Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide ***(Moradpour et al., 2007).*** It has been estimated that 180 million people are infected globally ***(Dreux et al., 2009).***

Apparently, the immune response against HCV is often not sufficient to lead to the elimination of the virus. A number of causes for this impairment have been proposed, such as hypervariability of the virus, inhibition of Natural Killer (NK) cell function, inefficient induction of T cell responses or dysfunction of Dendritic cells (DCs) ***(Piccioli et al., 2005).***

Clearance of acute HCV infection is associated with a strong HCV-specific CD4+ and CD8+ T-cell response directed against epitopes of the structural and nonstructural proteins. DCs play a key role in the induction and maintenance of antiviral T-cell immune responses. As antigen-presenting cells, DCs capture antigens, process them into peptides, and present them on molecules of the major histocompatibility complex (MHC) to T cells ***(Barth et al., 2005).***

During the innate response to many inflammatory and infectious stimuli, DCs undergo a differentiation process

termed maturation. Mature DCs activate antigen-specific naive T cells; however both immature and mature DCs activate resting NK cells. Within one week, the NK cells increase two to fourfold in numbers, start secreting interferon (IFN), and acquire cytolytic activity against the classical NK target, the DC-activated NK cells then kill immature DCs efficiently. The NK activating signal mainly involves the NKp30 natural cytotoxicity receptor, and not the NKp46 or NKp44 receptor. However, both immature and mature DCs seem to use a NKp30 independent mechanism to act as potent stimulators for resting NK cells ***(Ferlazzo et al., 2002).***

A subset of NK cells expresses the inhibitory killer cell lectin-like receptor G1 (KLRG1). KLRG1 expression is acquired during periods of NK cell division such as development and homeostatic proliferation. KLRG1+ NK cells are mature in phenotype, but show slower in vivo turnover rate, reduced proliferative response to interleukin (IL) -15, and poorer homeostatic expansion potential compared with mature NK cells lacking KLRG1 ***(Huntington et al., 2007a).***

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Aim of the Work

The aim of this work is to investigate the natural killer activation status in HCV infection and the role of this activation on the outcome of HCV infection whether clearance of the virus or inversely its persistence and chronicity of infection.

Natural Killer Cells

Natural killer cells (NK cells) are a type of cytotoxic lymphocyte critical to the innate immune system. NK cells are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor generating B and T lymphocytes, NK cells are known to differentiate and mature in the bone marrow, lymph node, spleen, tonsils and thymus where they then enter into the circulation ***(Lannello and Raulet, 2014).***

NK cells are an important component of the innate immune response against many viruses because of their ability to lyse virus-infected cells and to secrete cytokines that inhibit viral replication and activate and recruit cells of the adaptive immune response. The role of NK cells during the early antiviral immune response has been well described in mice, Murine cytomegalovirus activates myeloid dendritic cells through the engagement of toll-like receptor (TLR) 9, which results in interferon alpha and interleukin (IL)-12 production and activation of NK cells ***(Krug et al., 2004).***

- **NK cell; History:**

NK cells are LGL that constitute approximately 10% (5-15%) of the peripheral blood lymphocytes in humans. Most NK cells are found in the blood, liver and spleen, but they are also present in lymph nodes and have the capacity to migrate into

specific tissue sites upon infection, inflammation or tumor development ***(Gregoire et al., 2007).***

NK cells are distinct from B cells and T cells since they develop to mature effector cells without rearranging its cell surface receptors and without the requirement of clonal expansion, which give them the capacity to directly lyse targets without prior sensitization ***(Makrigiannis and Parham, 2008).***

NK cells are involved in the rejection of virally transformed and tumor transformed cells and play an important role as regulators of immune responses by linking and modifying innate and adaptive immunity ***(Lanier, 2008a).***

As an example of the later, NK cells have been reported to promote tolerance to graft transplants such as pancreatic islet as well as hematopoietic stem cells during transplantation ***(Beilke et al., 2005).***

In addition, data also indicate that NK cells are involved in autoimmunity as has been shown through the regulatory role of non-cytotoxic NK cells in the uterus during pregnancy ***(Croy et al., 2006).***

NK cells were first described by two independent groups ***(Hebermann et al., 1975 and Kiessling et al., 1975)*** as immune cells that were able to lyse target cells without prior sensitization of the host.

At that time, many groups had observed an unexplainable "background" killing of tumor cells in vitro by peripheral blood lymphocytes. The identification of the responsible lymphocyte subset was a result of thorough and systematic investigations of tumor cell killing in vitro by mouse and human lymphocytes that had not experienced tumor antigens prior to the assay ***(Karre, 2008).***

The "missing-self hypothesis", describing how NK cell activity is regulated, was first postulated in the thesis of Klas Kärre in 1981 and was later published in 1985 ***(Karre, 1985).***

Further studies in murine models revealed the major role for MHC class I in the protection of target cells from NK cell-mediated killing. Some years after the "missing-self hypothesis" was postulated, ***Chambers et al*** conducted experiments masking cell surface structures on rat NK cells by monoclonal antibodies (mAbs), which resulted in the identification of the first structure on the NK cell surface that negatively regulated NK cell activity ***(Chambers et al., 1989).***

However, ***Karlhofer et al.*** were the first to identify the inhibitory receptor Ly49 (expressed by murine NK cells) that specifically recognized MHC class I antigens and thereby inhibited NK cell activity ***(Karlhofer et al., 1992).*** The Ly49 receptor family was later localized to chromosome 6 in a region that was identified as the Natural killer genes complex (NKC) ***(Yokoyama and Seaman. 1993).***

The human equivalent to the murine MHC class I binding receptors are the killer cell Ig-like receptors (KIRs) that were first described in the beginning of the 1990ies by ***Moretta*** and colleagues ***(Moretta et al., 1993).***

In humans, the KIR locus constitutes a family of polymorphic genes that map to a region on chromosome 19q13.4 called the leukocyte receptor complex (LRC). The discovery of inhibitory natural killer receptors (NKRs) such as the KIRs has together with the subsequent identification of activating NKRs verified the role for both activation and inhibitory signals in the regulation of NK cell activity as was originally predicted in the "missing-self hypothesis" ***(Ljunggren and Karre, 1990).***

- **NK Cell Biology**:

I- NK Cell Receptors

The earliest insights into the molecular specificity of NK cells have later been complemented with additional studies that verified the need for positive stimulation to induce target killing ***(Bryceson et al., 2006a).***

NK cell activity is regulated by the integration of inhibitory and activating signals from MHC class I-restricted inhibitory receptors and a wide array of activating NKRs ***(Bryceson and Long, 2008).***

Specific combinations of NKRs expressed on a given NK cell lead to distinct NK cell subsets with a certain degree of target selectivity. The recent advances in the understanding of intracellular signaling have also given us deeper insights into receptor synergies that are involved in the control of NK cell activity. This section aims to introduce the NKRs, their specificity and their intracellular signaling pathways that regulate the NK cell activity ***(Carlsten et al., 2009).***

A- Types:

i. Inhibitory NK Cell receptors and their ligands:

The NK cell activity is under strict control of signals from inhibitory receptors that most often bind classical and/or non-classical MHC class I molecules. These molecules are normally expressed on most healthy cells in the body, but may be lost upon viral or malignant transformation and during tumor evolution. In humans, KIR and CD94/NKG2A play major roles as human leukocyte antigen (HLA) class I-specific inhibitory NKRs recognizing groups of HLA-A, -B, and –C alleles and HLA-E molecules, respectively ***(Moretta and Moretta, 2004).***

In contrast to most of the activating NKRs and the inhibitory CD94/NKG2A/B receptors, individuals differ in the number and type of KIRs expressed. This is partly explained by the identification of two major and divergent KIR haplotypes among the human population, which are composed of combinations of both activating and inhibitory KIRs. The

inhibitory and activating KIRs share the same structural features of their extracellular domain (2D or 3D reflecting the number of Ig-like domains), but have different cytoplasmic tails with either a long (L) or a short (S) tail mediating inhibition and activation, respectively ***(Bashirova et al., 2006).***

Non-functional KIR pseudogenes (P) have also been identified. The A haplotype harbors at least eight KIRs of which six are inhibitory (3DL3, 2DL4, 3DL2, 3DL1, 2DL1, 2DL2/3), one is activating (2DS4) and one is a KIR pseudogene (3DP1) ***(Martin et al., 2000).***

In contrast, the B haplotypes constitute up to fourteen KIRs, of which many are activating, with at least one additional gene not represented in the A haplotype ***(Moretta and Moretta, 2004).***

The set of KIR genes that represent the B haplotype most often include KIR3DL3, 2DL2, 3DP1, 2DL4, 3DS1, 2DL5, 2DS5, 2DS1, 2DS2, and 3DL2 ***(Wilson et al., 2000).***

The variegated expression pattern of KIR on NK cells may also be explained by the fact that specific KIR gene products are expressed randomly in distinct subsets of NK cells ***(Andersson et al., 2009).***

Despite a seemingly random expression pattern, most functionally mature NK cells express at least one inhibitory receptor (i.e., KIR and/or CD94/NKG2A) that is specific for a

self-MHC class I ligand. The clonal distribution of KIRs results in a system allowing NK cells to detect cells lacking expression of single MHC class I alleles ***(Parham, 2005).***

In addition to KIRs and CD94/NKG2A, the Leukocyte Immunoglobulin-like Receptor, subfamily B member 1 (LILR-B1) receptor, binding to a variety of HLA-class I molecules, including HLA-G, and virally-derived UL18 molecules, and also the KLRG1 receptor which binds to cadherins on epithelial and neural cells. These receptors may also contribute to inhibition of NK cell activity ***(Ito et al., 2006).***

In contrast to the KIRs that recognize polymorphic epitopes within the α1 and α2 domains of the HLA-class I heavy chain, the binding site for LILR-B1 has been mapped to the α3 domain and β2m, which is consistent with the broad-binding specificity of LILR-B1 since α3 domain is relatively conserved among HLA-class I molecules ***(Willcox et al., 2003).***

Importantly, under normal conditions, inhibition signals dominate over activation signals in NK cells. However in some situations, the activation signals may override the inhibitory signals as demonstrated for NKG2D-mediated killing of some MHC class I expressing tumor cell lines in mice ***(Long, 2008).***

ii- Activating NK Cell Receptors and their Ligands:

NK cells express the FcγRIII (CD16) that induce antibody-dependent cellular cytotoxicity (ADCC) upon binding to the

constant region (Fc) of IgG. CD16 occurs in 2 isoforms: CD16A, an integral membrane protein found on NK cells, and CD16B, a glycosyl phosphatidylinositol–linked protein expressed by granulocytes ***(Bryceson et al., 2006b).***

NK cells also express several other activation receptors which contribute to "natural cytotoxicity receptors" (NCRs), NKp30, NKp46 and NKp44 represent an important group of activating human NK cell receptors. Two of these, NKp30 and NKp46, are constitutively expressed on all peripheral blood NK cells, whereas NKp44 is induced on IL-2- activated NK cells ***(Moretta et al., 2001).***

The role of these receptors in NK cell-mediated target killing has been demonstrated by blockade of the receptor with anti-NCR mAbs. Indirect evidence for NCR ligand expression on several tumor types is provided by the use of soluble NCR fusion proteins ***(Byrd et al., 2007).***

However, despite considerable efforts to identify cellular ligands for the NCRs, only two candidate ligands binding to NKp30 the human leukocyte antigen-B associated transcript 3 (BAT3) and the B7-H6 ***(Brandt et al., 2009).***

In addition, data also suggest that hemagglutinin (HA) is a viral ligand for the NKp44 and NKp46 receptors ***(Arnon et al., 2001).***

The activating NK cell receptor NKG2D is particularly well characterized. It is constitutively expressed on all NK cells and recognizes the stress-inducible molecules major histocompatibility complex class I-related chain (MIC)A and MICB as well as the UL16-binding proteins (ULBPs) expressed by human cells ***(Eagle and Trowsdale, 2007).***

The NKG2D receptor has been shown to be involved in the rejection of both virally infected and tumor cells ***(Guerra et al., 2008).***

The DNAX adaptor molecule 1 (DNAM-1) receptor was first described on T cells. However, DNAM-1 is also constitutively expressed on all NK cells as well as on a subset of B cells and monocytes. The function of DNAM-1 is dependent on the physical association with lymphocyte-function associated antigen-1 (LFA-1; CD18/CD11a). Patients with leukocyte adhesion deficiency syndrome (LAD), lacking LFA-1, have defective DNAM-1 despite intact expression levels. However data indicate that cross-linking DNAM-1 with agonistic mAb can enhance the function of LAD-derived NK cells ***(Castriconi et al., 2007).***

Two ligands, CD155 (PVR) and CD112 (Nectin-2), have been identified for DNAM-1. CD155 appears to have a predominant role in inducing DNAM-1- dependent activation. The DNAM-1 receptor may also cooperate synergistically with NCR and NKG2D to trigger NK cell mediated cytotoxicity

(Bryceson et al., 2006b) and has been reported to be important in the protection from tumor cell development ***(Iguchi-Manaka et al., 2008).***

The 2B4 (CD244) receptor is expressed on the majority of human NK cells. It binds to CD48, which is commonly expressed by most hematopoietic cells. Interactions between 2B4 and its ligand results in induction of proximal activating signals but the magnitude of the signal is not sufficient to induce effective NK cell activation alone. In addition to these receptors, many other receptors, including CD2 (LFA-2), NKp80 and CD59, have been shown to be involved in activation. Several of these may have important co-activating or co-stimulatory functions in NK cell activation **Table (1)** ***(Bryceson et al., 2006a).***

Table (1): Specificity and signaling of human NK cell receptors ***(Bryceson et al., 2006a)***

Receptor	Signaling	Cellular ligand	Function
FcγRIIIa (CD16)	Activation	IgG	Elimination of antibody coated cells (ADCC)
NKp30 (CD337)	Co-activation	B7-H6	NK cell – myeloid cell cross-talk
NKp44 (CD336)	Activation	?	?
NKp46 (CD335)	Co-activation	?	Surveillance of mitotic cells
KIR (CD158a, b, etc.)	Activation	HLA class I	?
CD94/NKG2C (CD159c)	Activation	HLA-E	?
NKG2D (CD314)	Co-activation	ULBP, MICA, MICB	Surveillance of tumor cells and genotoxic stress
NKp80	?	AICL	NK cell – myeloid cell cross-talk
DNAM-1 (CD226)	Co-activation	CD112, CD155	Surveillance of tissue integrity
2B4 (CD244)	Co-activation	CD48	Interaction with hematopoetic cells
CRACC (CD319)	?	CRACC (CD319)	Interaction with hematopoetic cells
CD2	Co-activation	CD58	Interaction with hematopoetic and endothelial cells
KIR2DL4 (CD158d)	?	HLA-G (soluble)	Trophoblast-induced vascular remodelling?
LFA-1 (CD11a/CD18)	Granule polarization	ICAM	Recruitment and activation during inflammation, efficient cytotoxicity
KIR (CD158)	Inhibition	HLA class I alleles	Assess loss of MHC class I alleles
LIR1, LILR1 (CD85j)	Inhibition	HLA class I	Assess loss of MHC class I expression
CD94/NKG2A (CD159a)	Inhibition	HLA-E	Gauge MHC class I expression
KLRG1	Inhibition	E-cadherin	Assess loss of tissue integrity
NKR-P1 (CD161)	Inhibition	LLT1	?
LAIR-1 (CD305)	Inhibition	Collagen	Control activation in extracellular matrix
Siglec-7 (CD328)	Inhibition	Sialic acid	?
Siglec-9 (CD329)	Inhibition	Sialic acid	?
IRp60 (CD300a)	Inhibition	?	?

iii. Adhesion Receptors:

The adhesion receptors belong to different receptor families including the integrin, immunoglobulin, selectin, and cadherin families. The far most studied adhesion receptor expressed by NK cells is the integrin LFA-1 that besides adhesion also has many other functions. As an example, LFA-1 is critical for proper killing of NK cell targets by regulating the polarization of the cytolytic granules toward the target cell upon interaction with (Inter-cellular adhesion molecule 1) ICAM-1 ***(Bryceson et al., 2005).***

LFA-1 has also the capacity to induce NK cell activation when interacting with target cells expressing ICAM-1. Blockade of the LFA-1 receptor results in impaired NK cell cytotoxicity mediated by ADCC. Patients lacking the LFA-1 receptor due to mutations of CD18 (LAD syndrome type 1) experience severe infections and display impaired NK cell function ***(Etzioni, 2007).***

The expression and affinity of LFA-1 can be increased by cytokine stimulation (IL-2 and IL-15) and by local chemokine stimulation (CX3CL1) in the immunological synapse ***(Pallandre et al., 2008).***

In addition, co-receptors such as 2B4, CD2, CD44, and CD16 can also increase the adhesive properties of LFA-1 ***(Bryceson et al., 2005).***