due to absence of T cells, B cells, and natural killer cells (T cell-negative, B cell-negative, natural killer cell-negative). The T- and B-lymphocyte systems fail to develop. Lack of the enzyme adenosine deminase results in the accumulation of adenosine and toxic deoxyadenosine nucleotides. The latter can cause apoptosis of lymphocytes. Lymphocyte counts can be as low as 0.5 ×10 3/mm 3, affecting primarily T cells which are absent. There is severe lymphopenia and hypogammaglobulinaemia. There is atrophy of the thymus; the lymph nodes and spleen are small and devoid of lymphoid cells. Affected infants fail to thrive and die early in life from recurrent infections, such as Pneumocystis carinii, cytomegalovirus, other viruses, fungi and bacteria. While therapy of missing enzyme has been shown to effect improvement, bone marrow transplantation is the preferred treatment. Most recently, ADA deficiency has been treated successfully by 'gene therapy' – in which the ADA gene has been introduced in vitro into the patient's lymphocytes, which have then been reinfused. Milder forms of adenosine deaminase deficiency have been reported.

Deficiency of the enzyme, purine nucleoside phosphorylase, causes a more selective lack of T cells.

Another defect effecting B cells occurs in the gene that codes for the mRNA-editing enzyme known as activation-induced cytidine deaminase. As a result, hyper-IgM syndrome can arise due to an intrinsic B cell defect that prevents B cells from switching to production of immunoglobulins other than IgM.

In patients with X-linked agammaglobulinemia, a defect in a gene that codes for the Bruton tyrosine kinase, a cytoplasmic protein tyrosine kinase that is

necessary for the growth of B cell precursors and their maturation, results in the absence of circulating B cells (50).

In a very rare syndrome of lymphoreticular dysgenesis, there is failure of development of both the reticuloendothelial and lymphoid systems. Affected infants die soon after birth from overwhelming infection. Marked lymphopenia, and stigmata of splenic atrophy may be found in the peripheral blood.

Agammaglobulinemia or hypogammaglobulinemia is a defect in genes that code for immunoglobulin light or heavy chains or their associated signaling molecules.

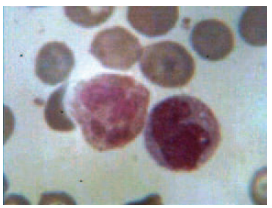
X-Linked hyper-lgM syndrome is a defect in gene coding for CD154 on activated T cell, which interacts with receptor on B cells (CD40). B cells make only IgM. Defect is affecting switching of IgM to other immunoglobulins.

X-Linked severe combined immunodeficiency is a defect in gene coding for y chain of IL-2 receptor.

Wiskott-Aldrich syndrome (WAS) is a defect on X chromosome involved in coding for protein regulating microvesicle formation. It is manifested with complete absence of antibodies to blood group antigens. Small defective platelets, thrombocytopenia and immunodeficiency.

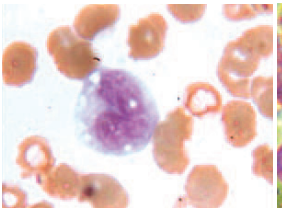
Ataxia telangiectasia is a defect in gene on chromosome 11, which codes for protein involved in signal transduction, DNA repair, and control of cell cycle. Defect appears in cell-mediated immunity. Cerebral ataxia and telangiectasias are hallmarks.

ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

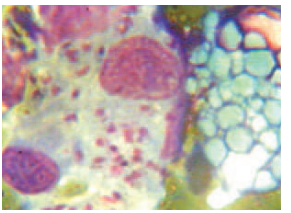


(Fig. 44)

Peripheral blood films of a healthy carrier of the (HIV virus) showing a lymphocyte with bilobed or convoluted nucleus. Note the hyper segmented Neutrophil in fig. 44 (Wright stain) (Mag. X400)



(Fig. 45)



(Fig. 46)

LD bodies (Leishman- Donovan bodies) in Patient with AIDS. (Wright stain) (Mag. X400)

AIDS remains a monumental problem, particularly in the developing world. There have been in the United States, more than 700,000 cases of AIDS diagnosed since 1981 and more than 400,000 deaths. More than 40 million people, According to the World Health Organization are currently living with AIDS globally. More than 25 million of these live in sub-Saharan Africa. Recently, many of the hematologic complications of HIV infections are less prominent because of the of highly active antiretroviral therapy (HAART) application.

This syndrome is caused by infection with the human immunodeficiency virus (HIV), a retrovirus of the lentivirus subgroup, first identified in 1983, is transmitted by sexual contact, transfusion or parenteral inoculation of infected blood, intravenous drug use, and vertical transmission from an infected mother to infant. AIDS is particularly common in homosexual men but is also seen frequently in heterosexual contacts of AIDS cases. Cytopenia, being one important hematological characteristic of the disease, once common, is now seen less frequently except in patients with advanced disease and in patients

undergoing chemotherapy. Non-Hodgkin lymphoma (NHL) still poses challenges in management and leads to death in most of those affected (9). The clinical outcome of infection has been classified into four stages or groups. Factors that determine the progression from one stage to another remain uncertain. A prodromal period of about 6 weeks follows initial infection. HIV initial infection causes an acute illness at the time of seroconversion. The acute illness can resemble infectious mononucleosis both clinically and haematologically but in general; the number of atypical lymphocytes (Figs. 44,45) is considerably less (27). A proportion of patients pass through the asymptomatic and persistent lymphadenopathy stages to the AIDS-related complex (ARC) and fully developed AIDS.

HIV produces its predominant effects through infection of T- helper (CD4+) cells. The Centers for Disease Control and Prevention (CDC) defines individuals as having AIDS using laboratory criteria: evidence of HIV virus and CD4 counts less than 200 cells/µl. The mechanism of infection is still not completely understood but is influenced by host factors, the presence of specific receptors on individual cell lines, and the strain of virus. Chemokine receptor expression can influence the susceptibility of the cell to viral infection and may account for the vulnerability of hematopoietic cells of different lineages to HIV infection. Intracellular signaling events after the interaction between chemokine receptors and chemokines and the HIV envelope may be important for viral entry. The CD4 antigen appears to be the main receptor for HIV, and CD4+ antigen-presenting cells are also an important site for viral replication. Direct infection by the virus of other cells, for example brain cells, also accounts for some of the pathology of the disease. Some CD4+ cells are lysed directly by replicating HIV, but the virus remains latent in most host cells, unrecognized by

the patient's immune system. When such latently infected T cells are activated, the virus replicates and cell death follows.

Currently, most HIV testing procedures rely on detecting HIV antibodies, to one or other HIV surface antigen, although more recent procedures that have been implemented for testing blood for transfusion rely on detection of HIV antigens or nucleic acids. The most widely used test, the enzyme-linked immunosorbent assay (ELISA), is used in conjunction with a confirmatory test, the Western blot (WB), for HIV antibody. A person is classified as HIV seropositive when a blood sample results in a positive result in two successive ELISAs and in one confirmatory WB or an equally sensitive and specific confirmatory test.

In HIV positive patients with chronic infection, there is a progressive decline in the number of CD4-positive lymphocytes and, usually, a decline in the total lymphocyte count and an alteration in the T lymphocyte subsets, with a fall in the CD4+/CD8+ (helper/suppressor) ratio from the normal value of 1.5–2.5:1 to <1:1.The declining CD4-positive lymphocyte count is associated with a progressive decline in immune function, which eventually leads to infection or neoplasia. The CD4 cells are destroyed through apoptosis and through direct cytolysis by the virus. Apoptosis of lymphocytes results from many factors, including cytokine dysregulation, continued immune stimulation, and exposure to viral proteins.

A polyclonal rise in serum immunoglobulins is often found in some cases with a paraprotein present. In HIV infection, some neutrophils may show reduced granulation, others may show binucleate macropolycytes and macropolycytes with an open chromatin pattern. Circulating giant metamyelocytes, cells that are characteristic of megaloblastic anemia and are usually seen only in the bone marrow may also be seen in the peripheral blood (27). Lymphocytes with

convoluted nuclei, resembling Sézary cells, may occur in HIV infection (Figs 44,45). Hematological abnormalities may include anemia, neutropenia or thrombocytopenia. Roughly 80% of patients develop anemia, at all stages of the disease. 20% of asymptomatic patients are anemic; 5%of patients with a CD4 count of > 700/mm3 are anemic, 50% of early symptomatic patients are anemic and 75% of later symptomatic patients are anemic (61). Hematological abnormalities in HIV individuals are often autoimmune in origin but are sometimes due to direct infection of haemopoietic stem and progenitor cells in the bone marrow. The cytopenias may be due to dysplastic changes, marrow lymphoma or fibrosis. A number of studies, including the MultiCenter AIDS Cohort study, demonstrated that anemia was an independent predictor of survival. Treatment with G-CSF or GM-CSF may be effective as an aid in management of chronic neutropenia due to HIV infection (59).

Wide spectrums of opportunistic organisms cause infections in AIDS patients, including Pneumocystis carinii, cytomegalovirus, atypical mycobacteria, Cryptococcus, histoplasmosis, and leishmaniasis (Fig. 46). Cytomegalovirus (CMV), B19 parvovirus, or Mycobacterium avium-intracellulare suppress erythropoiesis and are potentially treatable causes of anemia in this population.

Often non-specific granuloma is also found in AIDS patients. A proportion of patients develop Kaposi's sarcoma, a vascular skin tumor of endothelial cell origin; others may develop non-Hodgkin's lymphoma (NHL). NHL is the AIDS-defining condition in approximately 3% of HIV-infected persons, and is likely to be high grade and with a 20 percent incidence of lymphoma involvement in the central nervous system. The development of these malignancies is related to a number of factors, including immunosuppression and concurrent infections with other viruses such as human herpes virus-8 (HHV8) and Epstein-Barr virus (EBV), which foster malignant transformation.

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