INTRODUCTION

Liver cirrhosis (LC) is the end stage of chronic liver diseases, very difficult to treat and may end in liver failure which remains one of the life-threatening syndromes.

So, the demand for human liver cells, either for therapeutic interventions, for the restoration of reduced-size split liver grafts, for artificial liver devices, or for the study of drug metabolism during preclinical development is constantly increasing.

Cell-based hepatocyte transplantation is of particular interest and thought to hold great promise because of the simpler and less invasive procedure. It is often referred to as regenerative or reparative medicine. It may become possible to use the cells not just in cell-based therapies, but also for screening new drugs and toxins.

Recently, pluripotent stem cells prepared from human peripheral blood monocytes were induced to differentiate into liver cells by the use of hepatocytes growth factors. The accessibility and the proliferative capacity of these cells could make them eminently suitable for autologous transplantation. Moreover, this autologous cell transplantation would circumvent ethical and legal questions, problems of transmitting infections and immune rejection.

As such, monocytes and possibly bone marrow-derived stem cells may provide a realistic hope that adult stem cells

from one tissue lineage can transdifferentiate along the lineage of other tissues. This model opens new perspectives, it might even open a road to trigger cell fate and ‘‘trans’’-differentiate uncommitted cells from different tissues towards endodermal lineages.

AIM OF THE WORK

To evaluate the *in vitro* differentiation of a subset of human peripheral blood monocytes which behave as stem cells into hepatocyte- like cells, upon treatment with specific growth factors.

To assess the hepatocyte-like nature of the differentiated cells on the morphological, biochemical and immuno-histochemical levels.

CELLULAR TRANSPLANTS FOR LIVER DISEASES

I- Introduction:

A- Cellular Organization of the Adult Liver:

The liver is the largest organ, accounting for nearly 1/50 of the body entire mass or approximately 1.5 kg in the adult human. Specialized metabolic, secretory, and synthetic functions occur through interactions between blood and hepatocytes (parenchymal liver cells) in units referred to as liver lobules. Nearly 100,000 lobules constitute an adult liver. They are cylindrical structures between 0.8 to 2 mm in diameter. Each lobule, as depicted in **(Figure 1),** has three to seven sets of portal triads defined by a portal venule, hepatic arteriole, and bile duct on the periphery and a central vein at the center. The smallest functional unit of the liver is the acinus, which is defined by two central veins at the ends of the long axis and a portal triad at the center. Each lobule and acinus is composed of hepatocytes arranged in sheets called hepatic cellular plates, which radiate outward away from the central vein. Each hepatic plate has a thickness of two hepatocytes, with a space between adjacent cells where small bile canaliculi are located. Each bile canaliculus, is formed of epithelial cells called cholangiocytes, and empties into a bile duct in the fibrous tissue separating the adjacent liver lobules ***(Nicholas et al., 2007).***

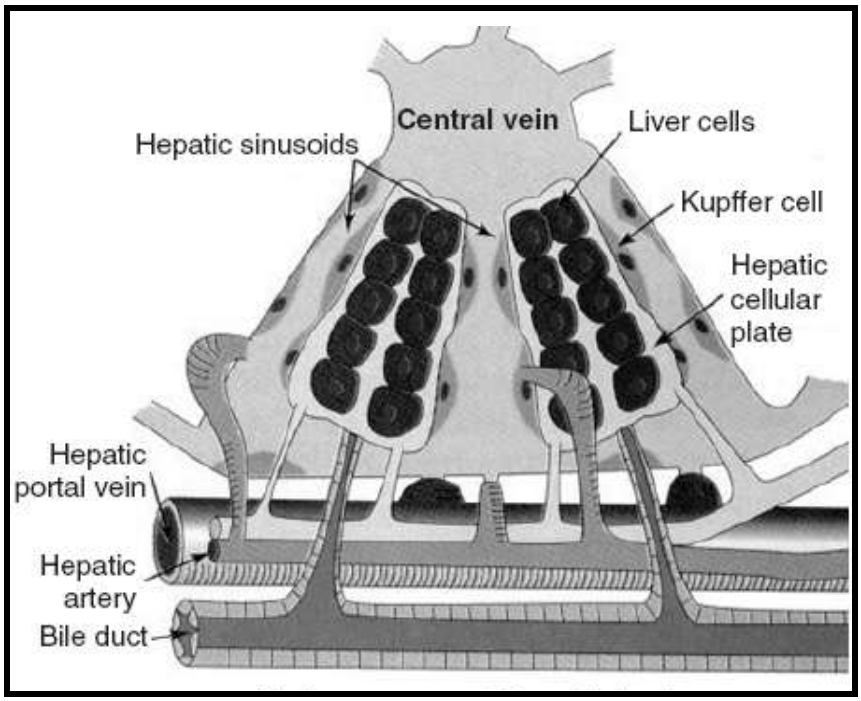

Figure (1): Liver lobule. Depiction of liver lobule showing essential components, including portal triad (hepatic artery, hepatic portal vein, and bile duct), hepatic sinusoids, hepatic plates, and parenchymal cells (hepatocytes) ***(Nicholas et al ., 2007).***

B- Liver Development and Regeneration:

During development, many organs such as the liver, lung, pancreas and intestine, bud from the foregut endoderm in response to signals from adjacent germ layers. The liver and ventral pancreas arise from neighbouring regions of the ventral foregut endoderm ***(Grapin-Botton and Melton, 2000)***, and fibroblast growth factor (FGF) signaling has been shown to direct the ventral pancreas to express genes for liver. It is now known that hepatic specification occurs in response to fibroblast growth factors and bone morphogenetic proteins secreted from the mesenchyme of the septum transversum ***(Craig and Markus, 2006)*** **(Figure 2)**.

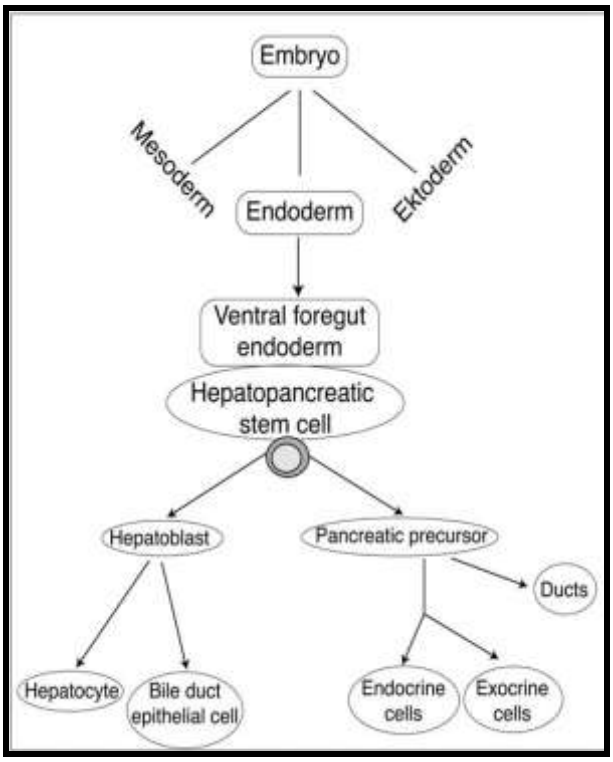

Figure (2): Hypothetical embryonic lineage relationships between liver and pancreas. The differentiation sequence from the totipotent endoderm to the liver and pancreatic lineages is depicted. The ventral foregut endoderm cell which gives rise to both pancreas and liver during development may persist as a hepatopancreatic stem cell in adult life ***(Craig and Markus, 2006).***

The strategic interposition of the liver between the gut and the systemic circulation, and its key role in biotransformation and detoxification, exposes liver cells continuously to many potential threats and toxic insults. The liver is therefore an organ with a tremendous capacity for self-regeneration. Despite the high gut renewal rate, the liver is considered a 'stable tissue', with a rate of turnover estimated to be only 1 in 20,000 – 40,000 cells, at any given time, and complete tissue replacement by normal tissue renewal happens no more than once a year. However, this mitotically dormant

state of liver parenchyma belies its tremendous proliferative potential in response to hepatic injuries ***(Menthena et al., 2004).***

The liver is an organ in which therapeutic intervention has a helping hand by virtue of the remarkable capacity for the liver to regenerate after damage. Centuries ago the Greek Gods noted this in the story of Prometheus, the mortal who stole fire from Zeus, was punished by having his liver plucked out by an eagle every night, only to have it regrow by morning. The ability to regenerate is a unique characteristic of the liver and offers an excellent model system to investigate the role of hepatocyte proliferation and stem cells as a source of renewal. Following surgical removal of 70% of the liver, there is a compensatory response and the remaining liver restores the liver mass and total parenchymal cell number within 14 days in the rat ***(Sandhu, et al., 2001).***

Replication of hepatocytes following liver regeneration has been well documented and differentiation of oval cells can contribute to the hepatocyte population usually when hepatocyte proliferation is impeded or hepatocyte destruction is severe ***(Michael et al., 2008).***

Hepatoblasts are considered to be bipotential committed cells and the precursors of foetal hepatocytes and biliary epithelial cells. Hepatoblasts migrate from the endoderm to form a tissue bud and then proliferate into the septum transversum. The hepatoblasts express alpha-fetoprotein (AFP) and albumin, and later start to express dual markers for both the hepatocyte AFP and biliary (cytokeratins 7 and 19) lineages ***(Lemaigre, 2003).***

So, the putative liver stem cell is 'bipolar': it co-expresses biliary and hepatocytic markers: cytokeratins CK8, CK14, CK18, CK19, AFP, OV-6 and the hepatocyte-specific HepPar1 antigen and can differentiate, *in vitro*, into both hepatocytes and bile duct epithelial cells (BDECs) ***(Vessey and de la Hall, 2001).*** OV-6, a monoclonal antibody raised against cells isolated from carcinogen-treated rat liver, remains one of the best available and most commonly used marker, it also reacts with normal biliary epithelial cells in both rat and human liver. The epitope recognized by OV-6 on rat liver is shared with cytokeratins 14 and 19. Cytokeratins are cytoskeletal intermediate filaments that provide structural support in the cytoplasm of higher eukaryotes and function to sustain the cells against mechanical and non-mechanical stresses. The antigen recognised by OV-6 in human liver has not yet been determined but the immunostaining pattern differs from that of CK19 ***(David and Alastai, 2005) (*Figure 3).**

The intrahepatic bile duct system derives from hepatoblasts that form a sleeve around the portal vein branches, called the *ductal plate*. This process starts at the liver hilum, at about the seventh week of gestation and then spreads throughout the rest of the organ. While the hepatoblasts progressively differentiate into BDECs (marked by CK7, CK18, CK19 OV-6 and gamma-GT) between the 14th and 16th weeks of gestation, other hepatoblasts differentiate into hepatocytes acquiring (ALB) and pyruvate kinase isoenzyme L (L-PK) as markers, losing CK14 and CK19 and serving HepPar1 and AFP ***(Vessey and de la Hall, 2001).***

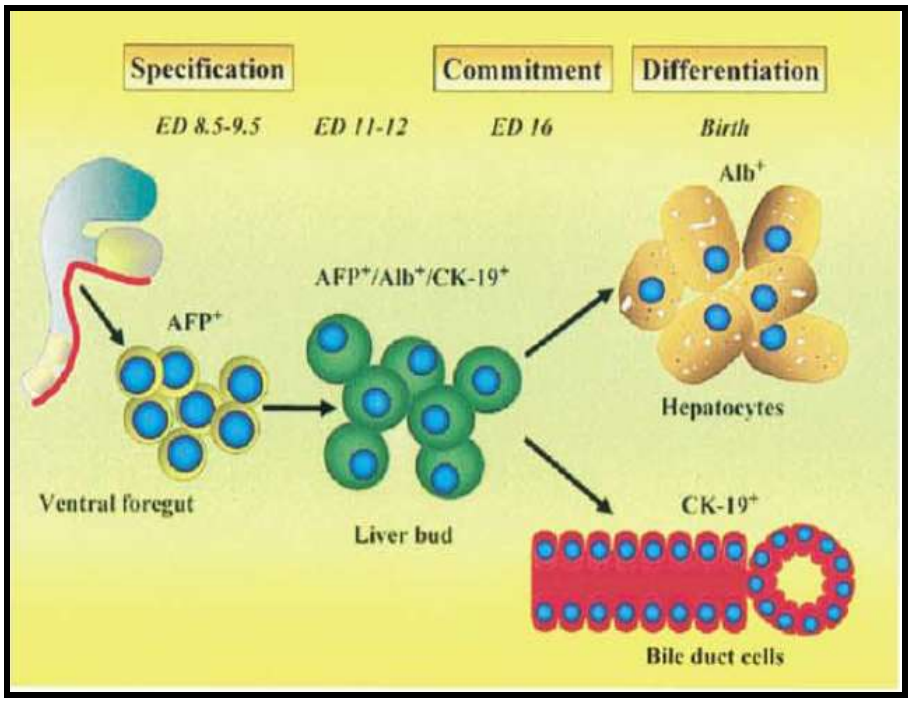

Figure (3): Model system for liver repopulation by transplanted fetal liver stem/progenitor cells: The fetal liver in mid gestation. Epithelial cells derived from the foregut have become specified to enter the hepatic lineage. They have begun to express hepatocyte-specific genes, such as AFP and albumin, and the cholangiocyte specific marker, cytokeratin-19, and are rapidly proliferating but are not yet committed to differentiate along the hepatocytic or cholangiocytic lineage ***(Lemaigre, 2003).***

In the 1960s, it was shown in rats that during liver regeneration, hepatocytes throughout the liver parenchyma are actively engaged in DNA synthesis, and it was estimated that 70–90% of hepatocytes undergo at least one round of cell division during this process. However, after two-thirds partial hepatectomy, only one or two divisions of each remaining hepatocyte are required to restore liver mass, so that the proliferative response is rather small. Under normal conditions, liver regeneration is achieved through proliferation of differentiated hepatocytes (including tetraploid cells) and does

not require the participation of stem cells. However, whether stem cells are involved in normal liver homeostasis or in maintenance of hepatic mass or function during chronic liver injury has not been determined ***(Philippe et al., 2008).***

II- Cellular Therapy for Liver Diseases:

Wide varieties of liver diseases lead to the impairment of liver functions and require medical intervention. In order to overcome these problems, alternative approaches have been proposed, such as auxiliary and split-liver transplantation, procurement from living donors, isolated cell transplantation, transgenic xenotransplantation or extracorporeal liver support. In the context of artificial livers, devices can be distinguished into mechanical devices, able to selectively remove toxins accumulated in the patient's blood and bioartificial devices, where isolated hepatocytes are used to simultaneously replace the missing synthetic and metabolic functions of hepatocytes ***(Boudjema, 2002).***

Liver transplantation is the primary treatment for end-stage hepatic diseases, with 4-year survival rate of 70% or greater for most clinical indications. Although effective, extensive clinical application is limited by the lack of "availability of donor organs", "operative damage", "rejection", and "high expense". Other adverse factors such as rejection problems associated with the long-term use of immunosuppressants, and perioperative morbidity and mortality contribute to additional complications. In view of these shortfalls, cell-based hepatocyte transplantation is of particular interest and thought to hold great promise because of the simpler and less invasive procedure. A single donor could serve multiple recipients, and excess cells could be cryopreserved for

future use. Also liver cells have been used clinically to "bridge" patients to whole organ transplantation or auto-recovery or as a "cell therapy," and an alternative to whole organ transplantation ***(François et al., 2007).***

However, studies have demonstrated that less than 20-30% of transplanted hepatocytes survive upon transplantation and that multiple transplantation procedures are required to achieve meaningful liver repopulation. Furthermore, the procurement of transplantable hepatocytes is hampered by the paucity of cadaveric liver, the limited replicative potential, the concomitant loss of characteristic hepatic functions upon *in vitro* culture and reduced numbers of viable and functional cells upon cryopreservation ***(Fox and Chowdhury, 2004).***

However, one major limitation of cell-based therapies for liver disease is the availability of human hepatocytes. A wider use of these techniques will not be possible until adequate numbers of functional cells for transplantation become more readily available. There are at least two possible sources that could meet the needs for transplantation, namely stem and precursor cell derived hepatocyte-like cells or replicating hepatocyte cell lines ***(Jang et al., 2004).***

Without doubt, the wide availability of human hepatocytes would be considered a major breakthrough and may open new perspectives for the treatment of liver disease. On the other hand, some studies presenting with far–reaching conclusions with respect to the capacity of stem cell therapy have not yet been reproduced or may have been interpreted in an over-optimistic manner ***(Popp et al., 2007).***

Initially, it was anticipated that liver cell transplants would be less likely to require vigorous immunosuppression than whole organ transplants, given that hepatocytes do not express MHC Class II and only weakly express MHC Class I antigens. Unfortunately, this laboratory finding has not proven to be totally useful in the clinical area, as immunosuppression is still required for patients receiving liver cell transplants ***(Allen et al., 2005).***

A- Candidate Stem Cells for Hepatic Replacement Therapy:

1- Liver Derived Stem Cells:

There are several different intrahepatic stem cells as well as potential sources of liver cells for transplantation ***(Weissman, 2002)***.

a- Foetal liver stem cells:

During development, hepatoblasts appear in the fetal liver bud, they give rise to the two epithelial cell populations of the liver, hepatocytes and biliary epithelial cells, and are therefore, classified as bipotential progenitor cells. These cells, which express α-fetoprotein as well as hepatocyte (albumin) and biliary (CK19) markers, have been described as fetal liver stem cells. They have also been used extensively in cell transplantation experiments and shown to successfully repopulate both cell types in animal livers (***Sandu et al., 2001).***

Retrorsine-stimulated progenitors in fetal rat liver exist as at least three distinct subpopulations of hepatoblasts (**bipotential, unipotential hepatocytic, and unipotential**

ductal) at embryonic days 12–14. After transplantation, the bipotential cells proliferate in retrorsine-treated recipients, but unipotent cells grow even in untreated rats. These fetal cells proliferate more readily than their adult counterparts, indicating an enhanced proliferative and differentiative potential ***(Craig and Markus, 2006).***

More recently human hepatoblasts have been demonstrated to undergo extensive replication *in vitro* and differentiation into hepatocytes again following transplantation. These exciting studies provided sufficient human foetal liver tissues, and this may ultimately offer the best source of mature hepatocytes for clinical studies ***(Michael et al., 2008).***

b- Adult liver stem cells:

The existence of adult stem cells in the liver is still controversial. As organ shortage is limiting the availability of this cell population, the conditions for their use in cell therapy are governed by two main characteristics, a high proliferation rate and/or a robust cell banking capacity. Regarding their promising hepatocyte-like functionality, some cell compartments could be promptly considered for toxicological assays. Even if identity and *in vivo* function of this cell population are currently under controversy, four main types of hepatic progenitors are described: oval cells, small hepatocytes, liver epithelial cells and mesenchymal-like cells ***(Philippe et al., 2008).***

Oval cells are small cells just at the periductular junction also called the canal of Hering. They have oval nuclei, scant cytoplasm. They are generated from the biliary tree in response to hepatic injury. Oval cells are examples of hepatic progenitor cells i.e. they are the progeny of the liver stem cell, they display

a bipotent differentiation potential (hepatic and biliary cells) and can be expanded *in vitro*. There is no consensus on whether these cells are originating in bone marrow (BM) or not ***(Oh et al., 2007).***

However, similar cell types were isolated from healthy liver. The first description of **small hepatocytes** isolation was made by ***Mitaka et al. (1995*)** from a non parenchymal fraction after centrifugation of isolated liver cells. These cells are smaller than hepatocytes, possess an *in vitro* proliferation capacity and can differentiate into mature hepatocytes *in vitro* ***(Fougere-Deschatrette et al., 2006).***

Liver epithelial cells are a population firstly described by ***Tsao et al (1984)*** in which diploid epithelial cell line (termed WB-F344) was isolated from the liver of an adult male Fischer-344 rat, the phenotypic properties of the liver epithelial cell line in culture mostly resemble those of the 'oval' cells. More recently, they investigate the presence and role of liver epithelial cells in the healthy human adult liver, they found that although different from oval cells, these cells are bipotential and are able to differentiate into hepatocyte-like cells *in vivo* ***(Khuu et al., 2007).***

A mesenchymal-like cell population has also been isolated from adult human liver. This population depicts high level of proliferation and as mesenchymal stem cells (MSCs), possesses a broad differentiation potential. How these cells share MSCs' immunological properties has to be determined. Other cell types were also isolated from manipulated livers, but the possibility of culture artifacts should always be considered before describing new 'liver progenitor cells' ***(Najimi et al., 2007).***

All stem cells have the unique ability to self-renew and to differentiate into progeny. Within the liver, stem cells are thought to reside in a niche or microenvironment. The niche is composed of cells, extracellular matrix and soluble factors released by the niche cells that help to maintain the characteristics of the stem cells. Two current views are held regarding the localization of the liver stem cell. The first is that liver stem cells are located in the canals of Hering. The second is that liver stem cells are found in the periductular / intraportal zone of the liver ***(David and Alastair, 2005)***.

The cells of the canal of Hering are those terminal bile ductular cells of the smallest branches of the biliary tree which are immediately adjacent to the periportal hepatocytes and hence have also been described as transitional cells. In rodent models of liver injury or treatment with hepatocarcinogens, the oval cells which are so prominent are thought to rise from this anatomical location. Over several decades, there is a substantial literature describing the isolation, culture differentiation and transplantation back into experimental animals ***(Fausto and Campbell, 2003).***

Despite the fact that isolation, purification and culture of rat oval cells were first described over 20 years ago. Their precursors, the cells of the Canals of Hering have not been characterized due to a lack of specific markers. The same problem prevails in the human liver, where oval cells arise during the pathogenesis of chronic liver disease. A variety of markers including OV6 have been used to identify human oval cells, but none of these are specific and therefore, are of limited use in defining a stem cell population which may be of clinical use ***(Fabris et al., 2000)***.