INTRODUCTION

Neutrophils are the most abundant white blood cells in circulation**.** They are phagocytes, capable of ingesting microorganisms or particles. For targets to be recognized, they must be coated in opsonins a process known as antibody opsonization. They can internalize and kill many microbes, each phagocytic event resulting in the formation of a phagosome into which reactive oxygen species and hydrolytic enzymes are secreted ***(Banerjee, 2011)***.

The consumption of oxygen during the generation of reactive oxygen species (ROS) has been termed the "respiratory burst", although unrelated to respiration or energy production.The respiratory burst involves the activation of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which produces large quantities of superoxide (O_2^-), a reactive oxygen species. Superoxide dismutates, spontaneously or through catalysis via enzymes known as superoxide dismutases (SOD), to hydrogen peroxide (H_2O_2), which is then converted to hypochlorous acid (HClO), by the green heme enzyme myeloperoxidase (MPO) ***(banerjee, 2011)***.

Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis is acute when it lasts less than six months and chronic when it persists

longer. A group of viruses known as the hepatitis viruses cause most cases of hepatitis worldwide ***(Dienstag, 2008)***.

Hepatitis C virus (HCV) is a major cause of liver disease. It affects nearly 170 million people worldwide ***(Lavanchy, 2009).*** Oxidative stress may play a role in the pathogenesis of chronic hepatitis C(CHC) and may regulate collagen synthesis, thus contribute to liver damage and the subsequent development of fibrosis ***(Ashfaq et al., 2012).***

Hepatitis B virus (HBV) infection is usually acquired parentrally leading to high prevalence of chronic infection .Chronic infection is usually associated with enhanced production of reactive oxygen species and reactive nitrogen species ***(Solomon et al., 2011).***

Some PMN cell functions are altered in patients with chronic liver disease. These observations include an impaired neutrophil chemotaxis function, a diminished phagocytic activity, and an altered capacity of PMN cells to produce oxygen derived free radicals ***(Engelich et al., 2001).***

Aim of the Work

The aim of the present study is to evaluate the phagocytic activity and oxidative burst of polymorph nuclear leucocytes in chronic hepatitis B and chronic hepatitis C virus patients.

Chapter One

PHAGOCYTOSIS

Introduction:

The immune system is designed to protect the body from pathogens. It begins to develop in the embryo and starts with hematopoietic stem cells which differentiate into granulocytes, monocytes, and lymphocytes. These stems cells also differentiate into erythrocytes and megakaryocytes and continue to be produced and differentiate throughout the lifetime ***(Lim et al., 2013).***

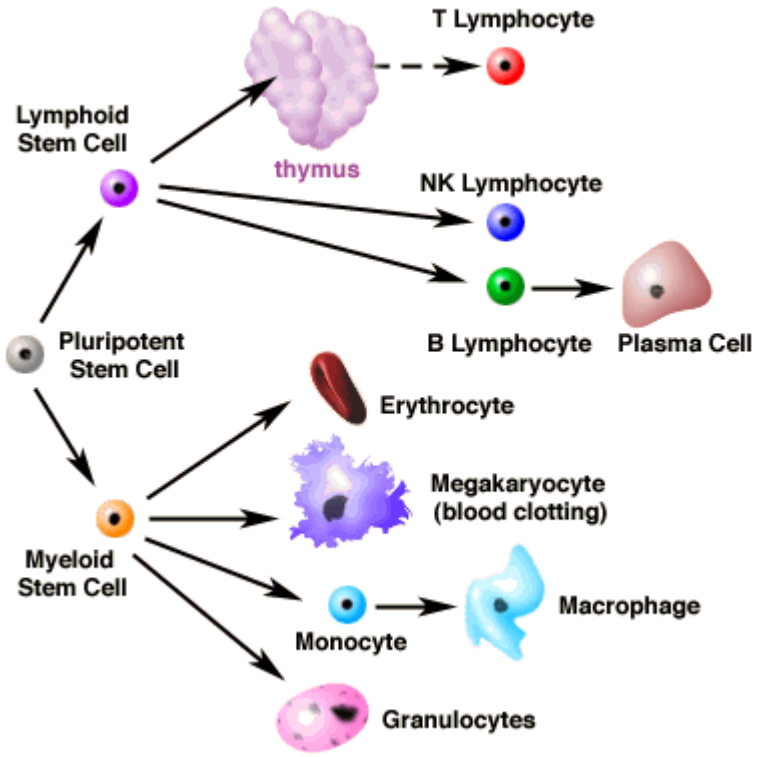

Figure (1): Stem cell differentiation ***(Muller-sieburg et al., 2002)***.

The immune system is divided into two categories: innate and adaptive.

Innate immunity:

It is nonspecific defense mechanisms that come into play immediately or within hours of an antigen's appearance in the body ***(Litman et al., 2005).***

Innate immunity includes:

- *Anatomic* (skin, mucous membrane)
- *Physiologic* (temperature, pH)
- *Cells:* mast cells, phagocytes (monocyte, neutrophil and macrophages), basophils and eosinophils, natural killer cells.
- *Complement* system and *inflammatory mediators* ***(Rus et al., 2005).***

Adaptive immunity:

It refers to antigen specific immune response. The antigen first must be processed and recognized. The adaptive immune system creates an army of immune cells specifically designed to attack that antigen. It includes a "memory" that makes future responses against a specific antigen more efficient ***(Pancer and Cooper, 2006).***

Cells of the adaptive immune system:

B cells and T cells are the major types of lymphocytes. B cells are involved in the humoral immune response, whereas T cells are involved in cell mediated immune response ***(Bonilla and Oettgen, 2010).***

B lymphocytes and antibodies:

B cell identifies pathogens when antibodies on its surface bind to a specific foreign antigen. This antigen antibody complex is taken up by the B cell and processed by proteolysis into peptides. The B cell then displays these antigenic peptides on its surface in association with MHC class II molecules. This combination of MHC and antigen attracts a matching helper T cell, which releases lymphokines and activates the B cell ***(Shlomchik and Weisel, 2012).***

As the activated B cell then begins to divide, its offspring (plasma cells) secrete millions of copies of the antibody that recognizes this antigen. These antibodies circulate in blood plasma and lymph, bind to pathogens expressing the antigen and mark them for destruction by complement activation or for uptake and destruction by phagocytes ***(Tung and Leonore, 2007).***

T cells:

Two major subtypes are identified, the killer T cell and the helper T cell.

Helper T-cells (CD4+) secrete cytokines that regulate the immune response and mobilize other cells to help eradication of a pathogen. Also they play a role in activating B-cells and selecting certain B-cells to become memory cells ***(Crotty, 2011).***

Cytotoxic T-cells (CD8+) efficiently lyse cells that produce target antigens. Importantly, this gives them the ability to destroy virus infected cells accomplished by production of perforin and granzymes. Killer T cells only recognize antigens coupled to Class I MHC molecules, while helper T cells only recognize antigens coupled to Class II MHC molecules ***(Chaplin, 2010).***

Another subtype of Tcell is T regulatory cells which is a component of the immune system that suppress immune responses of other cells. This is an important "self-check" built into the immune system to prevent excessive reactions. Regulatory T cells come in many forms with the most well-understood being those that express CD4, CD25, and Foxp3 (CD4+CD25+ regulatory T cells, or "T_{regs}"). These cells are involved in shutting down immune responses after they have successfully eliminated invading organisms, and also in preventing autoimmunity ***(Sakaguchi et al., 2009).***

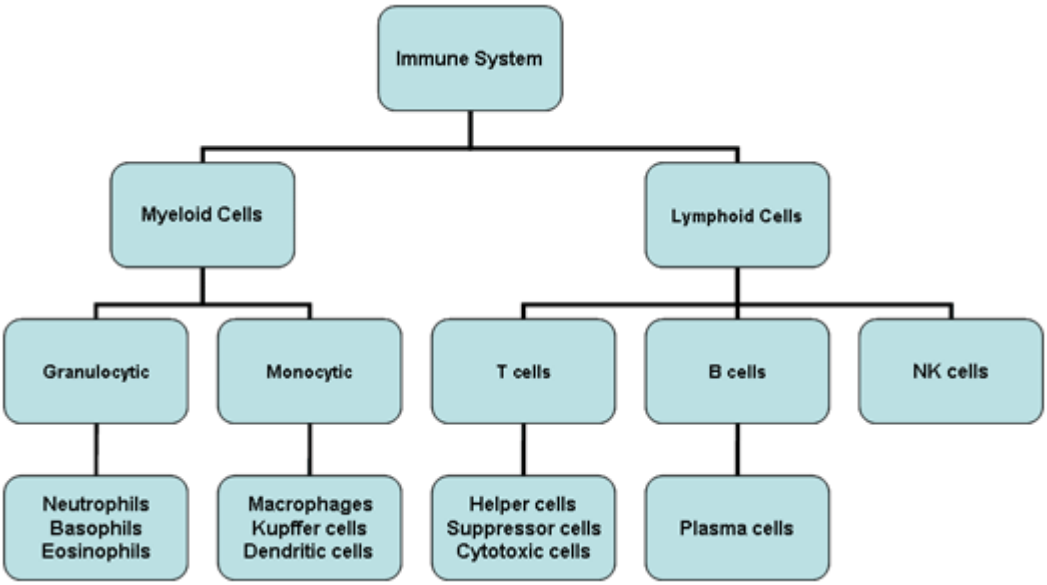

Figure (2): Cells of the immune system ***(Sakaguchi et al., 2009).***

Definition of Phagocytosis:

Phagocytosis is the process of taking in particles such as bacteria, parasites, dead host cells and foreign debris by a cell after the foreign body, has been bound to receptors on the surface of the phagocyte. Then the phagocyte stretches itself around the bacterium and engulfs it ***(Ravichandran and Lorenz, 2007).***

Once inside this phagocyte, the bacterium is trapped in a compartment called a phagosome then the phagosome fuses with a lysosome to form a phagolysosome in which lysosomal contents are released to destroy the pathogen ***(Nunes and Demaurex, 2012).***

Types of phagocytes:

Phagocytes are divided functionally according to the presence or absence of phagocytic receptors and how effective they are at phagocytosis into:

1. Professional

2. Non professional ***(Chaudhuri, 2013)***

Professional phagocytes:

The main difference is that professional phagocytes are almost exclusively involved in eating (phagocytosis), and have special receptors embedded on their surface that allow them to detect foreign objects not normally found in the body. The most important professional phagocytes are neutrophils and macrophage ***(Murray and Wynn, 2011).***

Neutrophils:

Neutrophils also called polymorphonuclear leukocytes (PMNs) are the most abundant type of phagocytes in the blood. Normally they represent 50% to 60% of the total circulating leukocytes, and are usually the first cells to reach the site of infection. The bone marrow of healthy adult produces more than 10^{11} neutrophils per day and more than 10 times per day during acute inflammation ***(Rich et al., 2008)***.

Cytoplasmic granules of the neutrophils are called lysosomes and contain the various bactericidal and digestive enzymes which can destroy bacterial cells after engulfment. The first granules formed at the promyelocyte stage are called primary or azurophilic granules. These primary granules contain microbicidal enzymes, including defensins, hydrolases and proteases. After the promyelocyte stage, secondary or specific granules form. The secondary granules are less dense and contain cytochrome b558, lysozyme, lactoferrin, and collagenase. The third type of granule, the gelatinase containing tertiary granule, probably forms after the metamyelocyte stage and can be detected in the band form and mature granulocyte ***(Turgeon, 2009)***.

Macrophages:

Monocytes are circulating precursors of macrophages that circulate briefly in the blood stream.Macrophages are mononuclear leukocytes that have left the blood stream to migrate in the tissues (eg., alveolar macrophages, kupffer cells and microglial cells) ***(Abbas et al., 2014).***

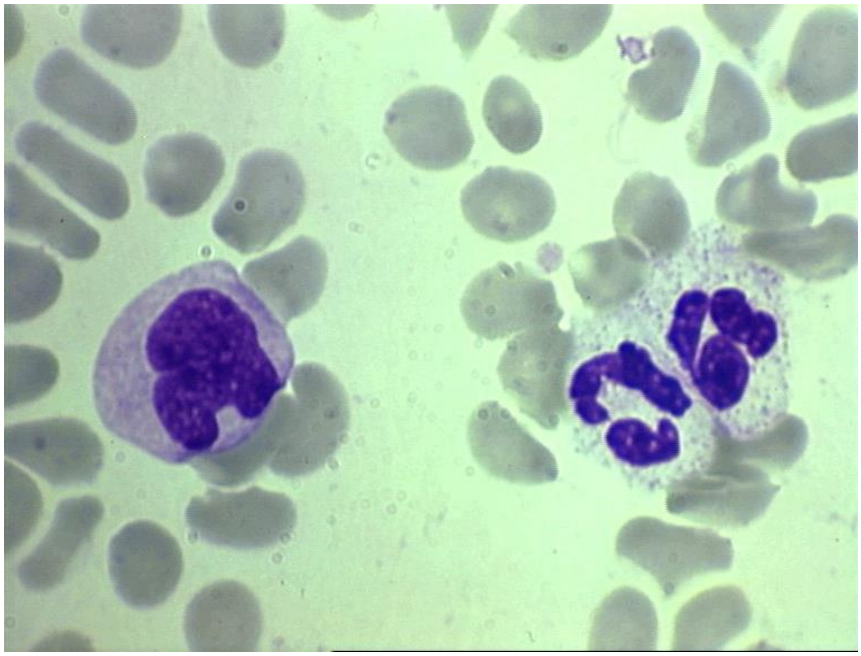

Figure (3): Blood film showing a monocyte (left) and two neutrophils (right) ***(Abbas et al., 2014).***

Non professional Phagocytes:

These cells include epithelial cells, endothelial cells, fibroblasts, and mesenchymal cells. Phagocytosis is not their principal function. Non-professional phagocytes are more limited than professional phagocytes in the type of particles they can take up, this is due to their lack of efficient phagocytic receptors. Most non professional phagocytes do not produce reactive oxygen containing molecules in response to phagocytosis ***(Visan, 2013).***

Initiation of phagocytosis

Phagocytic cells have a variety of receptors on their cell membranes through which infectious agents bind to the cells. These include:

1. Toll like receptors: phagocytes have a variety of Toll like receptors (Pattern Recognition Receptors or PRRs) which recognize broad molecular patterns called PAMPs (pathogen associated molecular patterns) on infectious agents. Binding of infectious agents via Toll like receptors results in phagocytosis and the release of inflammatory cytokines (IL-1, TNF-alpha and IL-6) by the phagocytes.
2. Fc receptors: bacteria with IgG antibody on their surface have the Fc region exposed and this part of the Ig molecule can bind to the receptor on phagocytes. Binding of IgG coated bacteria to Fc receptors results in enhanced phagocytosis and activation of the metabolic activity of phagocytes (respiratory burst).
3. Complement receptors: phagocytic cells have a receptor for the 3rd component of complement, C3b. Binding of C3b coated bacteria to this receptor also results in enhanced phagocytosis and stimulation of the respiratory burst.
4. Scavenger receptors: scavenger receptors bind a wide variety of polyanions on bacterial surfaces resulting in phagocytosis of bacteria ***(Murphy et al., 2011).***

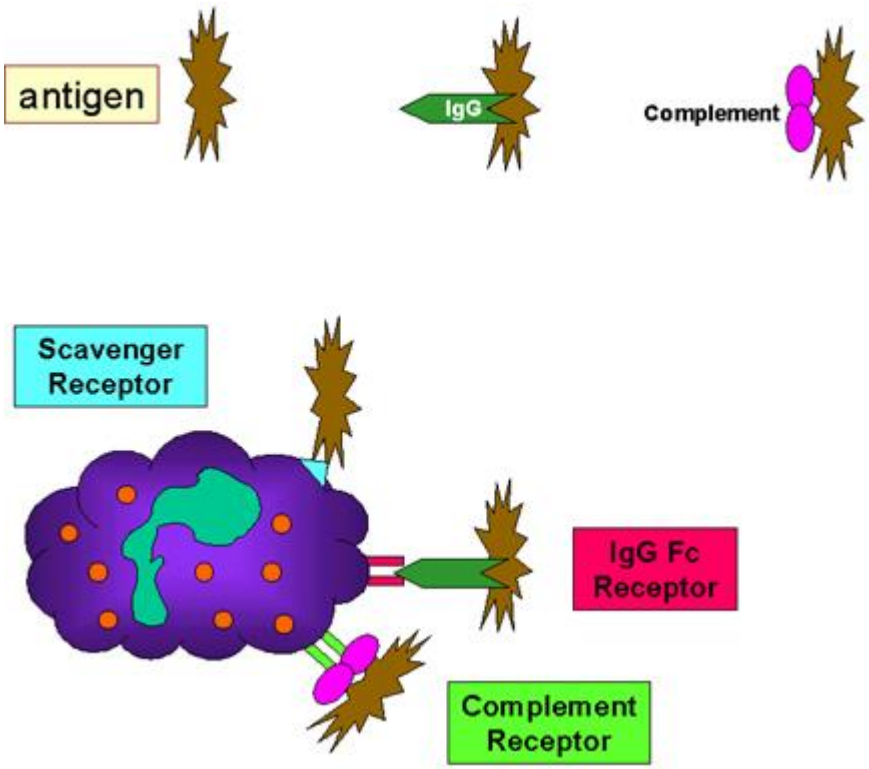

Figure (4): Adherence of bacteria via receptors ***(Murphy et al., 2011)***.

The Phagocytic Process

Phagocytosis and destruction of engulfed bacteria involves the following sequence of events:

1. Delivery of phagocytic cells to the site of infection.
2. Adherence.
3. Ingestion and phagosome formation.
4. Phagolysosome formation.
5. Killing and digestion.

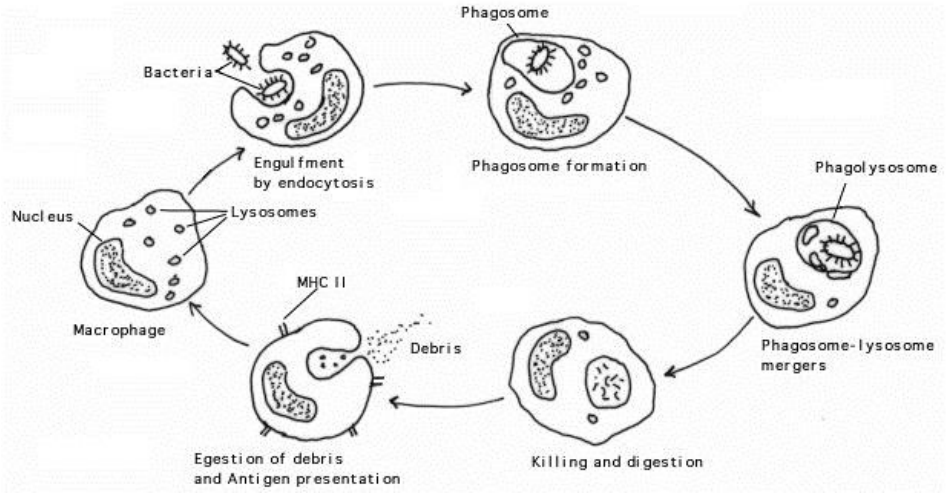

Figure (5): Phagocytosis by a Macrophage ***(Todar, 2014)*.**

Delivery of phagocytic cells to the site of infection

Cells are guided to the site of injury by chemoattractant substances. This event is termed chemotaxis. A chemotactic response is defined as a change in the direction of movement of a motile cell in response to a concentration gradient of a specific chemical, chemotaxin. These include bacterial products, cell and tissue debris, and components of the inflammatory exudate such as peptides derived from complement ***(Todar, 2014)*.**

Under certain circumstances of infection, bacteria or viruses, may become coated or otherwise display on their surfaces one or another of these substances (i.e., IgG, C3b, fibronectin or mannose). Such microbes are said to be opsonized and such substances as IgG or complement C3b bound to the surface of microbes are called opsonins. Opsonins

provide extrinsic ligands for specific receptors on the phagocyte membrane, which dramatically increases the rate of adherence and ingestion of the pathogen ***(Todar, 2014)***.

Adherence:

Neutrophils and monocytes migrate to extracellular sites of infection or tissue damage by binding to venular endothelial adhesion molecules. Leukocyte migration is a multistep process that consists of initial weak adhesive interactions of the leukocytes to endothelial cells, followed by firm adhesion and transmigration through the endothelium ***(Turgeon, 2009).***

Cytokines produced in response to microbial entry (eg., TNF and IL-1), act on the endothelium of venules near the site of infection. These cytokines stimulate the endothelial cells to rapidly express two adhesion molecules of the selectin family called E-selectin and P-selectin.Circulating neutrophils and monocytes express surface carbohydrates that bind weakly to the selectins result in rolling of the leukocytes along the endothelial surface ***(Abbas et al., 2014).***

Leukocytes express another set of adhesion molecules called integrins. Within a site of infection chemokines produced by tissue macrophages stimulate a rapid increase in the affinity of the leukocyte integrins for their ligands on the endothelium. The firm binding of integrins to their ligands arrest the rolling of leukocytes on the endothelium. As a result, the leukocytes begin to migrate between endothelial cells, through the vessel