INTRODUCTION

Acute myeloid leukemia is a malignant clonal disorder of immature cells in the hematopoietic hierachial system. On the other hand chronic myeloid leukemia is a clonal disease that results from an acquired genetic change in pluripotential hematopoietic stem cell. This altered stem cell proliferates and generates a population of differentiated cells that gradually displace normal hematopoiesis and leads to a greatly expanded total myeloid mass ***(Sonneveld and List, 2001; Burnett, 2002; Sanchez et al.,2004).***

Allogeneic stem cell transplantation has been established as an effective consolidation therapy in myeloid leukemias. Allogeneic BMT in first complete remission (CR), in patients under 65 years without major organ dysfunction (eg, renal pulmonary cardiac,or hepatic damage) who have an HLA-compatible related bone marrow donor or in those under age 55 years with an HLA compatible unrelated donor, results in cure in 40 to 60 percent of patients. However, toxicity is relatively high with treatment-related complications, including veno-occlusive disease, graft-versus-host disease (GVHD), interstitial pneumonitis and infections ***(Schiller et al.,1992)***.

Dendritic cells (DC) as key intiator and directors of the immune response are central to allogeneic

transplantation interactions. Preparative conventional conditioning (CC) regimens aim to control disease and ablates the host immune response to facilitate normal donor hematopoietic reconstitution.

The conditioning also unleashes a cytokine storm that activates the residual host immune system, driving host dendritic cells and donor T cell interaction that results in graft versus host disease. This led to a trend to reduce the intensity of the conditioning regimens (RIC). The reduced intensity conditioning regimens are used as alternatives in patients with co-morbid conditions. They maintain immune antileukemic activity of the T –replete hematopoietic stem cell transplantation (HSCT), which may lead to reduction of transplant-related mortality (TRM) and may delay the onset of GVHD, but the overall incidence of GVHD is unchanged ***(Turner et al., 2005).***

It was demonstrated that the circulating dendritic cell pool is rapidly reconstituted from both donor and recipient origins. Around day 28, donor engraftment of DC becomes predominant. On the other hand, the circulating dendritic cells are known to have an immunoregulatory role after allogeneic hematopoietic stem cell transplantation, and recipient DCs have been shown to be important in the development of GVHD in animal models. The successful application of DC depletion to control GVHD was

suggested to improve the safety of HSCT for patients with leukemia ***(Tabrizi et al., 2005).***

However, despite the importance of dendritic cells in immune reconstitution and GVHD, limited information is available about dendritic cells reconstitution after transplantation in patients receiving reduced intensity stem cell transplantation and its relation to outcome, which may influence the management decisions of transplantation - related complications**.**

AIM OF THE STUDY

The aim of this study is to determine the level of circulating mature dendritic cells in patients with acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) treated with reduced intensity hematopoietic stem cell transplantation after recovery,and to assess its relation to outcome.

THE IMMUNE SYSTEM & ANTIGEN PRESENTATION

The immune system

The immune system is an organization of cells and molecules with specialized roles in defending against infection.

There are two fundamentally different types of responses to invading microbes.

Innate (natural) response occur to the same extent however many times the infectious agent is encountered, where as acquired (adaptive) response improve on repeated exposure to a given infection. The innate responses use phagocytic cells (neutrophils, monocytes, and macrophage), cells that release inflammatory mediators (basophils, mast-cells, eosinophils), and natural killer cells.

The molecular components of innate response include complement, acute phase protein, and cytokines such as interferons. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, called antigen - presenting cells, display the antigen to lymphocytes and collaborate with them in the response to the antigen. B cells secrete immunoglobulins, the antigen - specific antibodies responsible for eliminating extracellular

microorganism. T-cells help B cells to make antibody and can also eradicate intracellular pathogen by activating macrophages and by killing virally infected cells. Innate and acquired responses usually work together to eliminate phathogens.

To establish an infection, the pathogen must first overcome numerous surface barrier. Any organism that breaks through this first barrier encounters the two levels of defense, the innate and acquired immune responses ***(Deleves and Roitt, 2000).***

The body can potentially respond to almost anything that can be bound by the receptors of either the innate or the acquired immune system. Molecules recognized by receptors on lymphocyte are generically to as antigens. Both the T cell receptor and the antibody that is embedded in the B-cell membrane have binding sites. Therefore, these receptors recognize only a small part of a complex antigen, referred to as the antigenic epitope ***(Garcia et al.,1999).*** Antigens that elicit immune response are termed immunogens. Not all antigens are naturally immunogenic. Small, nonimmunogenic antigens are called haptens and must be coupled to larger immunogenic molecules, termed carrier, to stimulate a receptor ***(Mitchison, 1971)***

Innate immune response

Cellular component of innate response

The innate immune system consists of all the immune defense that lack immunologic memory.

Maccrophages (derived from blood - borne monocytes) are important in the regulation of immune responses. They are often referred to as scavengers or antigen- presenting cells (APCs) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T -cells and B cells. It possess receptor for carbohydrates that are not normally exposed on the cells of vertebrates, and therefore can discriminates between "foreign" and "self" molecules ***(Fraser et al., 1998).*** In addition, both macrophages and neutrophils have receptors for antibodies and complement, so that the coating of microorganisms with antibodies, complement, or both enhances phagocytosis. The engulfed microorganisms are subjected to a wide range of toxic intracellular molecules to enhance its lysis ***(Aderem and Underhill, 1999).***

A key cellular component of innate immunity – and one of most intensely studied component during the past decade – is the dendritic cell ***(Bell et al., 1999).***

Eosinophils are the only weakly phagocytic and, on activation, probably kill parasites mainly by releasing

cationic proteins and reactivate oxygen metabolites into extracellular fluid. They also secrete leukotrienes, prostaglandins, and various cytokines ***(Wardlaw et al., 1995).***

Basophils and mast-cells have similar functional characteristics ***(Abraham and Arock, 1998),*** but there is little evidence that blood basophils develop into tissue mast-cells. Both types of cells possess high – affinity receptors for IgE and thereby become coated with IgE antibodies. These cells are important in atopic allergies ***(Kinet, 1999).***

Natural killer cells destroy infected and malignant-cells ***(Biron et al., 1999).*** They recognize their targets in one of two ways. Like many other cells, they possess Fc receptors that bind IgG these receptors link natural killer cells to IgG-coated targets-cells, which they kill by a process called antibody-dependent-cellular cytotoxicity ***(Moretta et al., 1997).*** The second system of recognition that is characteristic of natural killer cells relies on the killer-activating receptors and killer-inhibitory receptors of these cells. The killer-activating receptors recognize a number of different molecules present on the surface of all nucleated cells, whereas the killer-inhibitory receptors recognize MHC class 1 molecules, which are also usually present on all nucleated cells ***(Lanier, 1998).***

Soluble factors in innate defense:

Innate responses frequently involve complement, acute-phase proteins, and cytokines. The early events of complement activation can be triggered by one of three pathways. The classic pathway is activated by antigen-antibody complexes, the alternative pathway by microbial-cell walls, and the lectin pathway by the interaction of microbial carbohydrates with mannose-binding protein in the plasma the outcome is the generation of a number of immunologically active substances ***(Wallis and Drickamer, 1999).***

The molecules collectively referred to as acute-phase proteins enhance resistance to infection and promote the repair of damaged tissue. Plasma levels of these proteins change rapidly in response to infection, inflammation, and tissue injury. The acute-phase proteins include C reactive protein (a useful marker of inflammation, particularly in disease such as rheumatoid arthritis), serum amyloid A protein, proteinase inhibitors, and coagulation proteins ***(Gabay and Kushner, 1999).***

Cytokines constitute another group of soluble mediators. They act as messengers both within the immune system and between the immune system and other systems of the body, forming an integerated network that is highly involved in the regulation of the immune responses.In addition to acting as messengers, some cytokines have a

direct role in defense, for example, the interferons that are released by virally infected cells establish a state of viral resistance in the surrounding cells. Cytokines and their antagonists are increasingly being used as therapeutic agents ***(Keilholz et al.,1998; Feldmann et al.,1997).***

Aquired Immune Response

The development of lymphocytes and the myeloid lineage from primordial stem cells in the fetal liver and in bone marrow is guided by interactions with stromal cells (such as fibroblasts) and by cytokines. The initial stages of lymphocyte development do not require the presence of an antigen, but once these cells express a mature antigen receptor, their survival and further differentiation become antigen-dependent ***(Metcalf, 1995).***

B-cells (humoral immune response)

The major function of B lymphocytes is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. Antibodies are specialized proteins that specifically recognize and bind to one particular protein. Antibody production and binding to a foreign substance or antigen, often is critical as a means of signaling other cells to engulf, kill or remove that substance from the body. Antibodies consist of two identical heavy chains and two identical light chains that are held together by disulfide bonds. There are 5 types

(IgG, IgA, IgM, IgD, and IgE), each one has specific function ***(Edelman,1973).***

T-cells (cell mediated immune response)

Stem cells continuously migrate from the bone marrow to the thymus, where they develop into T-cells ***(Kruisbeek, 1999).*** T-lymphocytes are usually divided into two major subsets that are functionally and phenotypically (identifiably) different. The T helper subset, also called the CD4+ T-cell, is a coordinator of immune regulation. The main function of the T helper cell is to augment or potentiate immune responses by the secretion of specialized factors that activate other white blood cells to fight off infection ***(Ellmeier et al., 1999).*** Another important type of T-cell is called the T killer /suppressor subset or CD8+ T-cell. These cells are important in directly killing certain tumor cells, viral-infected cells and sometimes parasites. The CD8+T-cells are also important in down-regulation of immune responses.

Both types of T-cells can be found throughout the body. They often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they are also found in other tissues of the body, most conspicuously the liver, lung, blood, intestinal and reproductive tracts ***(Goodman and Lefrancois, 1998).***

Antigen presenting cells and phenomena of Antigen Handling and presentation

Antigen presenting cells (APCs) are accessory cells of antigen-inductive events; these cells function by handling and presenting antigen to lymphocytes. The interaction of APCs with antigens is an essential step in immune induction because it enables lymphocytes to encounter and recognize the antigenic molecules and to become activated. Both events, antigen recognition and cellular activation, are interrelated. The interaction between APCs and lymphocytes is usually reciprocal.

Types of APCs

Macrophages, dendritic cells and B-cells ***(Unanue, 1993).***

Mononuclear phagocytes

These cells are distributed throughout most tissues, playing a role in inflammation, in host defense, and in reaction against a range of autologous and foreign materials. In immunological reaction their function involve the uptake and presentation of protein to CD4 T-cells, the release of modulatory molecules such as interleukin-1(IL-1), tumor necrosis factor(TNF),IL-6, and their participation as an effector cell against microbes and tumors ***(Unanue, 1993).***

Macrophages represent the terminally differentiated cell that originates from a precursor found in various tissues ***(Volkman and Gowans, 1963).*** The precursor cell that gives rise to macrophages is monocytes. Monocytes have endocytic activity, circulate in blood for about 1 day, and then distribute into different tissues ***(VanFurth and cohn, 1968).***

In tissues, monocytes differentiate further into macrophage under control of CSF-1, M-CSF (macrophage - colony stimulating factor), GM-CSF (granulocyt macrophage colony stimulating factor), IL-3 and G-CSF (granulocyt colony stimulating factor) ***(Fung et al.,1984; Ladner et al., 1988).*** CSFs are produced by diverse cells and have been isolated from various body fluids and tissue extracts. They increase in amount during infection and strong immunological reactions ***(Bartocci et al., 1987).*** Macrophages are found in all tissues. Their turnover in a given tissue may vary between 4 and 15 days ***(Van furth, 1989).*** Macrophages in different tissues have distinctive properties and vary in their extent of surface receptors, oxidative metabolism and expression of class II MHC molecules ***(Howard, 1970).***

Kupffer cells are liver macrophages situated in the liver sinusoid in contact with endothelial cells ***(Lepay et al., 1985).*** They are the major cellular system responsible for

the clearance of particulate material or microbes from the circulation ***(Unanue, 1981).***

Alveolar macrophages remove unwanted particulate materials from alveolar spaces of the lung ***(Green and Kass, 1963).*** They are actively secreting proteases and bactericidal molecules such as lysozyme ***(Sibille and Reynolds, 1990).***

Macrophages in spleen shows evidence of compartmentalization.Those in the red pulp are less differentiated express high level of class II MHC molecules and take up anionic polysccharides such as that from the capsule of the pneumococcus ***(Humphrey and Grennan, 1981).*** In contrast, those of the marginal zone, which surrounds the lymphoid sheath, show limited expression of class II MHC molecules and take up selected neutral carbohydrates such as dextran or starch ***(Humphrey and Grennan, 1981).***

Macrophages are also found in many other important anatomical tissues. In kidney glomerulus, for example, a few are normally found in the mesangium, in close opposition to mesangial cells, where they trap antigen-antibody complexes ***(Schreiner et al., 1981).*** In the bone marrow, macrophages are an important component of the stromal cells and are found in close association with erythroid cells. Macrophages are also found in endocrine organs. Those in central nervous system are known as

microglia ***(Perry and Gordon, 1991).*** In bone the representative of the macrophages is the osteoclast ***(Ash et al., 1980)*** the specialized features of the osteoclasts are their multinucleation, Their close adhesiveness to bone matrix mediated most likely by integrin, and their secretion of acid proteases ***(Blair et al., 1986).*** Osteoclast have a key function in bone turnover ***(Marks, 1982).***Current attention centers on the possible role of osteoclast in osteoprosis ***(Yoshida et al., 1990).***

Recognition and internalization

Macrophages are active in absorptive endocytosis that is the uptake of a sample of the extracellular fluid. However, to effectively sample the environment the macrophage expresses surface receptors to a range of proteins, including hormones, immunoglobulins, complement (C) proteins, toxins, cytokines, polysccharides, and lipids. By way of these receptors, the macrophage take up microorganisms and responds to cytokines and foreign proteins. The response to many of the stimuli that bind to the macrophage is twofold, the first is to internalize the stimulus and subject it to the biochemical changes characteristic of endocytic processing and digestion, the second response is to express bioactive molecules that modulate the response of the macrophage to the environment ***(Ravetch and Kinet, 1991).*** Macrophages have receptors that bind to the Fc fragment of IgG (FcR) or