NEUTROPHIL CHEMOTACTIC ACTIVITY

IN

BILHARZIAL LIVER FIBROSIS

Thesis

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In

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By

Maher Milad Boushra

M.B.B.Ch.

Supervised By

Dr. Omar Fathy
Prof. of Int. Medicine

Dr. Salah Seif El-Din
Prof. of Tropical Medicine and Int. Medicine

Dr. EL Said Abou Gamrah
Ass. Prof. Of Internal Medicine

Dr. Fawzi Naguib
Prof. of Pathology

Internal Medicine Department
Ain Shams University

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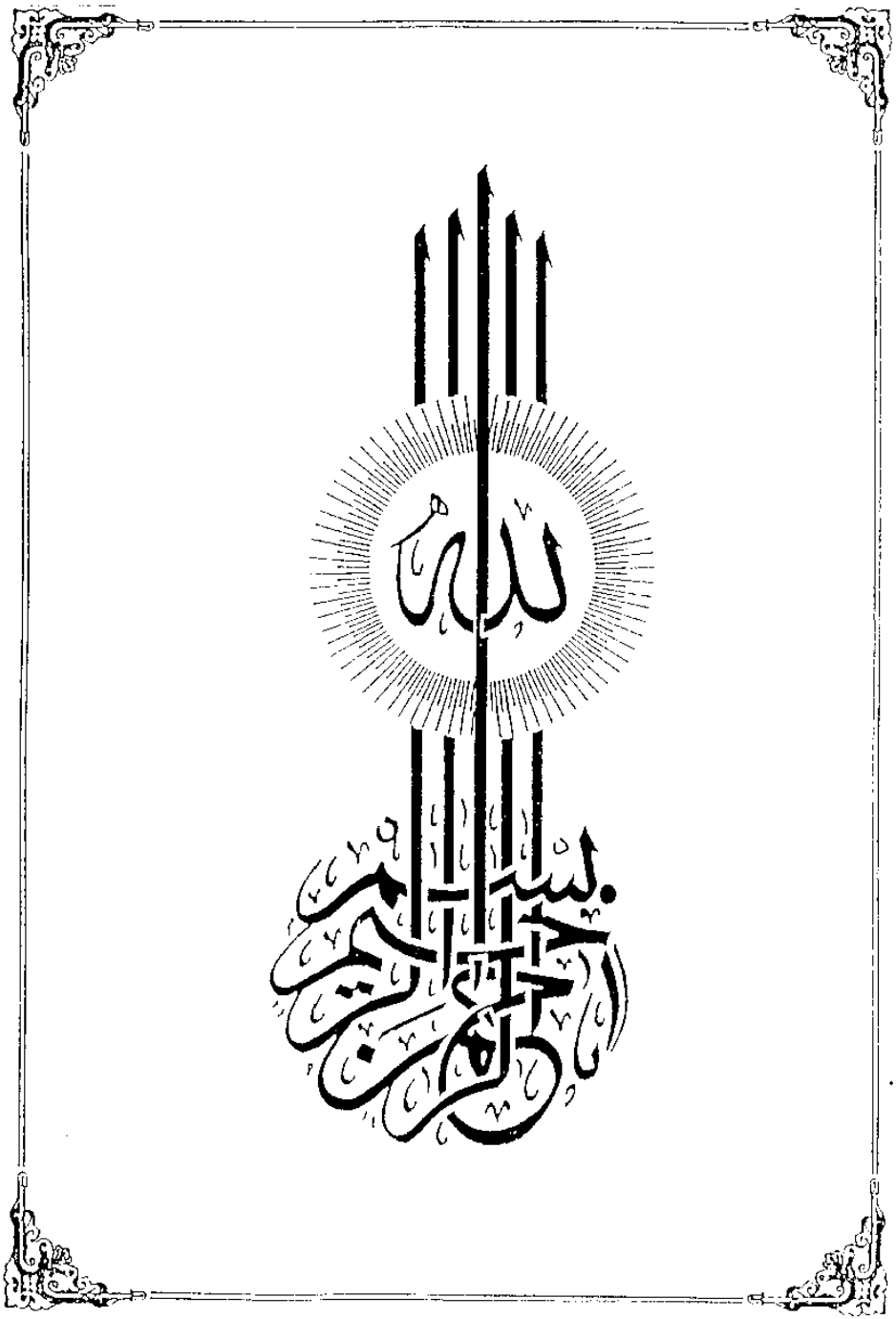

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It is well known that there is increased susceptibility to infection in cases of liver cirrhosis and the cirrhotic patients have limited ability to defend themselves against a variety of infectious agents including Gram negative and Gram positive bacteria.

Chemotaxis or leucocyte migration is one of the important mechanisms in immune reactions and it involves different types of leucocytes e.g. neutrophils, basophils eosinophils and lymphocytes.

Neutrophil chemotaxis plays a basic role in defending the body against different types of chemotactic factors e.g bacteria, viruses and candida.

It was proved that neutrophil chemotaxis is defective in liver cirrhosis.

The aim of this work is to study the chemotactic activity of neutrophils in patients with bilharzial hepatic fibrosis.

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REVIEW OF LITERATURE

SOME FUNCTIONS OF NEUTROPHIL LEUCOCYTES

There are two types of circulating phagocytes recognised long time ago and designated as microcytes and macrocytes on the basis of the size of the cells. We now refer to these cells as the polymorphnuclear leucocytes and monocytes respectively according to their nuclear configuration.

Van Oss (1979) had classified the mammalian phagocytic system into polymorphnuclear system, the first line of defence, comprising the the circulating polymorphnuclear leucocytes and the mononuclear phagocytic system, the second line of defence, comprising the circulating monocytes as well as the free and fixed macrophages (also known as the reticulo-endothelial system).

The mononuclear phagocytic system comprises, in the order of increasing phagocytic capacity, the promonocytes in the bone marrow which give rise to monocytes in the peripheral blood which in turn give rise to macrophages (free and fixed) localised in different tissues :

. Alveolar macrophages.
. Peritoneal macrophages.
. Skin macrophages. (Gadebusch,1980)

The polymorphnuclear leucocytes are subdivided into 3 types according to the staining characteristics named, eosinophils, basophils and neutrophils. Neutrophils play the major role in the defence against bacterial invasion while eosinophils and basophils contribute little to the host defence mechanisms against bacteria.

Gadebusch (1980) established that there is a general correlation between the degree of neutropenia and increased risk of bacterial infection, such that a patient with an absolute neutrophil count below 1000 / mm^3 has a moderately increased risk, while a patient with an absolute count below 500 / mm^3 there is a serious risk of bacterial infection.

The leucocytes unlike the red blood corpuscles don't carry out their functions in the circulating blood, for them the circulation is mainly a transport system through which they pass from the sites of its formation to the location where they are needed.

Functions of neutrophils :

The primary function of neutrophils granulocytes is localization and destruction of micro-organisms . There are several integrated activities which are very

important to acheive this function. Gadebusch (1980) had classified the factors affecting phagocytosis into two categories :

1- Factors related to leucocyte approach to bacteria such as, adequate production and mobilization of mature neutrophils into the blood stream, alteration of vascular permeability, adherence of neutrophils to blood vessel wall followed by migration of them through the endothelial gap (diapedesis) and attraction of neutrophils to the bacteria (chemotaxis).

2- Factors related to interaction between neutrophils and bacteria and these include :
Coating of bacteria with serum factors , the so called opsonization, adherence of neutrophils to bacteria followed by ingesion of bacteria by neutrophils (phagocytosis).

After ingesion of bacteria, there is a change in the metabolism of neutrophil (respiratory burst) , rupture of neutrophil granules followed by killing and digestion of bacteria within neutrophil.

ORIGIN AND MATURATION OF HUMAN NEUTROPHILS

The granulocytes arise from the undifferentiated mesenchymal cell called haemocytoblast which is a stem cell and it has the capacity of self renewal and of differentiation into more mature haematologic cells.

The stem cell may be pluripotent giving rise to cells of several haematopoietic lines or unipotent with maturation ability along a single line.

Under normal conditions the stem cell is assumed to be constant in size, while under differentiating stimulus a stem cell could divide assymetrically, one offspring remaining inside the stem cell compartment while the other leaves the compartment and provide progeny which mature along one or more haematopoietic cell lines depending on the nature of the stimulus whether hormonal or it is the environment surrounding the cell itself.

An alternative scheme, a stem cell may leaves the compartment under a differentiating stimulus and is replaced by the progeny of another stem cell.

(Cline, 1975)

Maturation of Neutrophil :

The earliest neutrophil precursor is the myeloblast which has a very large nucleus, little cytoplasm, numerous mitochondria with no granules and no phagocytic activity.

Granules begin to appear in the next or promyelocyte stage and are very clear in the myelocytes. These granules are reffered to as the primary granules or azurophil granules (because of their histochemical characteristics). They arise from the concave side of Golgi apparatus and are relatively large in size (800 nm in diameter) and are electron dense. These granules contain myeloperoxidase, acid phosphatase, B-glucoronidase, a portion of lysozymes and numerous cationic proteins.

When the cell is fully mature, the azurophil granules will make up only 10-20% of the total granule population of the cell.

(Bainton et al, 1966)

The secondary granules (specific granules) bud off the convex side of Golgi apparatus and they will be formed at a late myelocyte stage. They are smaller than the azurophil granules being only about 500 nm in diameter and are less dense. They contain lysozyme

and iron containing protein, lactofferin.

(Spitznagel et al, 1974, Baggiolini et al,1970)

In the metamyelocyte stage and next stages to mature neutrophil, there is full complement of azurophil and specific granules. The phagocytic ability begins from the metamyelocyte stage and increases as the cell matures. Mitochondria are abundant in the early stages of maturation and with increasing maturity they are replaced by deposits of glycogen (Scott & Horn,1970).

So that the mature cell is devoid of mitochondria, consequently the primary source of energy for neutrophil is glycolysis (Evans et al, 1962), as the cell is commonly required to function in the environment of tissue inflamation where the oxygen tension is quite low.

The mature neutrophil tend to be of uniform size, 12-15 um with granular cytoplasm. The nucleus is coarsly clumped and segmented into two to five lobes which are connected together by thin chromatin strands. Arneth (1904) believed that the nuclear lobulation continues as the cell ages and that granulocyte with three or four lobes is more mature than that with only two. The lobulation may enhance cell deformability and movement through the vessel walls or perhaps nuclear segmentation results from nuclear emptying and has no

function (Sinym,1965). The cytoplasm is faint pink and contain small number of primary granules and large number of specific granules. Golgi apparatus is atrophic with no granulogenesis. Mitochondria have been replaced by glycogen. The endoplasmic reticulum is less developed than in the earlier forms, free and bound ribosomes are much less common.

The maturation process requires about 9-11 days, but the half life span of a mature neutrophil in the circulation is only 6-7 hours. Granulopoiesis has three major cell pools (compartments) :

- Proliferating pool consisting of myeloblasts, promyelocytes and myelocytes.
- Maturing pool consisting of meta myelocytes, band and segmented forms.
- Functional pool consisting of neutrophils in the circulation. (Cronkite et al, 1970)

CHEMOTAXIS

Leucotaxis or chemotaxis of leucocytes is defined as a leucocyte directional migration in response to a diffusing gradient of chemical attractants without change in its speed (Ramsy, 1972). It has to be differentiated from chemokinesis and random movement.

Chemokinesis: is chemically or thermally stimulated, non directional cellular motility occuring in the absence of a chemotactic gradient.

Random migration: is cellular motility which occurs in the absence of any known chemical attractant and leads to leucocyte accumulation.

Chemoattractants for leucocytes:

Zigmond and Herish, 1973, had classified chemoattractants into :

1. Exogenous: including bacterial products and denaturated proteins.
2. Endogenous: which are humoral as complement activation products, kallikrin, thrombin and plasmin, and cellular as neutrophils, platelets and lymphocytes.

By another classification, chemotaxis can be mediated by two means :

(1) Cytotaxigens :

They are not chemotactic themselves but they