Natural Killer Cells in HCV Infection

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Introduction

Hepatitis virus C (HCV) infection is a main cause of viral hepatitis, liver cirrhosis and hepatocellular carcinoma [1]. Nearly 3% of world population are infected with HCV [1], and about half million people die from cirrhosis, hepatocellular carcinoma or other diseases caused by HCV infection each year [2]. Therapies for HCV infection include interferon (IFN) combined with ribavirin (RBV) [3], direct-acting antivirals (DAAs) [4] and host-targeting agents (HTA) [5]. Especially, DAAs therapy, a combination of Sofosbuvir and Ledipasvir with or without ribavirin, improves the sustained virological response (SVR) of several antiviral cytokines and chemokines, which eventually results in DNA fragmentation and apoptosis of the target cells [22-24].

NK cells are major effector cells of innate immunity, directly killing pathogen-infected cells and tumor cells [7]. According to CD56 and CD16 expression, NK cells can be divided into three subsets: CD56negCD16neg (~10%), CD56dimCD16~ (~90%) and CD56brightCD16+ (~10%) [8, 9]. CD56brightCD16+ subset produces cytokines such as IFNγ [10], CD56dimCD16+ subset presents high cytotoxicity but little cytokine secretion [11], and CD56negCD16+ subset has been shown functionally deficient and is increased in chronic HIV and HCV infection [12]. Activation of NK cells is determined by the balance between signals mediated by activating receptor and inhibitory receptor, while prior sensitization is not necessary. Inhibitory receptors expressed on NK cells can trigger inhibitory signal transduction by recognizing major histocompatibility complex (MHC) class I molecules, such as killer cell immunoglobulin-like receptors (KIRs) and CD94/NKG2A heterodimers [13-15]. Activating receptors consist of lectin-like receptors NKG2D, NKG2C [16], and natural cytotoxicity receptors (NCRs) [17], including Nkp30, Nkp46, and Nkp44. NKG2D recognizes MHC class I chain-related protein (MIC) A, B and UL16-binding proteins (ULBPs) [18,19]. NK cells also express FcγR III (CD16), which can bind to Fc portion of immunoglobulins to trigger antibody-dependent cell-mediated cytotoxicity (ADCC) [20,21]. Following cross-linking between CD16 and Fc portion, the C-terminal ITAM will be phosphorylated to activate Ca2+-dependent signaling pathway, leading to release of granzyme B and perforin and secretion of several antiviral cytokines and chemokines, which eventually results in DNA fragmentation and apoptosis of the target cells [22-24].

NK Cells and HCV Infection

NK cells are accounted for 5%–20% of peripheral blood mononuclear cells and comprise 30%–50% of liver-resident lymphocytes [25]. NK cells play an important role during HCV infection. In acute hepatitis C, a reduced frequency of CD56neg subset and an increased proportion of CD56bright subset are reported [26-28]. Receptors expressed on NK cell surface are also altered, with lower frequencies of Nkp44, Nkp46, CD161 and NKG2D on NK cells after acute infection [27]. Amadei et al. suggest an increase of NKG2DNK cells irrespective of outcome, and cytotoxicity of NK cells is increased only in individuals with KIRs combined with HLA-C1, especially in self-limited individuals [26]. Pavloset al. demonstrated that elevated frequencies of NKG2D and Nkp46 on NK cells may be related to self-limited HCV infection [29]. Werner et al. describe a strong NK cell response in healthcare workers with low-level HCV exposure but do not develop into acute HCV infection [30]. Notably, Pelletier et al. find an increased NK cell degranulation in early phase of HCV infection in a cohort of intravenous (IV) drug users who cleared HCV spontaneously [31]. Peter et al. also suggest that sustained NK cell activation protected highly-exposed uninfected injecting drug users from HCV infection. In chronic HCV infection, the distribution and phenotype of NK cell subsets are also changed [33,34]. The frequency of CD56dimCD16+ subset with defective function is elevated in chronic HCV infection [12,35,36]. Some controversies are existed in the expression of NCRs. One study shows that the levels of Nkp46 and Nkp30 expressed on NK cell surface are significantly decreased in chronic HCV infected subjects [37], while another study displays an increased proportion of Nkp44, Nkp46 and Nkp30 [38]. Furthermore, HCV non-structural proteins 5A(NS5A) can induce secretion of IL-10 by monocytes to promote production of transforming growth factor (TGF)β, resulting to lower surface expression of NKG2D which plays an important role in the control of HCV infection [39]. Several researchers report that the function of NK cells appears to be polarized during development of chronic HCV infection. Cytotoxicity of NK cell is up-regulated while IFN-γ production by NK cells is down-regulated [33,36,38]. This functional polarization of NK cells may contribute to persistence of HCV virus and increased liver damages [38]. Nkp46highNK cells are enriched in liver and exert stronger cytotoxicity...
against hepatic stellate cells (HSCs) [38,40]. Glässneret et al. demonstrate that NK cells from HCV-infected patients can induce apoptosis of activated HSCs, and perform an anti-fibrotic role in chronic hepatitis C [41]. Taken together, NK cells play a pivotal role in the control of HCV infection despite of some discrepancies existing in some studies.

Fewer studies have been done on the ADCC activity mediated by NK cell in HCV infection. It is demonstrated that spontaneous clearance of HCV occurs most often after the induction of an anti-HCV humoral immune response [42,43], indicating that antibody-mediated immune responses, such as NK-ADCC, may contribute to the spontaneous clearance of HCV. Jacob et al. demonstrate that specific antibodies to HCV E2 in serum can mediate ADCC activity, while the pathological mechanisms have not yet been studied in chronic HCV infection [44]. Alter et al. stimulate NK cells with the P815-Ab antigen-antibody complex, and find that the proportion of IFN-γ-producing NK cells and CD107a-positive NK cells is lower in chronic HCV infected individuals than in subjects with spontaneous recovery from HCV infection [27]. Barbara et al. report an impaired antibody-dependent cytotoxicity induced by metzincin-mediated CD16 cleavage due to hepatitis C virus-induced NK cell activation [45]. Our research also reveals a dysfunctional characteristic of antibody-dependent NK cell responses in chronic HCV carriers [28]. Our study reveals that non-neutralizing epitopes can induce robust hepatitis C virus (HCV)-specific antibody-dependent CD56+ NK cell responses in chronic HCV-infected patients [28]. Of note, ADCC activity in cancer immunotherapies and protective immune responses in several virus infections is becoming more and more important. ADCC is utilized in the treatment of various cancers that involve tumor-antigens targeting by monoclonal antibodies (mAbs), such as Rituximab for non-Hodgkin's lymphoma [46] and Obinutuzumab for chronic lymphocytic leukemia [47].

**Conclusion**

Nowadays, more and more studies turn to focus on induction of ADCC-mediating Abs during vaccine research and development [48-50]. ADCC displays a remarkable role in protecting against HIV infection in the RV144 Thai trial for HIV vaccine, resulting to an approximately 31% protection [48]. ADCC response is also used in universal influenza vaccines, since ADCC-mediating Abs can target more conserved regions of influenza virus and recognize a broader array of influenza strains [51]. It is anticipated that the strategies of inducing ADCC-mediating Abs may be used broadly in vaccine development and immunotherapies.

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