Monocytes

The initial cell in development is **monoblast**, which is indistinguishable from myeloblast. The next cell is promonocyte which has an oval or clefted nucleus with fine chromatin pattern and 2 to 5 nucleoli. The monocyte is a large cell (15-20 um) with irregular shape, oval or clefted (often kidney-shaped) nucleus, and fine delicate chromatin. Cytoplasm is abundant, blue-grey with ground glass appearance and often contains fine azurophilic granules and vacuoles.

Monocytes circulate in blood for about 1 day and then enter and settle in tissues where they differentiate into macrophages. Macrophage phagocytosis is slower as compared to neutrophils. In some organs, macrophages have distinctive morphologic and functional characteristics. Examples include Kupffer cells in the liver, Alveolar macrophages in the lungs, osteoclasts in the bone, and others.

Lymphocytes

These are of two types- small and large. Most of the lymphocytes in peripheral blood are small (7-8 um). The nucleus is round or slightly clefted with coarse chromatin and occupies most of the cell. The cytoplasm is basophilic, slight and visible as a thin border around the nucleus.

Around 10 to 15% of lymphocytes in peripheral blood are large (10-15 um). Their nuclei are similar to those of small lymphocytes but their cytoplasm is relatively more and contains few azurophilic granules.

On immunophenotyping, there are two major types of lymphocytes in peripheral blood: B lymphocytes (10-20%) and T lymphocytes (60-70%). About 10 to 15% of lymphocytes are of natural killer (NK) cell type.

B Lymphocytes

B lymphocytes arise from the lymphoid stem cells in the bone marrow (common lymphoid progenitor). Initial development occurs in primary lymphoid organ (bone marrow) from where B cells migrate to the secondary lymphoid organs (lymph nodes and spleen) where further differentiation occurs on antigenic stimulation. On activation by antigen, B cells undergo differentiation and proliferation to form plasma cells and memory cells. Plasma cells secrete immunoglobulins while memory cells have a life span of many

years and upon restimulation with the same antigen undergo proliferation and differentiation. Plasma cell is a round to oval cell with eccentrically placed nucleus and deeply basophilic cytoplasm. Nuclear chromatin is dense and arranged in a radiating or cartwheel pattern. The function of B lymphocytes is production of antibodies after differentiation to plasma cells. Antibodies can cause destruction of target cells/organisms either directly or by opsonisation.

T Lymphocytes

T lymphocytes originate from the progenitor cells in the bone marrow and undergo maturation in thymus. After their release from thymus, T cells circulate in peripheral blood and are transported to secondary lymphoid organs (i.e. paracortex of lymph nodes and periarteriolar lymphoid sheaths in spleen).

There are two major subsets of mature T cells: T-helper cells and T cytotoxic cells. Helper T cells regulate the functions of B cells and cytotoxic T cells. They recognize antigens presented by antigen presenting cells (APCs) in association with MHC class II molecules. Cytotoxic T cells recognize antigens in association with MHC class I molecules and play an important role in cell-mediated immunity. T lymphocytes secrete cytokines such as interferon-gamma, GN-CSF, TNF, and some interleukins.

Natural Killer (NK) cells

About 10 to 15% of peripheral blood lymphocytes are natural killer cells. These cells do not require previous exposure or sensitization for their cytotoxic action. They play a significant role in host defense against tumor cells and virally-infected cells. Morphologically, these cells are large granular lymphocytes.

The human leukocyte antigens (HLA) system

The human leukocyte antigens are encoded by a cluster of genes on short arm of chromosome 6 called as major histocompatibility complex (MHC). There are numerous allelic genes at each locus which makes the HLA system extremely polymorphic. The antigens are called as HLA because they were first detected on white blood cells, although they are present on several other cells also.

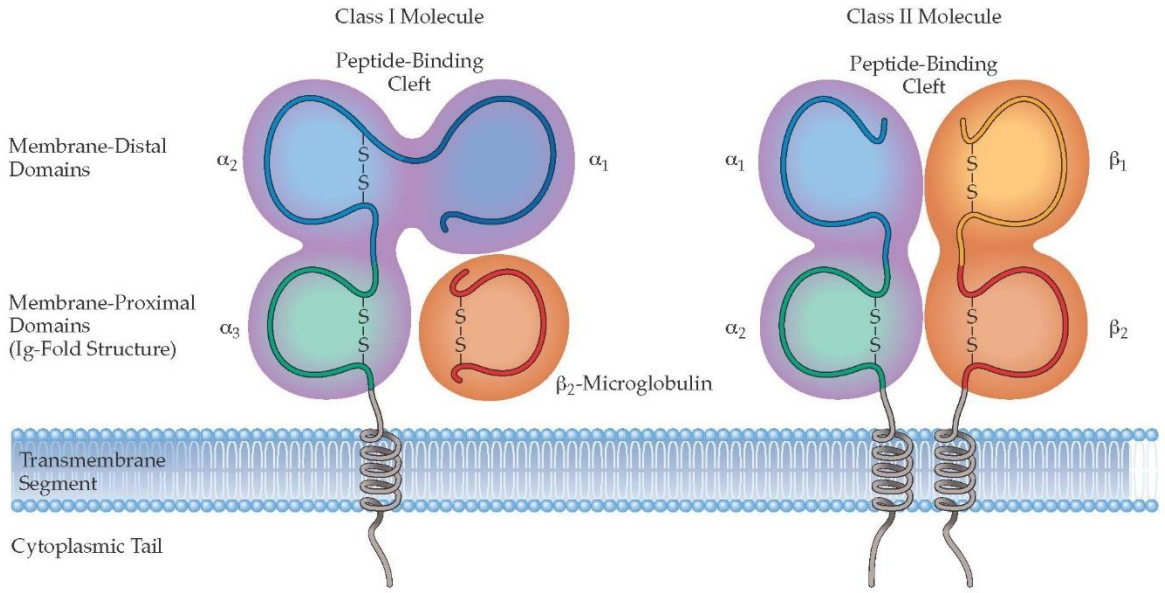
Types of HLA antigens:

There are three types of HLA antigens: class I, class II, and class III.

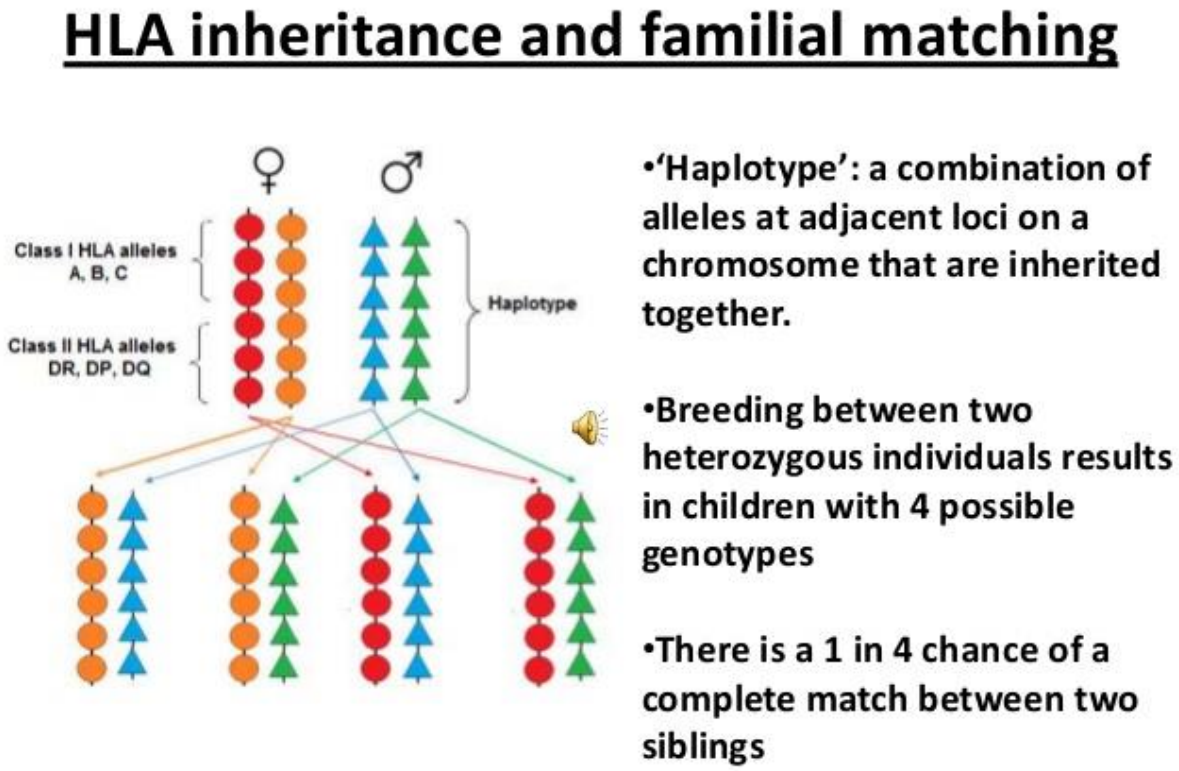
Class I antigens: Genes at HLA-A, HLA-B, and HLA-C positions specify class I antigens. Class I antigens are glycoproteins which are associated non-covalently with β2 microglobulin. Almost all nucleated cells possess class I antigens.

Class II antigens: HLA-D region (HLA-DR, HLA-DQ, and HLA-DP) encodes class II antigens. These consist of two glycoprotein chains α and β which are bound noncovalently. Class II antigens are present on monocytes, macrophages, B-lymphocytes, and stimulated T lymphocytes.

Class III antigens: genes specifying class III antigens are situated between genes which specify class I and class II antigens. Class III antigens encode certain complement components and cytokines.



The HLA genes are closely linked and are inherited by an individual as a haplotype from each parent.



Significance of HLA antigens

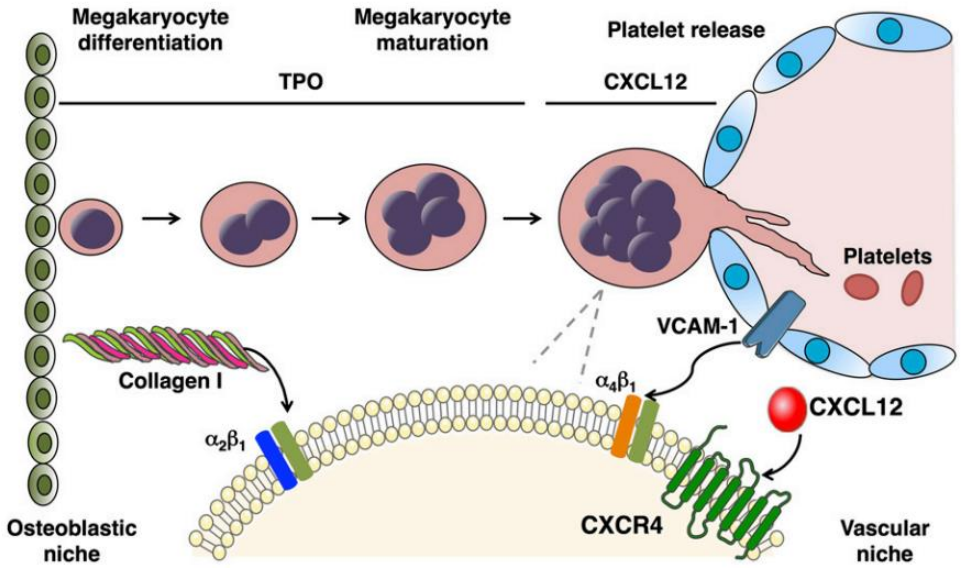
(1) They are important as histocompatibility antigens in organ transplantation.
(2) HLA antigens play a major role in recognition of foreign antigens and in immunity.
(3) In transfusion medicine, HLA antigens are responsible for alloimmunizaztion and graft-versus-host disease.
(4) A relationship exists between some HLA antigens and susceptibility to certain diseases.
(5) HLA antigen typing can be used for paternity testing.

Megakaryopiesis

The process of development of megakaryocytes and platelets in bone marrow is known as megakaryopoiesis. It is divided into four stages: Megakaryoblast, promegakaryocyte, granular megakaryocyte, and mature megakaryocyte. It is stimulated by thrombocytopoietin.

Megakaryoblasts are the earliest morphologically recognizable precursors; they are 6 to 24 um in diameter, contain a single, large, oval, kidney-shaped, or lobed nucleus with loose chromatin and multiple nucleoli, and have deeply basophilic agranular cytoplasm. **Promegakaryocytes** are larger than megakaryoblasts (15-30 um), have lobulated or horseshoe –shaped nucleus, more abundant and less basophilic cytoplasm which may contain azurophilic granules. **Granular megakaryocytes** are 40 to 60 um in diameter, contain a large multilobed nucleus with coarsely granular chromatin, and have abundant mildly basophilic cytoplasm containing numerous azurophilic granules. **Mature megakaryocyte** are of similar size, contain a tightly packed mutilobed and pyknotic nucleus, and have acidophilic cytoplasm; granules are arranged as 'platelet fields' (groups of 10-12 azurophil granules).

Mature megakaryocytes extend long and slender cytoplasmic processes (proplatelets) between endothelial cells of sinusoids in bone marrow and platelets are released from fragmentation of these processes. Each megakaryocyte produces 1000 to 5000 platelets leaving behind a 'bare' nucleus which is removed by macrophages.



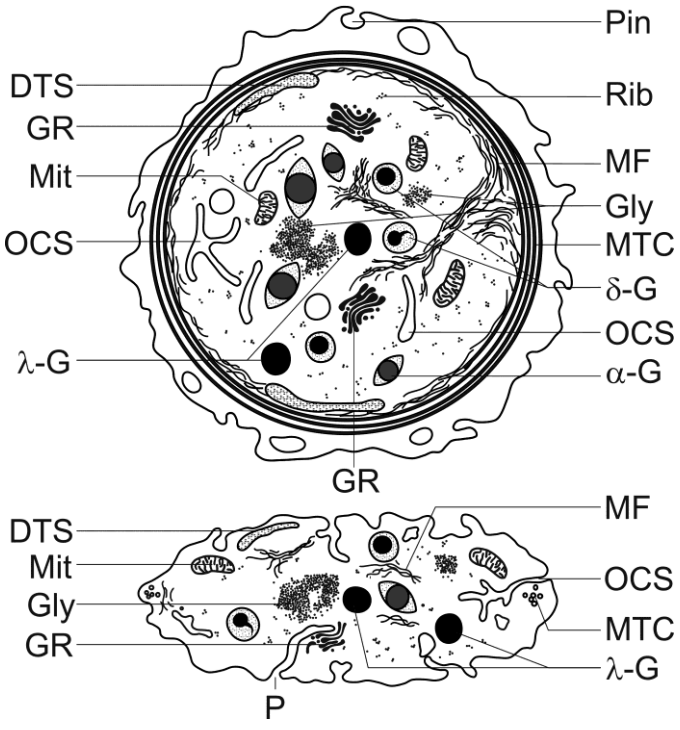
A unique feature of thrombocytopoiesis is endomitosis. This refers to nucleus division with cytoplasmic maturation but without cell (cytoplasmic) division. As the cell matures from megakaryoblast to the megakaryocyte, there is gradual increase in cell size, number of nuclear lobes, and red-pink granules and gradual decrease in cytoplasmic basophilia.

Thrombocytes (Platelets)

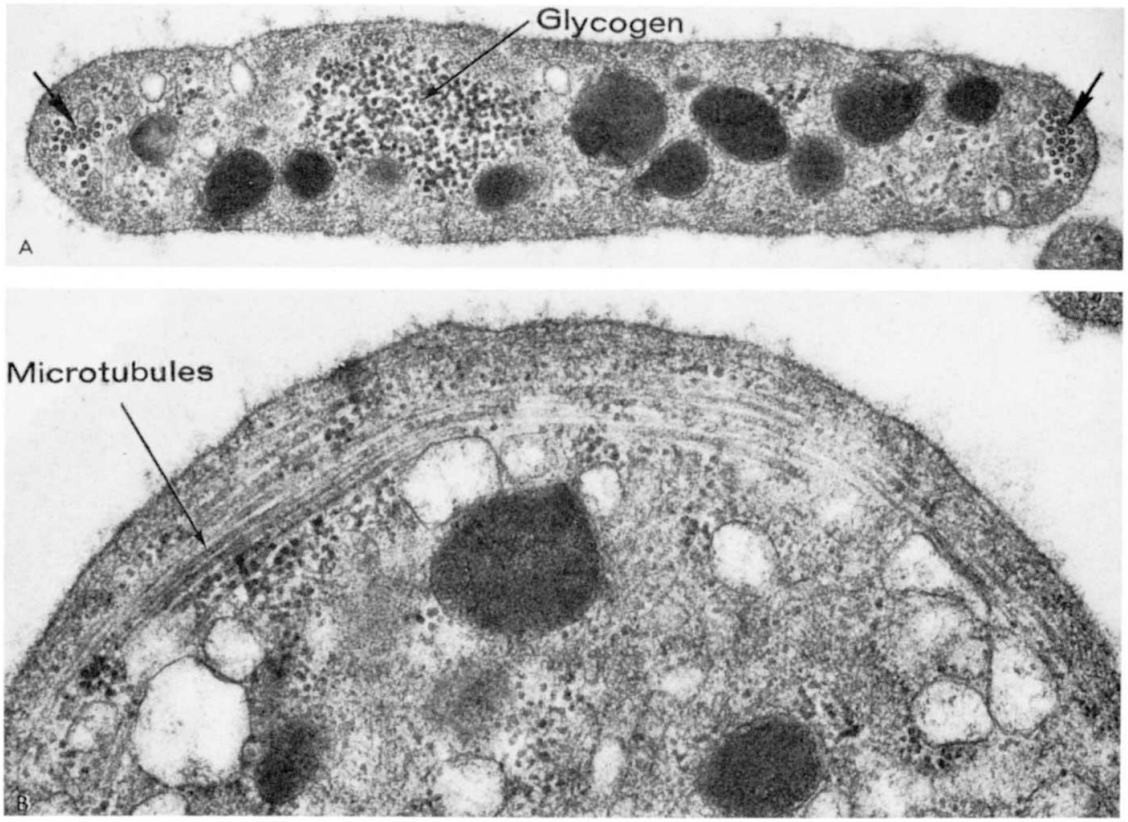
Platelets are derived from cytoplasmic fragmentation of megakaryocytes. They measure 2 to 3 um in diameter. Platelets remain viable in circulation for approximately 5 to 9 days. About one-third of the total platelets in the body are in the spleen. Under light microscope, in peripheral blood smear stained with one of the Romanowsky stains, platelets appear as small, irregular fragments, with fine cytoplasmic processes.

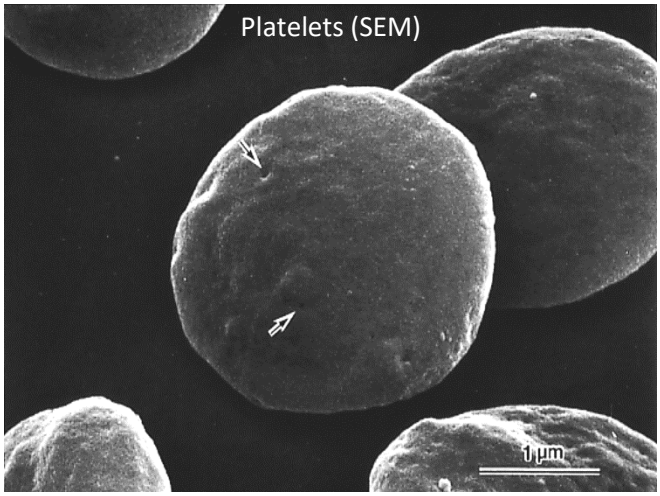
Ultrastructure of platelets

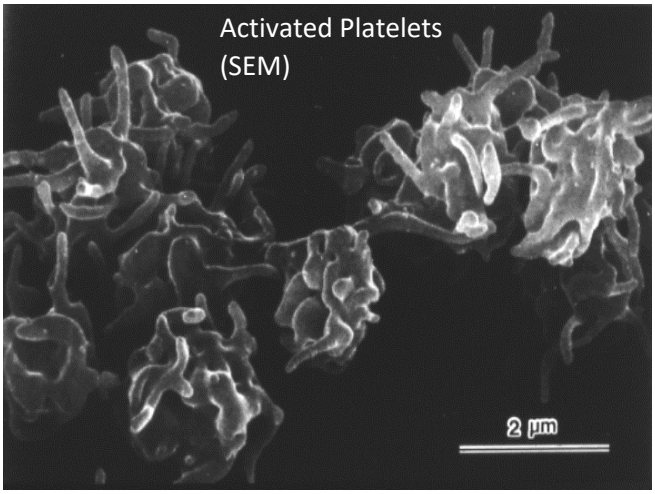
Ultrastructurally, the following three zones can be distinguished: (1) Peripheral zone: exterior coat (glycocalyx), cell membrane, open canalicular system; (2) Sol-gel zone: microfilamnets, circumferential microtubules, dense tubular system (Ca^{++}); (3) Organell zone: alpha granules, dense granules, mitochondria, lysosomes.



A scheme of a platelet in the equatorial plane (upper image) and in cross section (image at the bottom). Abbreviations: DTS *dense tubular system*, Gly *glycogen*, α *α-granules*, δ *δ-granules or dense bodies*, λ *λ-granules or lysosomes*, GR *Golgi remnants*, MF *microfilaments*, Mit *Mitochondria*, OCS *open canalicular system*, P *pores of the OCS*, Rib *ribosomes*.







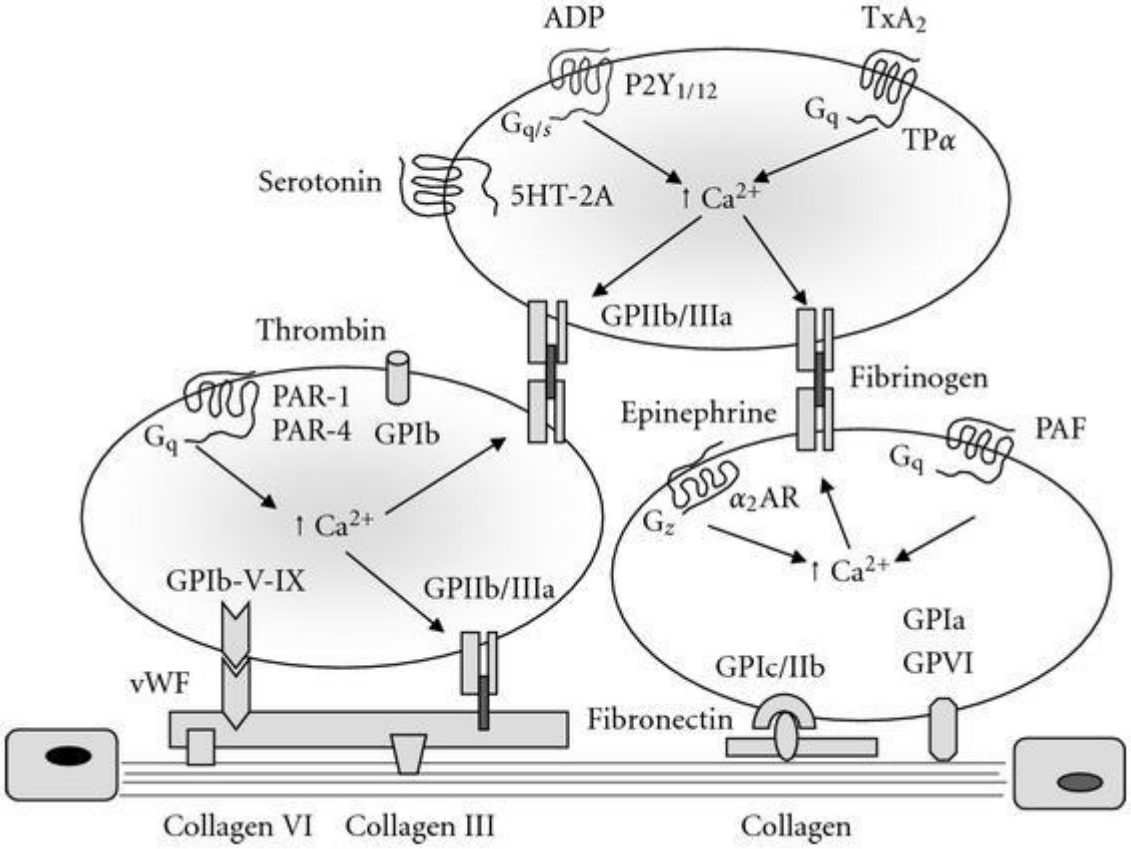
Platelet membrane glycoproteins

The cell membrane contains integral membrane glycoproteins (Gp), which play an important role in hemostasis. Important platelet membrane glycoproteins and their functions are as follows:

Gp Ib-IX-V: This is a constitutively active receptor that mediates vWF-dependent adhesion of platelets to subendothelial collagen.

Gp IIb/IIIa: On activation, serves to bind fibrinogen and thus mediates aggregation. Also receptor for vWF, fibronectin, and thrombospondin.

Gp Ic-IIb: Constitutively active receptor for collagen and mediates platelet adhesion independent of vWF.



Platelet function

Platelets are necessary for **hemostasis**, which means prevention of blood loss. There are three mechanisms, and platelets are involved in each.

1. **Vascular spasm**: when a large vessel such as an artery or vein is severed, the smooth muscle in its wall contracts in response to the damage. Platelets in the area of the rupture release serotonin, which also brings about vasoconstriction. The diameter of the vessel is thereby made smaller, and the smaller opening may then be blocked by a blood clot. If the vessel did not constrict first, the clot that forms would quickly be washed out by the force of the blood pressure.
2. **Platelet plugs**: when capillaries rupture, the damage is too slight to initiate the formation of a blood clot. The rough surface, however, causes platelets to become sticky and stick to the edges of the break and to each

other. The platelets form a mechanical barrier or wall to close off the break in the capillary. Capillary ruptures are quite frequent, and platelet plugs, although small, are all that is needed to seal them.

3. **Chemical clotting**: the stimulus for clotting is a rough surface within a vessel, or a break in the vessel, which also creates a rough surface. The more damage there is, the faster clotting begins, usually within 15 to 120 seconds. The clotting mechanism is series of reactions involving chemicals that normally circulate in the blood and others that are released when a vessel is damaged.

The chemicals involved in clotting include platelet factors, chemicals released by damaged tissues, calcium ions, and the plasma proteins prothrombin, fibrinogen, Factor VIII, and others synthesized by the liver. Vitamin K is necessary for the liver to synthesize prothrombin and other clotting factors. Most of our vitamin K is produced by the bacteria that live in the colon; the vitamin is absorbed as the colon absorbs water.

The clot itself is made of **fibrin**, which is a thread-like protein. Many strands of fibrin form a mesh that traps RBCs and creates a wall across the break in the vessel.

Once the clot has formed and bleeding has stopped, **clot retraction** and **fibrinolysis** occur. Clot retraction requires platelets, ATP, and factor XIII and involves folding of the fibrin threads to pull the edges of the rupture in the vessel wall closer together. This will make the area to be repaired smaller. As repair begins, the clot is dissolved, a process called fibrinolysis.

Blood Conditions (Terminology)

Hemorrhage (bleeding): Blood leaking out of blood vessels may be obvious, as from a wound penetrating the skin. Internal bleeding (such as into the intestines, or after a car accident) may not be immediately apparent.

Hematoma: A collection of blood inside the body tissues. Internal bleeding often causes a hematoma.

Leukemia: A form of blood cancer, in which white blood cells multiply abnormally and circulate through the blood. The abnormal white blood cells make getting sick from infections easier than normal.

Multiple myeloma: A form of blood cancer of plasma cells similar to leukemia. Anemia, kidney failure and high blood calcium levels are common in multiple myeloma.

Lymphoma: A form of blood cancer, in which white blood cells multiply abnormally inside lymph nodes and other tissues. The enlarging tissues, and disruption of blood's functions, can eventually cause organ failure.

Anemia: An abnormally low number of red blood cells in the blood. Fatigue and breathlessness can result, although anemia often causes no noticeable symptoms.

Hemolytic anemia: Anemia caused by rapid bursting of large numbers of red blood cells (hemolysis). An immune system malfunction is one cause.

Hemochromatosis: A disorder causing excessive levels of iron in the blood. The iron deposits in the liver, pancreas and other organs, causing liver problems and diabetes.

Sickle cell disease: A genetic condition in which red blood cells periodically lose their proper shape (appearing like sickles, rather than discs). The deformed blood cells deposit in tissues, causing pain and organ damage.

Bacteremia: Bacterial infection of the blood. Blood infections are serious, and often require hospitalization and continuous antibiotic infusion into the veins.

Malaria: Infection of red blood cells by Plasmodium, a parasite transmitted by mosquitos. Malaria causes episodic fevers, chills, and potentially organ damage.

Thrombocytopenia: Abnormally low numbers of platelets in the blood. Severe thrombocytopenia may lead to bleeding.

Leukopenia: Abnormally low numbers of white blood cells in the blood. Leukopenia can result in difficulty fighting infections.

Disseminated intravascular coagulation (DIC): An uncontrolled process of simultaneous bleeding and clotting in very small blood vessels. DIC usually results from severe infections or cancer.

Hemophilia: An inherited (genetic) deficiency of certain blood clotting proteins. Frequent or uncontrolled bleeding can result from hemophilia.

Hypercoaguable state: Numerous conditions can result in the blood being prone to clotting. A heart attack, stroke, or blood clots in the legs or lungs can result.

Polycythemia: Abnormally high numbers of red blood cells in the blood. Polycythemia can result from low blood oxygen levels, or may occur as a cancer-like condition.

Deep venous thrombosis (DVT): A blood clot in a deep vein, usually in the leg. DVTs are dangerous because they may become dislodged and travel to the lungs, causing a pulmonary embolism (PE).

Myocardial infarction (MI): Commonly called a heart attack, a myocardial infarction occurs when a sudden blood clot develops in one of the coronary arteries, which supply blood to the heart.