LECTURE: 15

Title **NEUTROPHIL, BASOPHIL, EOSINOPHIL, AND PLATELETS *SURFACE RECEPTORS***

LEARNING OBJECTIVES:

The student should be able to:

- **Determine the relative percentages in blood for the various types of polymorphonuclear granulocytes PMNs (e.g., Neutrophil, Basophil, and Eosinophil), platelets, and mast cells.**
- **Determine the locations where PMNs are mainly found.**
- **Enumerate some of the granulocyte's cell surface molecules and their importance such as:**

- **Receptor for Fc portion of the immunoglobulin G (FcγR).**
- **CD10 (CALLA, neut5ral endopeptidase).**
- **CD11a lymphocyte functional antigen [LAF-1 (alpha-chain)].**
- **Complement receptor 3 "CR3" CD11b (alpha-chain).**
- **Complement receptor 4 "CR4" CD11c (alpha-chain).**
- **Immunoglobulin E receptor (FcεRI) on eosinophil and basophil.**
- **CD45RB, CD45RO, CD46, CD47, and CD48.**

- **Describe the roles that platelets play in immunity.**
- **Enumerate some important receptors expressed by platelets and their importance such as:**

- **Class I major histocompatibility MHC.**
- **Receptor for Fc portion of the immunoglobulin G (FcγRII).**
- **Low affinity receptor for FcεRII (CD23).**
- **Receptor for Factor VIII.**
- **CD 41 GpIIb/IIIa complex.**
- **CD 42 GpIb/GpIx complex.**
- **CD107a (LAMP-1), and CD107b LAMP-2).**

- **Enumerate some important receptors expressed by mast cells and their importance such as:**

- **Receptor for immunoglobulin E (FcεR I).**
- **Receptor for complement protein C3a, and C5a anaphylatoxins.**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 2. pp. 15-23.

POLYMORPHONUCLEAR GRANULOCYTES AND MAST CELLS

These cells are produced in the bone-marrow, at a rate of 80 million per minute and are short-lived (2-3 days) compared to mono/and macro which may live for months or years. PMNs represents about 60-70% of blood leukocytes. Like monocytes PMNs can adhere to endothelial cells lining the blood vessels and squeeze between them to scape from the blood vessels (this process is called diapedesis). The adhesion is mediated by the receptors on the granulocytes and ligands on the endothelial cells, and promoted by the chemo-attractant such as IL-8. Granulocytes play an important role in the acute inflammation; their predominant role is the phagocytosis.

Granulocytes {Polymorphs (PMNs)} are produced in the bone marrow at a rate of ≈ **80 million per minute**.

What you should generally know about granulocytes?

♦ PMNs are short-lived (2-3 days) compared to monocytes / macrophages which may live for months or years.
♦ They represent about 60-70% of the total normal blood leukocytes.
♦ PMNs are found mainly in the extravascular sites.
♦ They travel to the out the vascular system through the process of diapedesis {like monocytes, PMNs can adhere to the endothelial cells lining the blood vessels and extrvasate (squeeze between them to escape from the blood vessels)}
♦ The adhesion is mediated by receptors on the granulocytes and ligands on the endothelial cells and is promoted by chemo-attractant such as IL-8.
♦ PMNs play important role in acute inflammation through phagocytosis (usually synergizing with abs and complement in protection against microorganisms).
♦ The importance of these cells can be recognized in individuals with reduced granulocytes numbers, or with rare genetic defects with prevents Extravasation in response to chemotactic stimuli. Both defects dramatically increase susceptibility to infection.

What are the several different types of polymophonuclear granulocytes?

PMNs granulocytes are classified into:

1. Neutrophils
2. Eosinophils
3. Basophils & mast cells

NEUTROPHILs

These cells represent about 90% of the circulating granulocytes. They have a characteristic multilobed nucleus and are 10-20 μm in diameter. The activated protein fragments of the complement are the chemotactic agents of the neutrophils (e.g., C5a) Chemotactic agents for neutrophils include factors derived from Fibrinolytic and kinin system, the products of other leukocytes and platelets, and the products of certain bacteria. Chemotactic stimuli result in neutrophil migration (adhesion to endothelial cells) and diapedesis. These events occur in three of more steps:

*** Rolling and arrest on endothelial cells mediated by selectins and their ligands.**
*** Activation mediated by cytokines released by T cells and macrophages.**
*** Firm adhesion established by leukocyte integrins and their ligands expressed on the activated endothelial cells (e.g., CR3/ICAM-1), this lead to extravasation of the activated leukocyte.**

Neutrophils contain two types of granules. The primary (azurophilic) granules are lysosomes containing acid hydrolases **(Figure-1)**. Myeloperoxidase and muramidase (lysozyme). The secondary specific granules, contains lactoferrin in addition to the lysozymes. The mico-organisms are destroyed within the phagolysosome, which is formed from the fusion of lysosome into phagosomes. Extracellular release of granules and cytotoxic substances by neutrophils can also occurs when they are activated through the Fcγ receptors by immune complexes. The cell surface molecules present in neutrophils are; CR1, CR3, FcγRII, and FcγRIII. Neutrophils do not express FcεRI, FcεRIII.

1. Neutrophils have a characteristic multilobed nucleus.
2. They are 10-20 μm in diameter.
3. Neutrophils possess two types of granules:

A. The primary (azurophilic) granules are lysosomes containing acid hydrolases, myeloperoxidase and muramidase (lysozyme).
B. The secondary (specific) granules contain lactoferrin in addition to lysozyme.

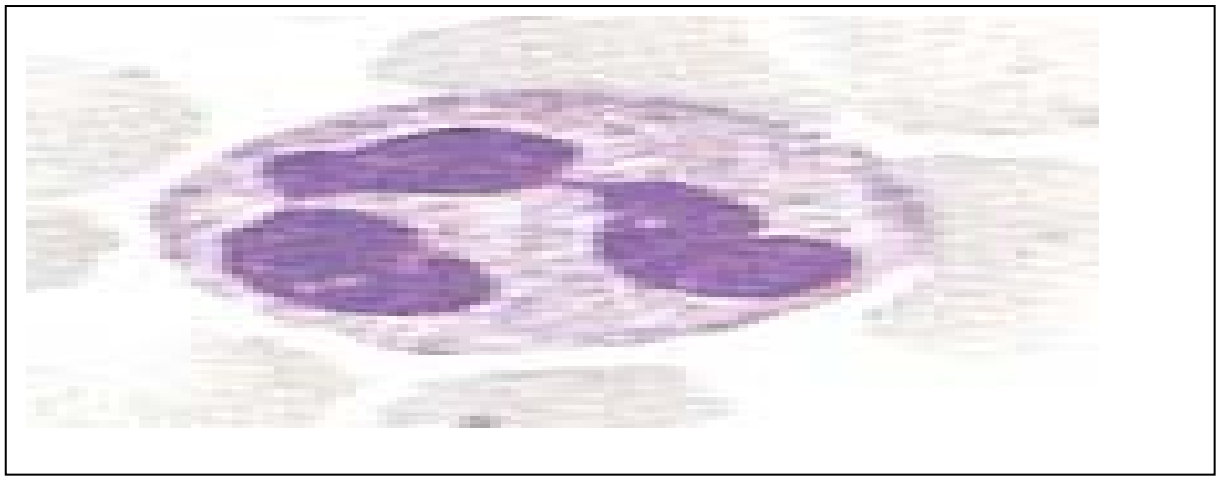

Figure-1 Blood neutrophils show the azurophilic (primary) granules are larger than the secondary (specific) granules. The majority are specific granules. Neutrophils represent about 95 % of the circulating granulocytes. They have a characteristic of multilobed nucleus.

Ingested organisms are contained within vacuoles termed phagosomes, which fuse with the lysosomes to form phagolysosome. Neutrophils migrate into the inflamed are via the effect of the chemotactic agents, these agents includes:

1. Proteins fragments released when complement is activated (e.g., C5a).
2. Factors derived from the Fibrinolytic and kinin systems.
3. The products of other leukocytes and platelets.
4. The products of certain bacteria.

Chemotactic stimuli result in neutrophil margination (adhesion to endothelial cells) and diapedesis.These events occur in three or more sequential steps:

♦ Rolling and arrest on endothelial cell mediated by selectins and their ligands.
♦ Activation mediated by cytokines released by per vascular T cells and macrophages.
♦ Firm adhesion established by leukocyte integrins and their ligands expressed on activated endothelial cells (e.g., CR3/ICAM-1). This last step leads to Extravasation of the activated leukocyte.

EOSINOPHILs

These cells are usually bilobed nucleus and many cytoplasmic granules. The comprise 2-5 % of blood leukocytes in healthy non-allergic individuals. Eosinophils are particularly prevalent in parasitic infections. They are capable of phagocytosis and kill parasites and a variety of micro-organisms. It is about 12 micrometer in diameter. The granules are membrane-bound organelles with crystalloid cores. Degranulation will occurs after the stimulation of the eosinophils with certain stimuli, which cause the granules fuse into the cytoplasmic membranes and release their contents into the surrounding area when the object is large to be phagocytosed. Eosinophils are attracted to their targets by the chemotactic factor of anaphylatoxins **"eosinophils chemotactic factor of anaphylaxis (ECF-A**) released from T cells, mast cells and basophils. They bind to schistosomules coated with IgG or IgE and degranulate releasing toxins known as "major basic protein". Eosinophils release histamine and arylsulphatase, which inactivate the mast cell products histamine and slow reactive substance of anaphylaxis (SRS-A). The effect of the eosinophil factors is to dampen down the inflammatory response and reduce granulocyte migration into the site of invasion. Eosinophils express on their surface cell membrane CR1, CR2, LFA-1, FcγRII, FcγIII, and FcεRII. It is the only cell which expresses the FcεRII comparing to the other granulocytes such as neutrophils, basophils, and mast cells. While basophils and mast cells express FcεRI

Human blood eosinophils usually have a bilobed nucleus and many cytoplasmic granules **(Figure-2)**. They comprise 2-5% of blood leukocytes in healthy, non-allergic individuals. Although it is not their primary function, they appear to be capable of phagocytosing and killing ingested microorganisms. The granules in mature eosinophils are membrane-bound organelles with crystalloid cores that differ in electron opacity from the surrounding matrix. Certain stimuli will cause eosinophils to degranulate.

Figure-2 Eosinophil shows granules with crystalloids, the azurophilic (primary) granules are larger than the secondary (specific) granules. Eosinophils represent about 2-5 % of the circulating blood leukocytes in healthy non-allergic persons. Human blood eosinophil usually has bilobed nucleus and many cytoplasmic granules, stains with acidic dyes such as eosin.

Degranulation involves:

A. Fusion of the intracellular granules with the plasma membrane.
B. Release of the granule contents into the surrounding area.

This type of reaction is the only way that these cells can use their granules against large targets, which can not be phagocytosed. Eosinophils are thought to play a specialized role in immunity to parasitic worms using this mechanism. Eosinophils are attracted by products such as eosinophil chemotactic factor of anaphylaxis (ECF-A), released from T cells, mast cells and basophils.

- They bind schistosomules (worm larvae) coated with IgG or IgE and degranulate, releasing a toxin known as "major basic protein".
- Eosinophils also release histamine and aryl sulphatase, which inactivate the mast-cell products histamine and slow reactive substance of anaphylaxis (SRS-A).
- The effect of the eosinophil factors is thus to dampen down the inflammatory response and reduce granulocyte migration into the site of invasion.

BASOPHILs AND MAST CELLs

Basophils are found in very small numbers in the blood circulation (<0.2%) of leukocytes and are characterized by deep violet blue granules **(Figure-3)**. The mast cell which is not found at all in the circulation is often indistinguishable from basophil in a number of its properties **(Figure-4)**. There are two different types of mast cells; the mucosal mast cell (MMC) associated with mucosal epithia, and the connective tissue mast cell (CTMC). The stimulus for eosinophil or mast-cell degranulation is often an allergen (an antigen causing allergic reaction). To be effective an allergen must cross-link IgE molecules bound to the surface of the mast cell or basophil via its high affinity Fc receptors for IgE (FcεRI). The release of histamine causes the adverse of the allergy. On the other hand they may play an important role in immunity against parasite. Both cells "basophils and mast cells" express CR1, CR3, LFA-1, C5aR, FcγRIII, and FcεRI. Also both cells do not express FcγRII, and FcεRII. The mast cell, which is not found at all in the circulation, is often indistinguishable from the basophil in a number of its properties. There are two different kinds of mast cell:

A. The mucosal mast cell (MMC). They are associated with mucosal epithelia. These cells appear to be dependent on T cells for their proliferation.
B. The connective tissue mast cell (CTMC). They appear to be independent from T cells for their proliferation**.**

Both types can be visualized under light microscopy with Alcian blue staining. Mature blood basophils have randomly distributed granules surrounded by membranes. The granules in both basophils and mast cells contain:

♦ Heparin.
♦ SRS-A (slow reactive substance of anaphylaxis).
♦ ECF-A (eosinophil chemotactic factor of anaphylaxis).

The stimulus for eosinophil or mast-cell degranulation is often an allergen (an antigen causing allergic reaction). To be effective, an allergen must cross-link IgE molecules bound to the surface of the mast cell or basophil via its high-affinity Fc receptors for IgE (FcεRI). Characteristically, the degranulation of a basophil or mast cell is substantial, with all the granules being released simultaneously. This is made possible by intracytoplasmic fusion of the granules, followed by rapid expulsion of their contents to the exterior. Mediators such as histamine that are released by degranulation cause the adverse symptoms of allergy. On the positive side, they may also play a role in immunity against parasites.

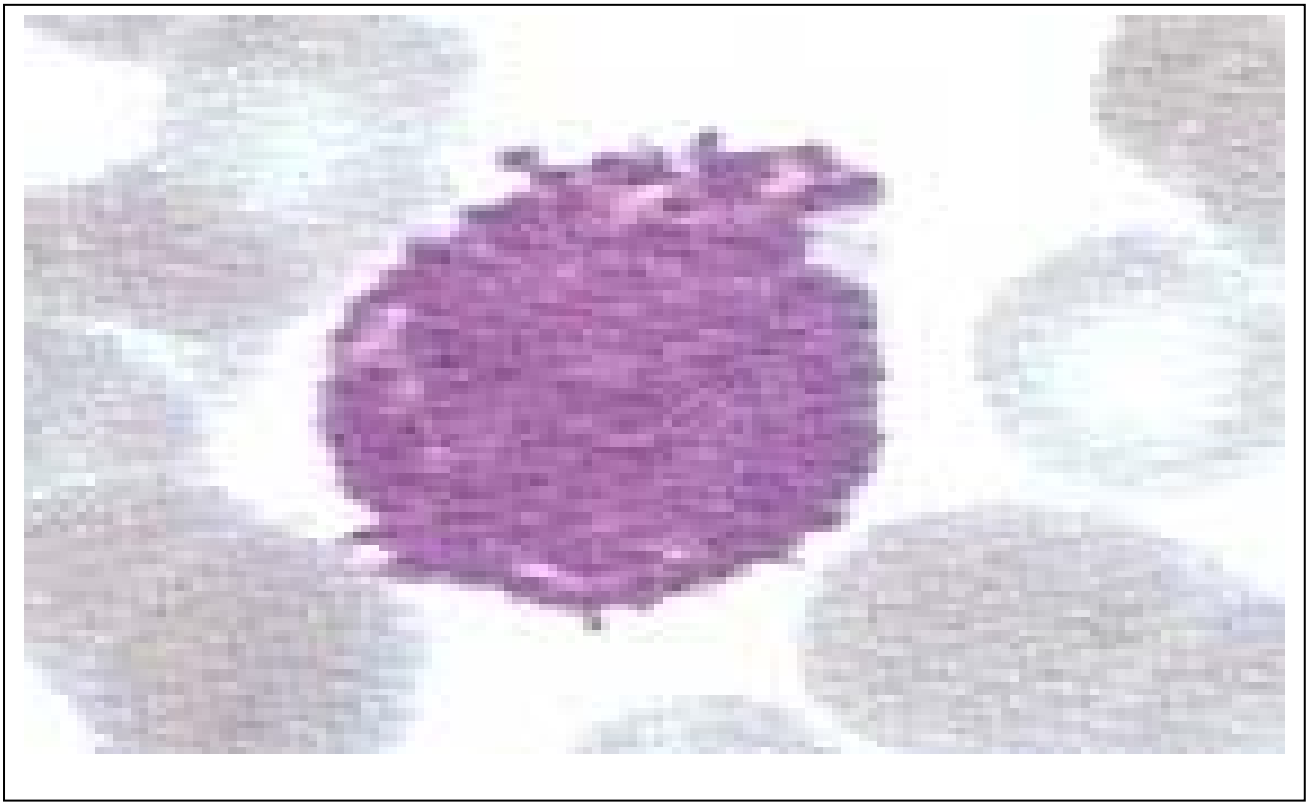

Figure -3 Basophil plays a role with the mast cell in immunity against parasites. They are found in very small number in the circulation and account for less than 0.2 % of leukocytes. The up above figure shows segmented nucleus and the large cytoplasmic granules.

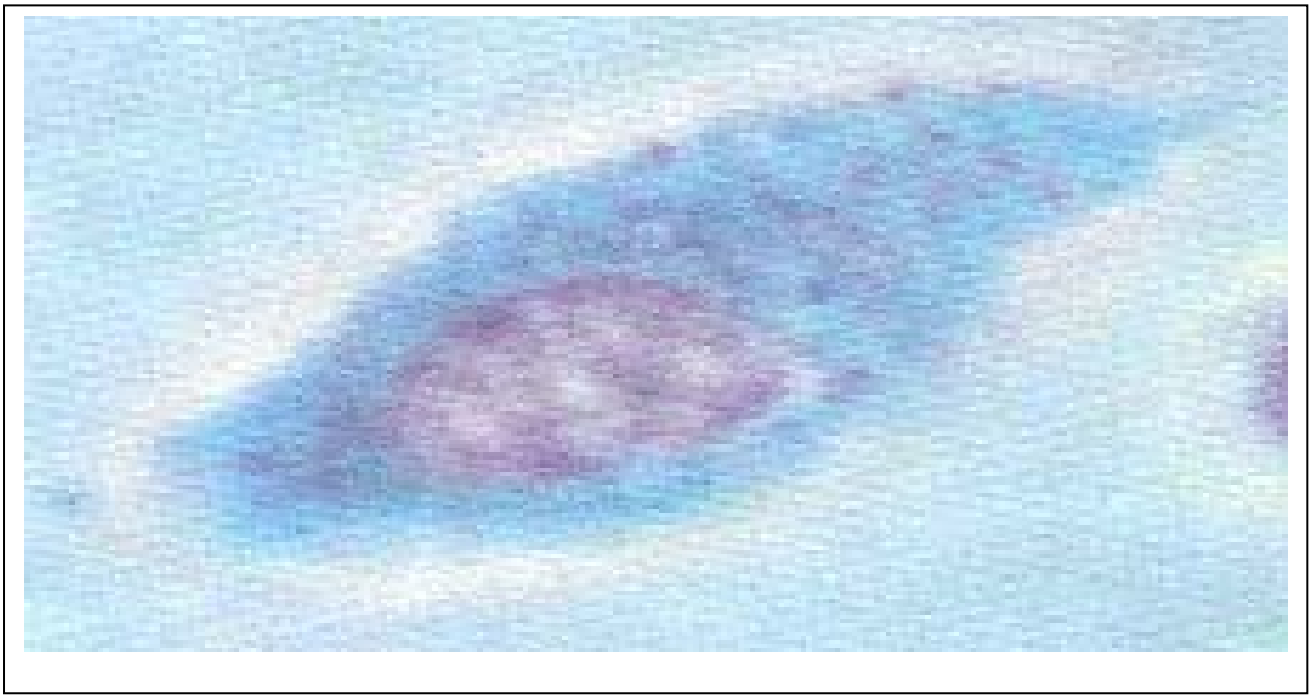

Figure -4 The connective tissue mast cell is not found in blood circulation, and it is indistinguishable from basophil in a number of characteristics, but displays some distinctive morphological features. It plays a role with parasitic infections. The micrograph shows dark blue cytoplasm with purple granules. Alcian blue and safranin stain.

PLATELETs

Blood platelets participate in the blood clotting and immune response (especially in inflammation). They are derived from the megakaryocytes in the bone marrow and contain granules **(Figure-5)**. They express class I MHC and receptor for IgG (FcγRII) and low affinity receptor for IgE (FcεRII). They carry receptor for factor VIII. The aggregated platelets release substances that increase permeability, as well as factors that activate complement and hence attract leukocytes.

Platelets express:

1. Class I MHC products
2. Receptors for IgG (FcγRII)
3. Low-affinity receptors for IgE (FcεRII; CD23).

Megakaryocytes and platelets carry:

A. Receptors for factor VIII.
B. Molecule important for their function, such as:

1. GpIIb /IIIa complex (CD41).
2. GpIb/GpIx complex (CD42).

The GpIIb/IIIa complex is a cytoadhesion, and is responsible for binding to:

1. Fibrinogen.
2. Fibronectin.
3. Vitronectin**.**

In addition, both this complex (GpIIb/IIIa), and the GpIb/GpIx complex are receptors for von Willebrand factor. There is an additional vitronectin receptor, CD51. Both receptors and adhesion molecules are important in activation of platelets.

Following injury to endothelial cells, platelets adhere to the surface of the damaged tissue. The aggregated platelets release substances that increase permeability, as well as factors that activate complement and hence attract leukocytes.

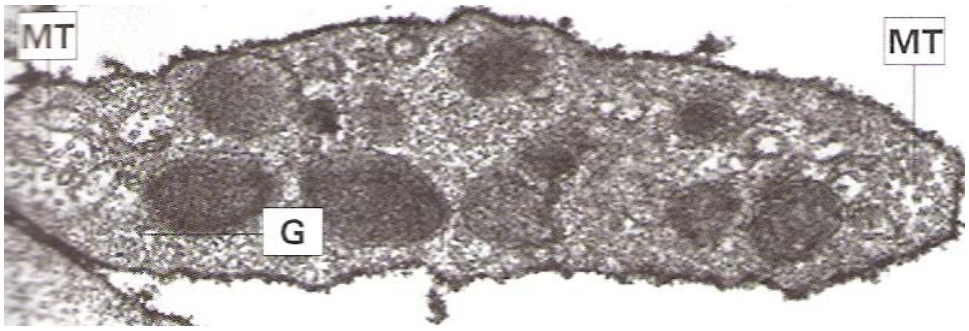

Figure-5 Platelet cross section of a platelet showing two types of granule (G) and bundles of microtubules (MT) at either end

DR. MUSTAFA HASAN LINJAWI