INTRODUCTION

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. The long term impact of HCV infection is highly variable, from minimal changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma (HCC). The number of chronically infected persons worldwide is estimated to be about 160 million, and the number of newly infected individuals is increasing ***(European Association for the Study of the Liver, 2014).***

Antigen-specific adaptive T cell immunity plays an important role in determining the outcome of acute HCV infection. The development of early, vigorous, and multi-epitope CD4 and CD8 T cell responses is crucial for HCV clearance. Because adaptive immunity requires weeks and even months to develop, early host control of HCV infection relies on the innate immune response ***(Li and Lemon, 2013).***

Dendritic cells (DCs) are unique antigen presenting cells (APCs) of the immune system that are bridging innate and adaptive immunity. They are responsible for the initiation of immune responses and are involved in regulation of central and peripheral tolerance ***(Volchenkov et al., 2013).*** Two major subsets of DCs are readily purified from human peripheral blood: plasmacytoid **(p)**DCs and conventional or myeloid **(m)**DCs. Each subset represents 0.3–0.5% of the normal human peripheral blood mononuclear cell (PBMC) population ***(Losikoff et al., 2012).***

Plasmacytoid dendritic cells are specialized in rapid and massive secretion of type I interferon (IFN-α/β) following virus infection in response to foreign nucleic acids. Combined with their antigen presentation capacity, this powerful functionality enables pDCs to orchestrate innate and adaptive immune responses ***(Reizis et al., 2011a).*** PDCs produce large amounts of type 1 IFN via Toll-like receptor 7 (TLR7) signaling that is induced by direct cell-to-cell contact with HCV-infected cells ***(Takahashi et al., 2010),*** they also upregulate HLA-DR and the cell surface expression of co-stimulatory molecules (e.g., CD80 and CD86), acquire T cell stimulatory activity, and induce Th1 cell polarization and the production of IFN-γ ***(Losikoff et al., 2012).***

Myeloid dendritic cells, on the other hand, recognize viral ligands (e.g., HCV core and NS3) via TLR-2, exhibit elevated HLA-DR levels, specialize in IL-12 production, polarize $CD4^+$ T cells toward Th1 and demonstrate potent T cell reactivity ***(Losikoff et al., 2012).***

DCs also play a crucial role in the activation of natural killer (NK) cells representing important effectors in the innate immune defense against viruses. There is a bidirectional crosstalk between human DCs and NK cells where DCs enhance CD69 expression, proliferation, (IFN-γ) and cytotoxic activity of NK cells. Reciprocally NK cells either enhance the maturation of DCs and markedly augment their capacity to produce proinflammatory cytokines and to stimulate T-cell responses ***(Wehner et al., 2011)***, or it could enhance immature DCs apoptosis, depending on the activation status of both players ***(Rizzello et al., 2011).*** NK cells can also kill immature DCs. Thus, NK cells regulate DC homeostasis ***(Lee et al., 2013).*** The NK cell activating receptor NKp30 appears to play a central role in DC maturation or apoptosis induced by NK cells ***(Walzer et al., 2005).***

Despite extensive investigation, there is no general consensus regarding the effects of HCV on DC function. The numbers comprising both the pDC and mDC subsets circulating in the peripheral blood were found to be reduced substantially in chronic HCV infected patients. Contradictory data indicate that functions of pDCs and mDCs are either intact or impaired and phenotypically immature in patients with chronic hepatitis C**.** Functional impairments include: decreased IFN-α and IL-12 secretion, increased IL-10 production, lowered expression of HLA-

DR and co-stimulatory molecules decreased stimulatory activity and increased ability to prime regulatory T-cells, (T reg) and consequently, decreased anti-viral potency and skewing the immune response toward tolerance ***(Losikoff et al., 2012).*** However, other studies mentioned that pDCs from HCV-infected patients seem to have a ‘‘normal’’ phenotype, judged by the expression of CD40, HLA-DR, CD80, CD83 and CD86 ***(Dolganiuc and Szabo, 2011).***

Persistent levels of IL-10 associated with chronic viral infections were found to play a central role in progressive immune dysfunction in the form of immature DCs exhibiting aberrant resistance to NK cell mediated elimination, whereas mature DCs exhibit increased susceptibility to NKG2D-dependent NK elimination ***(Alter et al., 2010).***

AIM OF THE WORK

This work is an experimental design to study the level of expression of different maturation markers of DCs in chronic hepatitis C infection.

DENDRITIC CELLS

Dendritic cells were first identified in the mouse spleen based on their unique morphology. They are the most potent stimulators of the mixed lymphocyte reaction, and thus became a scope of years of research highlighting the importance of DCs in initiating adaptive immune responses ***(Shraml and Sousa, 2015).***

The name dendritic cell has become correspondent to motile cells with stellate morphology, showing elevated levels of major histocompatibility complex (MHC) class II molecules and CD11c. They can migrate from non-lymphoid to lymphoid organs and have a distinguished capacity to stimulate T lymphocytes ***(Shraml and Sousa, 2015).***

DCs consist of classical DCs (cDCs) and plasmacytoid DCs (pDCs) which have different abilities to respond to external stimuli and stimulate effector lymphocytes ***(Reizis et al., 2011a)*****.** They recognize and present antigens (Ag) to start antigen specific adaptive immune responses, and thus they bridge the innate and adaptive immune systems ***(Beiber and Autenreith, 2014).***

DCs develop from pluripotent stem cells in the bone marrow (BM) that give rise to progenitor cells which follow a variety of differentiation pathways to differentiate into mature cells ***(Beiber and Autenreith, 2014).***

Immature DCs (iDCs) are sentinels of the immune system patrolling the periphery and taking up different kinds of antigens and change into mature DCs that have a distinct ability to stimulate T-cells. Maturation is accompanied by increased expression of surface MHC class II and co-stimulatory molecules. They then migrate in a CCR7-dependent fashion to the draining lymph nodes (LNs) where antigens are processed and loaded as peptides on MHC ***(Beiber and Autenreith, 2014).***

DCs present antigens to $CD8^+$T cells (*cross presentation*) and $CD4^+$T cells (*direct presentation*) leading to peripheral tolerance in the steady state or leading to effector immunity when they prime $CD4^+$T cells to T helper 1 (Th1) cells, Th2, Th17 cells, or T reg ***(Steinman and Banchereau, 2007).***

Ontogeny of DCs

Many dendritic cell subsets exist that are specialized in morphology, function, and location in order to effectively take up, process, and transport antigens to lymph nodes for presentation to T cells ***(Belz and Nutt, 2012)***.

The classification of DCs based on phenotypic and functional properties is difficult because of sharing some of these properties with other cell types which even led to debate over the existence of DCs as a discrete cell lineage (***Shraml and Sousa, 2015)***.

Four main categories of dendritic cells are mainly recognized: (1) conventional dendritic cells, (2) Langerhans cells (LCs), (3) plasmacytoid dendritic cells, and (4) monocyte-derived dendritic cells (moDCs). An alternative subset of dendritic cells, the myeloid DCs (mDCs), is also included. The myeloid DC subset is comprised of both cDCs and moDCs ***(Mbongue et al., 2014).***

DC development begins in the BM from CD34$^+$ Fms-like tyrosine kinase 3$^+$ (FLT3$^+$) common myeloid progenitor (CMP) cells which differentiate into macrophage dendritic cell progenitors (MDP). The MDPs branch to: the monocyte and the common dendritic cell progenitor (CDP). Monocytes are found in the peripheral circulation and during inflammation they differentiate into CD11b$^+$, CD11c$^+$, and MHCII$^+$ moDCs ***(Mbongue et al., 2014).***

The CDPs differentiate in the BM into plasmacytoid DCs and into pre-DCs which exit the bone marrow and migrate through the blood to lymphoid and non-lymphoid organs to give rise to conventional DCs in peripheral tissues ***(Belz and Nutt, 2012).*** The only DCs that do not originate in the BM are the Langerhans cells which are derived from a local Ly6C$^+$ myelomonocytic precursor in the skin that originates from macrophages during early embryonic development ***(Mbongue et al., 2014).***

Producing DCs from human progenitors is dependent on Flt-3 ligand, granulocyte- monocyte colony stimulating factor (GM-CSF) and IL-4 in vitro and Flt-3, macrophage colony-stimulating factor (M-CSF),GM-CSF signaling via signal transducers and activators of transcription 3 and 5 in vivo ***(Collin et al., 2013).***

In humans, DCs lack lineage markers as CD3 (T cell), CD19/20 (B cell) and CD56 (NK cell) and express high levels of MHC class II (HLA-DR) ***(Ziegler-Heitbrock et al., 2010).***

mDCs express myeloid antigens CD11c, CD13, CD33 and CD11b. Both monocytes and mDCs express CD11c, but DCs lack CD14 or CD16 and may be split into CD1c$^+$ and CD141$^+$ fractions. These two fractions share homology with mouse classical DCs expressing either CD11b (CD1c$^+$ DCs) or CD8/CD103 (CD141$^+$ DCs) ***(Collin et al., 2013).***

Dendritic cell subsets:

A- Conventional dendritic cells:

Conventional dendritic cells are highly efficient at antigen processing and presentation. They are categorized as migratory or lymphoid tissue resident DCs ***(Liu and Nussenzweig, 2010).***

Migratory DCs take up and process antigens and migrate to the draining lymph nodes to present sequestered antigens to naïve T cells. This migration process is highly enhanced during inflammation. On the contrary, lymphoid tissue resident dendritic cells are present in the spleen, thymus, and lymph nodes. In contrast to migratory DCs, they do not migrate but develop from DC precursors found in lymphoid tissues ***(Naik et al., 2006).***

According to *Collin et al., 2013,* conventional DCs could be further subdivided into:

1- *CD1c$^+$ myeloid DCs*

2- *CD141^{high} myeloid dendritic cells*

3- *CD14$^+$ DCs*

1- ***<u>CD1c$^+$ myeloid DCs</u>***

CD1c$^+$ mDCs are the major population of human mDCs that were first described in the blood as a fraction of HLA-DR$^+$ lineage$^-$ cells expressing; CD11b, CD11c, CD13, CD33, CD172 (SIRPα) and CD45RO. They comprise about 1% of mononuclear cells, slightly lower than pDCs. CD1c was recognized as a useful marker by the commercial antibody blood dendritic cell antigen-1 (BDCA-1) ***(Collin et al., 2013).***

Dermal CD1c$^+$ DCs were described as the HLA-DR$^+$ ‘indeterminate’ cell by electron microscopy (EM) and later as CD1a$^+$ DCs migrating from in vitro explants. Both dermal CD1c$^+$ DCs and LCs express CD1a but LCs are identified by Langerin, epithelial cell adhesion molecule (EpCAM) and higher CD1a expression ***(Collin et al., 2013)***.

Human tissue CD1c$^+$ DCs are more activated than their blood counterparts regarding expression of CD80, CD83, CD86 and CD40 with the absence of homing receptors; cutaneous leucocyte-associated antigen (CLA) and CD62L but up-regulated CCR7 ***(Bocian et al., 2016).***

CD1c$^+$ DCs are also found as ‘interdigitating cells’ of T-cell areas of the lymph nodes. Skin-draining nodes show variable expression of CD1a and Langerin suggesting that both CD1c$^+$ DCs and LCs contribute to this population ***(Van de Ven et al., 2011).*** Tonsil and spleen also contain CD1c$^+$ DCs ***(Mittag et al., 2011).*** Both of them do not receive afferent lymph, thus it is concluded that some CD1c$^+$ DCs are ‘resident DCs’ originating directly from the blood ***(Jongbloed et al., 2010).***

CD1c$^+$ DCs have a wide range of lectins, TLRs as well as other pattern recognition receptors (PRRs) for antigen uptake, transport and presentation ***(Van der Aar et al., 2007).***

CD1c and CD1a present glycolipid antigens of mycobacteria and other pathogens. Dectin-1 (CLEC7A) and Dectin-2 (CLEC6A) are highly expressed, which play a role in fungal recognition. DEC205 (CD205; CLEC13B) and macrophage mannose receptor (CD206; CLEC13D) are variable ***(Collin et al., 2013).***

CD1c^{+} DCs stimulate naive CD4^{+} T cells but have an inferior ability to cross-present antigens to CD8^{+} T cells in comparison with CD141^{+} DCs ***(Crozat et al., 2010).*** They secrete tumor necrosis factor-α (TNF- α), IL-8 and IL-10 when they are stimulated and produce IL-12 when TLR7/8 are ligated by R848. A small amount of IL-23 can be found after stimulation **.**This implies an important dual role in Th1 and Th17 sensitization and highlights the plasticity of DCs in different contexts ***(Collin et al., 2013).***

2- CD141^{high} myeloid dendritic cells

CD141 (BDCA-3) or thrombomodulin is expressed on approximately 10% of human blood mDCs (0-1% of mononuclear cells) ***(Collin et al., 2013).***

CD141^{+} DCs were found among resident DCs of lymph node, tonsil, spleen and BM ***(Mittag et al., 2011)*** and non-lymphoid tissues, skin, lung and liver ***(Haniffa et al., 2012).***

The small numbers as well as the wide expression of CD141 on other cells have rendered identification of

$CD141^+$ DCs difficult ***(Jongbloed et al., 2010)***. CD141 is found on migratory $CD14^+$ DCs and on $CD1c^+$ DCs and monocytes cultured with vitamin D ***(Chu et al., 2012).*** Flow cytometry is used to differentiate them from the $CD1c^+$ DC where $CD141^+$ DCs express less CD11b and CD11c ***(Haniffa et al., 2012).***

$CD141^+$ can ‘cross-present’ Ags in comparison with other myeloid or classical DCs ***(Joffre et al., 2012).***

DEC205 which is a marker of $CD8^+$ mouse DCs hasn’t been found to be restricted to human $CD141^+$ DCs. XCR1 and CLEC9A were discovered as important markers of cross-presenting DCs in many species ***(Poulin et al., 2012).***

$CD141^+$ mDCs take up necrotic cells via CLEC9A, viral nucleic acids with TLR3 and TLR8 and they cross-present antigens to $CD8^+$ T-cells in vitro. The latter is a function homologous to mouse $CD8^+$/$CD103^+$ DCs. $CD141^+$ DCs secrete TNF-α, CXCL10 and IFN-α and surprisingly little IL-12 p70, opposite to mDCs and $CD1c^+$ DCs ***(Collin et al., 2013).***

3- <u>$CD14^+$ DCs</u>

$CD14^+$ DCs are found in tissues and lymph nodes. They are a third subset of $CD11c^+$ myeloid cells that were first described as ‘interstitial DCs’. They are monocyte-like or macrophage-like and can arise from monocytes ***(Collin et al., 2013).***

B- Langerhans cells:

Langerhans cells’ function is similar to migratory conventional DCs. They are found in the skin and are identified by the expression of the monocyte/macrophage and endothelial cell differentiation antigen Ly6C. They capture, identify, and present antigens to naïve T cells**.** Both cDCs and Langerhans cells maintain immunological homeostasis during the steady state ***(Mbongue et al., 2014).***

Langerhans cells are found in the supra-basal epidermis and stratified squamous epithelia (bronchus, oral and genital mucous membrane). They highly express the C-type lectin Langerin and CD1a. Antigen capture and presentation molecules are found in a specialized endosomal compartment, visible by EM known as the Birbeck granule. Other markers include CD36, ATPase and FcεR1 ***(Collin et al., 2013).***

Langerhans cells can be easily detected in skin-draining lymph nodes, especially in skin inflammation. They occupy the lymph node paracortex as high interdigitating langerin$^+$ CD1a$^+$ cells ***(Geissmann et al., 2002).***

The function of LCs is difficult to pin down. They can mature into potent cross-presenting DCs but also lack critical TLRs and can induce regulatory T cells and IL-22 production by CD1a-restricted antigen to autologous T cells. LCs maintain epidermal tolerance to commensals, while keeping the capacity to respond to selected intracellular pathogens and viruses ***(Collin et al., 2013).***

LCs development depends on transforming growth factor-β (TGF-β), whereas langerin$^+$ DC develop from a BM precursor depending on Flt3. LCs are positioned to provide the first line of defense of the immune system against external pathogens. LCs are the only cells expressing MHC II in the epidermis in the absence of inflammation and they are able to migrate through the dermis to the lymph nodes that drain the skin, that's why LCs have been thought to be critical for immunity after skin infection. Although the biological function of LCs *in vivo* is still a matter of debate, it is now thought that LCs have a major role in maintaining immune tolerance ***(Chopin et al., 2012).***

C- Plasmacytoid Dendritic Cells:

Plasmacytoid dendritic cells comprise about 0.3–0.5% of the human peripheral blood cells. They arise from lymphoid progenitors and are widely distributed in the