INTRODUCTION

Hepatitis C is the disease that has affected around 200 million people globally. Hepatitis C virus (HCV) is a life threatening human pathogen, not only because of its high prevalence, but also because of the potentially serious complications of its persistence ***(Bostan and Mahmood, 2010)***. HCV chronically infects 3% of the global population and represents a major global health problem. Although adaptive immune responses contribute to controlling and clearing acute HCV infection, it is clear that other branches of the immune response are important for viral control ***(Pembroke et al., 2014).***

Natural killer (NK) cells are major effector cells of the innate immunity and are generally thought to play a fundamental role in antiviral response ***(Spaggiari et al., 2008)***. Their function is primarily regulated by a number of activating and inhibitory receptors, some of which recognize MHC class I molecules ***(Morandi et al., 2012)***. They also could be triggered by cytokines, including interleukin (IL)-2, IL-12, IL-15, and IL-18 ***(De Maria et al., 2010)***. Activated NK cells operate through direct cytolysis of target cells and through cytokine secretion ***(Kassim et al., 2009)***. They are able to recognize and lyse target cells without previous sensitization ***(Moretta et al., 2008)***.

During inflammation, NK cells, after extravasation and recruitment into tissues in response to chemokine gradients, can interact with other immune cell types ***(Moretta et al., 2005).*** These interactions may play a critical role not only during early innate immune responses but also in the initiation, amplification, and polarization of adaptive responses; for example, a bidirectional crosstalk occurs between NK and dendritic cells (DCs) ***(Bellora et al., 2010).***

It was found that NK/DC interaction is bi-directional and complex, as it could result not only in NK cell activation but also in DC maturation or apoptosis, depending on the activation status of both players ***(Rizzello et al., 2011).*** 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).***

Viruses have developed mechanisms to escape from the antiviral response of NK cells and establish persistent infection ***(Lodoen and Lanier, 2005).*** HCV is characterized by an extraordinary ability to establish viral persistence, which it achieves by evading intracellular responses of infected hepatocytes and humoral and cellular responses of the adaptive immune system ***(Yoon et al., 2009).***

During chronic viral infections, an aberrant DC susceptibility to NK cell mediated lysis results in an accumulation of poorly immunogenic DCs in lymph nodes, causing progressive immune dysfunction ***(Alter et al., 2010).*** On the other hand, DCs lysis by NK cells could also negatively regulate the duration of virus specific T cell responses in-vivo by limiting exposure of T cells to infected antigen presenting cells (APCs) ***(Andrews et al., 2010).***

Aim of the Work

This work is an experimental design to investigate the interaction between NK cells and DCs in hepatitis C infection and to identify the effect of DCs on activation/ inhibition status of NK cells.

Natural Killer Cells

Introduction:

Natural killer cells are a subset of lymphocytes that belongs to the T-cell lineage. They recognize and kill infected cells, cancer cells and cells lacking self-major histocompatibility complex (MHC) class I molecules, with no previous sensitization ***(Abdel-Hady et al., 2014).***

Natural Killer Cell Subtypes:

Natural killer cells are classified as a member of group 1 innate lymphoid cells (ILC), which secretes interferon gamma (IFN-γ). Human NK cells are classically characterized as $CD56^{+}CD3^{-}$ cells, differentiating them from $CD56^{+}CD3^{+}$ NK-like T (NKT) cells ***(Campbell and Hasegawa, 2013).*** They also express CD16, which is important to exert antibody dependent cell mediated cytotoxicity (ADCC) ***(Abbas et al., 2015).***

Two main subsets of NK cells are present in humans, according to their levels of CD56 expression, including $CD56^{dim}$ and $CD56^{bright}$. $CD56^{dim}$ NK cells (90% of the NK cells in peripheral blood) are fully mature and predominantly mediate cytotoxicity responses. In contrast, $CD56^{bright}$ cells (5-15% of total NK cells) are more immature and are mainly considered as cytokine producers ***(Campbell and Hasegawa, 2013)*** (Fig. 1) ***(Zalli, 2012).***

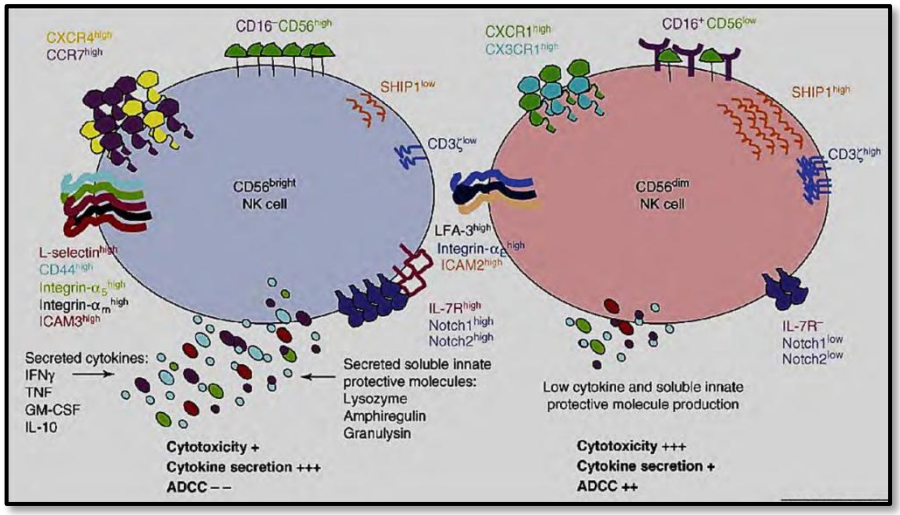

Figure (1): Two main NK cell subsets**,** including their surface phenotype and functional properties ***(Zalli, 2012).***

With the consideration of CD16 human peripheral blood NK cells can be divided into five distinctive subpopulations according to their cell surface density. These are (1) $CD56^{dim}$ $CD16^{bright}$, (2) $CD56^{dim}$ $CD16^{-}$, (3) $CD56^{bright}$ $CD16^{dim}$, (4) $CD56^{bright}$ $CD16^{-}$ and (5) $CD56^{-}$ $CD16^{bright}$. Out of these diverse NK cell subpopulations, the greater functional subsets include $CD56^{dim}$ $CD16^{bright}$ and $CD56^{bright}$ $CD16^{dim/-}$ ***(Poli et al., 2009).***

In the presence of IL-12 or IL-4, human NK cells can differentiate into NK cell subsets secreting distinct cytokine patterns similar to T cell subsets. NK cells grown in IL-12 (NK1) produce IFN-γ, whereas NK cells grown in IL-4 or IL-10 (NK2) produce IL-5 and IL-13 and have regulatory functions ***(Aktas et al., 2008).***

Natural Killer Cell Receptors:

The NK cell function is managed by a harmony between signals that are created from activating and inhibitory receptors. The activating receptors act through protein kinases that phosphorylate downstream signaling substrates, while inhibitory receptors act through phosphatases that balance the kinases ***(Abbas et al., 2015).***

- **Inhibitory receptors:**

They recognize self MHC class 1 molecules on all healthy nucleated cells in the body making NK cells "tolerant to self". When NK cells experience cells with diminished expression of MHC class 1 molecules (i.e. virally-infected or tumor cells), they are no more controlled by inhibitory signals, and NK cell cytotoxicity and cytokine production will be advanced, this phenomenon is known as "missing self-hypothesis" (Fig. 2) ***(Zalli, 2012).***

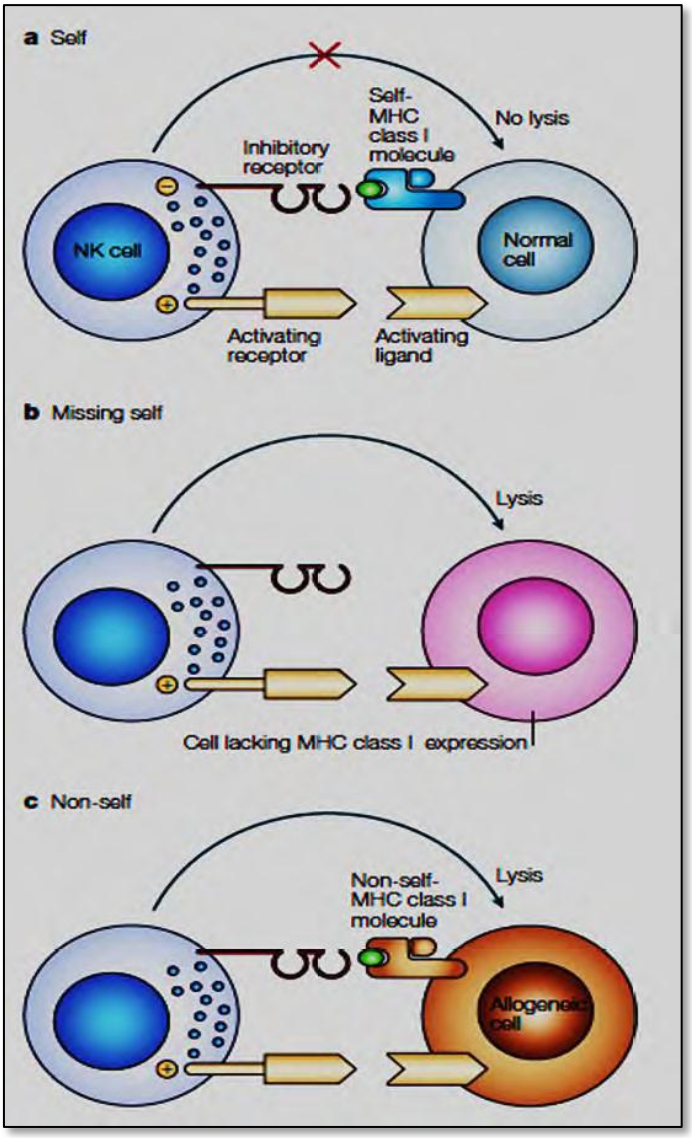

Figure (2): NK cell self-tolerance and the "missing self-hypothesis" ***(Zalli, 2012).***

Natural killer cell inhibitory receptors include 1) the killer cell immunoglobulin (Ig) -like receptors (KIRs), (KIR2DL and KIR3DL) which bind a variety of class I MHC molecules; 2) the C-type lectin-like family of

receptors, such as the CD94/Natural killer group 2A (NKG2A) heterodimer, which recognizes a class I MHC molecule called human leukocyte antigen E (HLA-E) and the killer cell lectin-like receptor G1 (KLRG1) recognizing E-and N- cadherins; and 3) the leukocyte Ig-like receptors (LIRs), that bind class I MHC molecule with low affinity ***(Abbas et al., 2015)*** (Fig. 3) ***(Vivier et al., 2011).***

Despite the structural diversity, all inhibitory NK receptors have long cytoplasmic domains with an immuno-receptor tyrosine-based inhibitory motifs (ITIMs) which induce inhibitory signals after being phosphorylated ***(Zalli, 2012).***

- **Activating receptors:**

They are involved in activation of NK cell effector functions. Several NK cell activating receptors have been identified, including 1) the natural cytotoxicity receptors (NCR); NKp46, NKp30, and NKp44 recognizing viral hemagglutinins and membrane associated heparin sulfate proteoglycans; 2) the C-type lectin-like family of receptors such as CD94/NKG2C, CD94/NKG2E heterodimers recognizing the nonclassical MHC class I molecule HLA-E and NKG2D recognizing the polymorphic MHC class I-related chain (MICA and MICB) molecules; 3) the activatory KIRs; KIR2Ds and KIR3Ds recognizing

classical MHC-I molecules; and 4) the low affinity immunoglobulin gamma fragment crystallizable (Fc) region receptor III-A (FcγRIIIA) (CD16) recognizing the Fc portion of IgG antibodies ***(Gleason et al., 2012)*** (Fig. 3) ***(Vivier et al., 2011).***

All activating receptors lack ITIMs and have short cytoplasmic domains with immuno-receptor tyrosine-based activation motifs (ITAMs) which enable the transmission of the activating signals (with exception of NKG2D which couples to the non-ITAM-containing transmembrane adaptor DAP10) ***(Zalli, 2012).***

Both the C-type lectin receptor CD69 and KLRG1 are highly upregulated on NK cells following activation by a variety of stimuli, including viral infections. CD69 induces NK cell mediated cytolytic activity and is not expressed on resting NK cells. On the other hand, 30-40% of resting NK cells express KLRG1. $KLRG1^{+}$ NK cells are less activated and more prone to apoptosis following viral infection than are $KLRG1^{-}$ NK cells ***(Fogel et al., 2013).***

Other NK cell receptors include: cytokine receptors (e.g. IL-2 and IFN-α receptors), chemotactic receptors (e.g. C-C chemokine receptor (CCR)-2, CCR7 and CCR5) and adhesion receptors (e.g. integrins) (Fig. 3) ***(Vivier et al., 2011).***

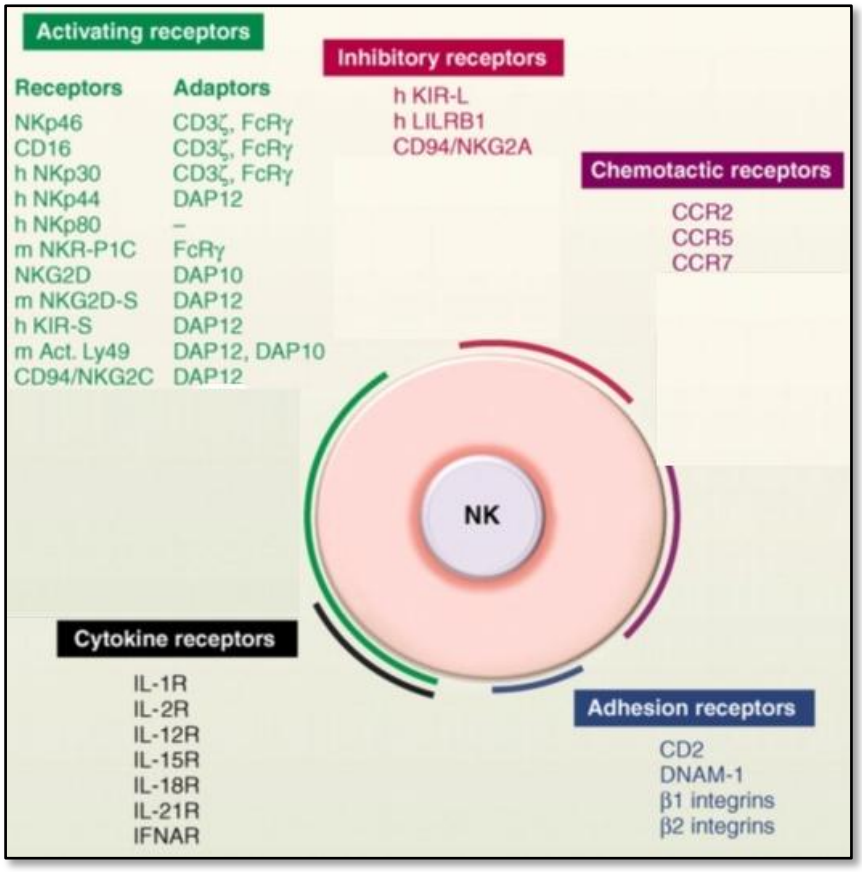

Figure (3): NK cell receptors ***(Vivier et al., 2011).***

Natural Killer Cell Function

1. Natural killer cell cytotoxicity:

Two mechanisms of NK cell cytotoxicity exist. The first includes the introduction of lytic granules into target cells. Perforin facilitates the entry of other granule proteins, called granzymes, that cause death of the target cells by apoptosis. The second mechanism includes the expression of death receptor ligands, for example, tumor necrosis factor (TNF) and Fas ligand, that lead to activation of

caspases 8 and 10 promoting target cell death by activating caspase 3 and the release of cytochrome C ***(Hazeldine and Lord, 2013)*** (Fig. 4) ***(Cheent and Khakoo, 2011).***

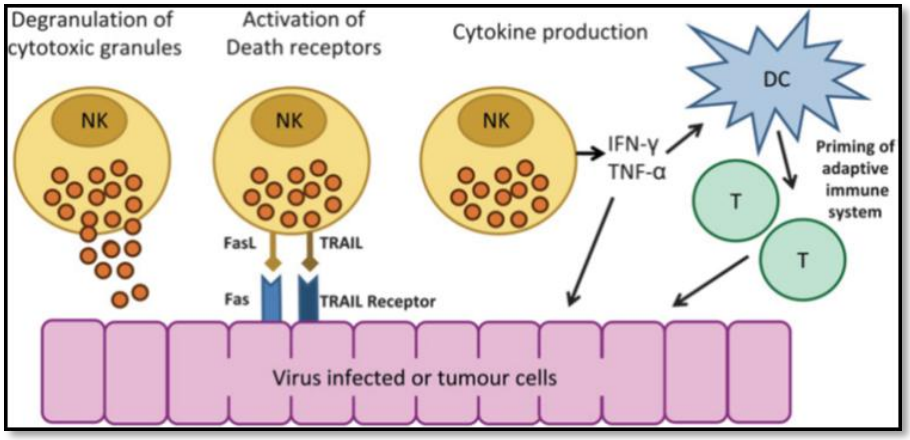

Figure (4): Effector mechanisms of natural killer (NK) cells ***(Cheent and Khakoo, 2011).***

2. Antibody - dependent cell - mediated cytotoxicity (ADCC):

Natural killer cells, the cytotoxic $CD56^{dim}$ $CD16^{+}$ subset mainly, are the main mediators of ADCC as they do not express the inhibitory FcγRIIb. They only express the activating low affinity FcγRIIIa (CD16) that binds IgG1 and IgG3 inducing a potent activating signal which results in both cytotoxicity and a cytokine response ***(Seidel et al., 2013).***

3. Cytokine and chemokine production:

Activated NK cells secrete many immunoregulatory cytokines and chemokines such as TNF-α, IFN-γ, IL-8 and

macrophage inflammatory protein-1-alpha (MIP-1α). Via the production of these cytokines, NK cells can amplify the on-going innate immune responses and impact the early stages of an adaptive immune reaction by stimulating DC maturation and T cell differentiation ***(Hazeldine and Lord, 2013).***

The Bidirectional Crosstalk between Human Natural Killer Cells And Dendritic Cells

Dendritic cells and NK cells control each other's function during early immune responses. NK cells can be activated by the cytokines secreted from DCs and also by direct cell-cell contact. On the other hand, NK cells can cause DC maturation which is important in anti-viral and anti-tumor immunity. They can also kill immature DCs. Thus, NK cells might regulate DC homeostasis ***(Chijioke and Münz, 2013).*** This reciprocal crosstalk between DCs and NK cells can occur in the periphery or in secondary lymphoid tissues ***(Harizi, 2013).***

- **Dendritic cells induce NK cell activation:**

Dendritic cells are the main professional APCs that initiate adaptive immune responses ***(Schmidt et al., 2012).*** They also participate in phagocytosis and the secretion of specific cytokines, including type I IFN, IL-15 and IL-12 ***(Lee et al., 2013a).***

A hallmark of DCs is their heterogeneity. There are different subtypes that differ in their origin, anatomical localization, life span, and function. Four major populations

of DCs have been described, namely plasmcytoid DCs (pDCs), myeloid DCs (mDCs), Langerhans cells (LCs), and the monocyte-derived DCs (MoDCs). During DC maturation, they upregulate specific molecules on their surface like MHC class II, CD80, CD83 and CD86 necessary for antigen presentation and interaction with T cells ***(Ferlazzo and Morandi, 2014).***

Dendritic cells stimulate the release of cytokines by NK cells (mainly TNF and IFN-γ) and enhance NK cell proliferation and cytolytic activity through the release of soluble factors and cell-to-cell contact ***(Ferlazzo and Morandi, 2014).*** Interleukin-12 which is secreted by mDCs efficiently stimulates IFN-γ secretion by NK cells. Whereas, pDCs release type I IFN which activates NK cell cytotoxicity ***(Zhang et al., 2013).*** Interleukin-15, which is produced also by DCs can stimulate NK cell proliferation and survival. Moreover, the interaction of CXC3 chemokine ligand 1 (CXC3CL1) on DCs with CX3C chemokine receptor 1(CX3CR1) on NK cells results in IFN-γ release by NK cells ***(Anguille et al., 2015)*** (Fig. 5a) ***(Chijioke and Münz, 2013).***

- **Natural killer cells induce activation and editing of DCs:**

Following activation, NK cells produce large amounts of TNF and IFN-γ, which are involved in DC maturation. TNF improves the expression of costimulatory molecules on DCs and, synergizing with IFN-γ, contributes to the DC production of IL-12 ***(Chijioke and Münz, 2013).***

On the other hand, the exposure of NK cells to innate cytokines released by mDCs such as IL-12 and IL-18 can promote T helper-1 (Th1) polarization ***(Saïdi et al., 2016).*** INF-γ can induce the expression of a membrane-bound form of IL-15 on DCs. This maintains both T and NK cells survival and activation ***(Jayaraman et al., 2014)*** (Fig.5b) ***(Chijioke and Münz, 2013).***

Interleukin-18 activated NK cells stimulate DCs to release IL-12, up-regulate CCR7 expression and migrate in response to its ligand, chemokine (C-C motif) ligand (CCL)-21. Furthermore, NK cells also stimulate DCs to produce chemokines, mainly chemokine (C-X-C motif) ligand (CXCL)-9, CXCL-10, and CCL-5, which influence the attraction of effector T cells. Therefore, NK cells can stimulate DCs to home to secondary lymphoid tissues and prime Th1 responses ***(Chijioke and Münz, 2013).***

During initiation of an anti-viral or anti-tumor immune response, interactions happening between DCs and NK cells can bypass $CD4^+$ T cell helper signals by the production of IFN-γ, which in turn, can stimulate IL-12 production by DCs leading to a protective cytotoxic T lymphocyte (CTL) response. In a human in-vitro system, it has been displayed that cross-presentation of antigens to $CD8^+$ T cells by DCs needs NK cells. Catch of tumor cells and development status of DCs are not sufficient to induce cross-priming of T cells without further NK-mediated activation and IL-18 release ***(Ferlazzo and Morandi, 2014).***