3. K vitamin antagonists

4. Thrombolytic drugs

The first group – **substances influencing the thrombocytes** are used in profylaxis of arterial thrombosis. In the initiation of arterial thrombosis the most important role play the thrombocytes. The acetylsalycilic acid is used the most frequently for this purpose. It inhibits the enzyme cyclooxygenase thus reducing the thromboxan A_2 production in thrombocytes. Activated thrombocytes are the TXA_2 producers. TXA_2 is a potent vasoconstrictive agent. It influences the thrombocyte aggregation and the release of further substances from thrombocytes.

Heparin is used to prevent both the venous and arterial thrombosis. The parenteral administration of heparin inhibits the thrombin formation (heparin is not a homogenous substance, it is a mixture of polysacharides with molecular weight 3000 to 4000 daltons. For the first time it was isolated form the liver therefore it was termed heparin. It activates the antithrombin III).

Coumarin anticoagulants inhibit the synthesis of coagulation factors. This is why their effect appears only in some days. They are administered as prevention of both, arterial and venous thromboses. Following oral application they are absorbed in the intestines. In plasma they are bound with albumin and are transported into the liver, where they are inhibiting the vitamin K – dependent coagulation factors (II,VII,IX,X).

Fibrinolytic substances (the tissue plasminogen activator) and fibrinolytic enzymes (streptokinase and urokinase) activating plasminogen into the plasmin are used in last occurs. Fibrinolytic substances can dilute thrombi in few hours.

The greatest **danger of thrombolytic treatment** is the bleeding. Patients with thrombocytopenia and chronic alcoholism, uraemia, and those taking the acetylsalicylic acid tend to this complication. Except of the drugs mentioned, there exist many substances having the anticoagulation as side effect.

2.6 Disorders of leucocytes

The main function of leucocytes is to defend the organism against diseases, especially against the infection. Mononuclear phagocytes (monocytes and the tissue macrophages) and granulocytes perform this function by swallowing up the microorganism and digesting the tissue detritus. The lymphocytes participate in activating of immune system functions.

The polymorphonuclear leucocytes form the highest percentage of leucocytes. They do not divide themselves or differentiate further in circulating blood. They have the ability to move actively and to phagocytise various microorganisms and niert particles. They release enzymes from cytoplasmatic granules into the phagocytic vacuoles and in certain circumstances into the extracellular space. The neutrophils use their ability of chemotaxis (motion in direction of ascending concentration of the substance of target organism), of phagocytosis (ingestion of the microbe into the phagocytic vacuole) and the capability to scarve the microbe chemically by releasing the content of granules into the phagocytic vacuoles (bactericidal proteins, myeloperoxidase, cathepsins) and by forming of free oxygen radicals as superoxide and hydroxylated anion. In presence of halogens e.g. Cl-toxic substances are formed killing the swallowed up microbes.

Chemotaxis of neutrophiles is extraordinarly important. They are able to force the neutrophils to become attached to the vascular endothelium and thereafter to penetrate across the vascular wall (throngh the intercellular junctions and to migrate into the extravascular space. The migration in sense of concentration gradient may be influenced significantly by bacterial peptides, components of complement system and leucotrien B_4.

The ability of leucocytes to distinguish microorganisms or other particles facilitates the phagocytosis. So do the opsonization or the proteins bound to the surface of microorganisms. Fragments of complement C3b, the plasmatic protein fibronectin and immunoglobulins are involved. These substances adhere to surface of microorganism enhance the binding with the receptors of neutrophil plasmatic membrane.

During the phagocytosis the phagocytic vacuole fuses with intracellular vacuoles containing enzymes. The neutrophils contain two types of cytoplasmatic granules. The azurophil granules contain lysozyme, acid hydrolases, neutral proteases including the cathepsin G and the elastases, myeloperoxidase and basic proteins. The specific granules contain lysozyme, transcobalamin 3, apolactoferin, collagenase, C5 protease. The azurophil granules connect with the phagocytic vesicules and the plasmatic membrane. During this connection the content of azurophil granules is discharged into the acid milieu of vesicules. The specific granules are connected with phagocytic vesicules and plasmatic membrane so that their content is released into the vacuoles also arround the neutrophils. These enzymes facilitate the bacterial covers, dissolve the connective tissue, decompose the tissue detritus, or bind with specific substances, useful for bacterial metabolism, e.g. the iron.

Granulocytes have a short biological half-life, about 6 h. They circulate early after their release in to the blood, enter the tissue and there they can survive even several days.

Circulating monocytes and fixed macrophages (phagocytes) have a broader functional adaptability then the neutrophiles. They survive much longer than neutrophiles. They contain granules not divided in subtypes. The circulating monocytes have less chemotactic activity than the neutrophils and appear later in the focus of inflammation. Their phagocytic effects are directed against the intracellular microorganisms. Lipopolysaccharides and gama interferon activate the monocytes. It is manifested by their higher motility, metabolic activity, proportions and microbocide potency.

The monocytes have three basic functions: 1 secretion, 2 phagocytizing capability, 3 interaction with lymphocytes. Monocytes synthesize 50 various protein mediators and enzymes and release interleukins which influence the lymphocytes and cause the fever. They have a very active metabolism of arachidonic acid leading to the prostaglandin production (PGE_2). PGE_2 regulates the bone marrow and the cells involved in immune response proliferation. A further product are leukotrienes and thromboxane. Activated monocytes have tumoricide effect. Interaction between monocytes and leucocytes are necessary for maintaining normal function of immune system. Monocytes and macrophages activate the T cells. This function performs a special monocyte subpopulation – the dendritic cells and the Langerhans's cells in the skin. The dendritic cells form a very small subpopulation of monocytes with long projections. They act like antigen presenting cells in blood. Langerhans's cells have similar function in the skin.

Lymphokinins secreted by activated T-cells induce the accumulation and activation of monocytes. One of these leukokinins – interleukin-2 acts as growth factor of T-cells enabling them the expansion of T-cell population and the immune response. Moreover the macrophages stimulate the proliferation and differentiation of B lymphocytes by interleukin-1 secretion. Prostaglandin E2 secreted by monocytes and macrophages inhibits, in contrast, the lymphocytic reactions.

The participation of leucocytes in many haematologic, infections, inflammatory and neoplastic processes is evident. This is why the leucocyte count determination and their histological examination is considered to be the fundamental laboratory estimation of pathologic alterations in organism.

All five types of leucocytes (neutrophils, eosinophils, basophils, lymphocytes, monocytes) originate in a common stem cell. In spite of the common origin their development, function and distribution are quite different. Basal laboratory examination determines the leucocyte count and their percentual distribution according to the types.

2.6.1 Leucocytosis

The leucocyte count elevation – the leucocytosis is in organism atteined by several mechanisms. **Under physiological circumstances about a half of all leucocytes adheres freely to the vascular endothelium** or remains in the microcirculation. If the state of organism is altered, e.g. during exertion or after adrenaline application, leucocytes are released and circulate in the whole organism. This process of leucocyte release from the marginal pool is named demargination. Neutrophils are not involved. **Neutrophil stores are in bone marrow** – it is the so-called medullar reserve of neutrophils, from where they are released in stress, infection and corticosteroid administration. If the stimulus is very intense – e.g. the bacterial infection apart from mature neutrophils the immature forms – the metamyelocytes may be released. This type of leucocytic respons to

the infection is named the shift to the left. Leucocytosis can be due to increase in leucocyte production. It may reach very intense stimulation 25 000 to 50 000 Le in microliter. Such an alteration is named leukemoid reaction.

The leucocyte release from the marginal pool in to the circulation occurs during some minutes. The release of neutrophils from bone marrow lasts several hours. Increase in leucocyte production in bone marrow becomes manifest in the peripheral blood only in several days.

The leucocyte production is controled. Under physiological circumstances there is the local control in bone marrow, thymus, lymphatic glands and spleen. Interleukins and CFS (colony stimulating factor) are involved in the control. Interleukin-2 (IL-2) and IL-4 can cause increase in T and B lymphocytes. G-CSF (granulocyte colony stimulating factor), GM-CSF (granulocyte-macrophage colony stimulating factor), M-CSF (macrophage colony stimulating factor) and four interleukins: IL-1, IL-2, IL-3, IL-5 and IL-6 enhance the production of neutrophils, monocytes and eosinophils. In leucocytosis mostly prevail the neutrophils. Leucocytosis with prevailing lymphocytosis is observed in chronic leukaemia, infectious mononucleosis, infectious hepatitis, infectious lymphocytosis, pertussis (whooping cough), tuberculosis and lues. A moderate increase in lymphocyte count is usually associated with thyrotoxicosis and Addison's disease.

Increase in circulating monocytes is observed in chronic inflammatory processes like tuberculosis, bacterial endocarditis, brucellosis, malaria, sarcoidosis, Crohn's disease and collagenoses. It can be important in myeloproliferative syndromes.

2.6.2 Leucopenia

If the leucocyte count falls there is mostly dicrease in neutrophil leucocytes involved. There is an asymetric decrease in all types of leucocytes.

Neutropenia. The severity of neutropenia is expressed by the absolute neutrophil count (from percentual participation of neutrophils in differential blood picture the absolute number of neutrophils is counted of the number of all leucocytes in 1 μl of blood. Under physiological conditions are 2 500 to 3 500 leucocytes present in 1 microliter of blood). Natable neutropenia represent a condition with less than 500 neutrophils in 1 microliter of blood. In moderately severe neutropenia 1 μl of blood contains 500 to 1 000 neutrophils and in a mild form 1 000 to 2 000 neutrophils. The risk of infection and its severity is augmented if the neutrophil count is less than 1 000 in 1 microliter of blood.

Neutropenia may be due to **ineffective neutrophil production** or to increased margination, eventually to enhanced neutrophil utilization.

Probably commonly the neutropenia appears owing to the **bone marrow suppresion** following cytostatic treatment. Neutropenia appears in conditions affecting haemopoietic stem cells, i.e. in leukaemia, myelodysplastic syndrome or in pernicious anaemia. In B_{12} and folic acid deficiency is the neutrophil production ineffective. Defective neutrophils are destroyed in bone marrow earlier than they get into the blood flow.

In cyclic neutropenia fever, weakness, ulcerations of oral mucosa and absence of neutrophils in peripheral blood are observed. This condition returns in 21 day intervals. Between these episodes is the patient very well. In the phase of impairment the thrombocyte and reticulocyte fall also occurs. A defective regulation of stem cells is supposed to be the underlying cause. The G-CSF application increases the neutrophil production and moderates the infection course.

In chronic idiopatic neutropenia is the neutrophil count substantially reduced, nevertheless the monocyte count is elevated. Bone marrow is not considerably altered. Decrease in count of mature neutrophils is observed only. In some cases antibodies against the neutrophils are found. The role of these antibodies is not known.

Neutropenia may be induced by many drugs. In these cases fever, sore throat and perianal ulcerations are present. The mechanism of drug action may be toxic or immune. After interruption of treatment the neutrophil count returns to normal values. Neutropenia is sometimes observed in relation with increasing of drug doses (chloramphenicol, propylthiouracyl).

Isoimmune neonatal neutropenia is transient condition due to the transplacental transfer of antibodies from the mother's body into the fetal organism. Antibodies directed against neutrophils are involved. In this condition spontaneous recovery occurs during some month after the birth.

In systemic lupus erythematosus occurs usually

neutropenia of medium severity. In some patients with rheumatoid arthritis severe form of neutropenia with splenomegaly and high levels of rheumatic factors (Felty's syndrome) occur.

Neutropenia may develop in bacterial, viral, parasital, rickettsial diseases. Abnormal production, margination or utilization of neutrophils may be involved. In infectious mononucleosis, infectious hepatitis and HIV infection the neutrophil production is impaired. In influenza and in rickettsial infections increased neutrophil margination occurs. In gram-positive infections the utilization of neutrophils is elevated. Neutropenia may develop in splenomegaly due to hypersplenism owing to the increased uptake of neutrophils in the spleen.

Lymphocytopenia (lymphopenia) is a condition the count of lymphocytes in 1 μl of blood falls to less than 1 500 in adults and to 3 000 in young people and children.

It occurs most commonly in congenital immunodeficiency syndrome. It is observed also following irradiation, cytotoxic and corticosteroid treatment. Lymphopenia accompanies the lymphomas, aplastic anaemia, renal failure, right cardial failure, and cachexia.

Monocytopenia appears in acute infections, in stress and following glucocorticoid application. It is developing in aplastic anaemia, acute myeloid leukaemia, after myelotoxic, immunosuppressive treatment.

Eosinopenia occurs in stress, infections anf following glucocorticoid application.

Eosinophilia can appear during chronic myeloic leukaemia. It may be severe in parasitic and allergic diseases. The underlying causes of eosinophilia are usually chronic inflammatory and malignant diseases.

In idiopathic hypereosinophilic syndrome there is hepatosplenomegaly, peripheral neuropathy, and congestive heart failure.

2.7 Malignant haematologic diseases

The haematologic malignancies comprise a group of conditions originating in bone marrow and lymphatic glands. Primary bone marrow diseases are the leukaemias, the immunoproliferative and the myeloproliferative syndromes. It is evident in all these diseases, that the origin lies in cell mutation being the basis for development of malignant clone. The malignant clone exhibits an abnormal growth potency. In chronic lymphocytic leukaemia the malignant lymphocytes produce an identical immunoglobulin. In chronic myelogenous leukaemia all cells of myeloid series, the erythroid precursors, the megakaryocytes and B lymphocytes have an identical Ph[1] Philadelphia chromosome. Many further facts indicate that the malignant cells arise from one cell and by their expansion the clone of malignant cells is formed.

2.7.1 Myeloproliferative disorders

Four diseases may be included in this group, each of them being an independent clinical unit:

1. Polycythaemia vera
2. Myelofibrosis with myeloid metaplasia
3. Essential thrombocytopenia
4. Chronic myelogenous leukaemia

In all these conditions are an uncontrolled expansion of bone marrow elements included. Increase in erythroid, myeloid and megakaryocyte cells production is due to malignant transformation of pluripotent stem cell. Fibrosis of bone marrow is frequently present owing to growth stimuli affecting normal fibroblasts. The origin of these growth stimuli is in neoplastic cells. They are produced most probably by megakaryocytes in bone marrow. In myeloproliferative disorders the megakaryocytes count is usually extremely elevated.

Polycytaemia vera is a myeloproliferative neoplastic disease. The stem erythroid cell is primarily affected. Hyperplasia of all bone marrow components