The Egyptian Journal of Hospital Medicine (October 2016) Vol. 65, Page 592- 597

Natural Killer Cells And Their Interaction With Dendritic Cells in Hepatitis C Infection

Aisha Yassin Abdel Ghaffar, Manal Zaghloul Mahran, Hossam Mostafa Fahmy, Dina Elsayed Elshennawy, Dalia Youssef Samaha, Sara Ibrahim abdel Fattah Taha

Clinical pathology department-Faculty of medicine - Ain shams University

ABSTRACT:

Background: Hepatitis C is a viral infection of the liver that has affected around 200 million people globally. The immune response against HCV infection includes both the innate and adaptive arms of immunity, with crosstalk between liver inhabitant and infiltrating cells. In the current study, we aimed to investigate the natural killer cells activation and inhibition status, and their role in interaction with DCs utilizing different combinations between NK cells and DCs in the presence of HCV peptides in a ratio of 5 NK: 1DC.**Results**: HCV NK cells upregulated both activation and inhibition markers. This could be attributed to HCV infection and their interaction with DCs especially healthy DCs. Moreover, apoptosis of DCs and NK cells occurred more in HCV NK cultures due to their higher frequency of NKp30 and KLRG1. The death of NK cells was more than DCs despite DCs maturation defect due to HCV infection, suggesting that the inhibitory marker KLRG1 took the upper hand over the upregulated activation markers leading to impaired cytotoxic activity and apoptosis of NK cells.

Conclusion: The bidirectional crosstalk between NK cells and DCs is important in both potentiating mechanisms of the innate immune responses and the subsequent adaptive immune responses in the immune surveillance of cancer and infections. HCV infection impairs this crosstalk which may be a leading cause in viral persistence and chronicity.

Keywords: natural killer cells and dendritic cells crosstalk, natural killer cells and dendritic cells in HCV, natural killer cells and dendritic cells co-culture

INTRODUCTION:

It was found that NK / DC cell 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 [(1)]. The NK cell activating receptor NKp30 appears to play a central role in DC maturation or apoptosis induced by NK cells [(2)].

Viruses have developed mechanisms to escape from the antiviral response of NK cells and to establish persistent infection [(3)].During chronic viral infections, an aberrant DC susceptibility to NK cell-mediated lysis resulted in an accumulation of poorly immunogenic DCs in lymph nodes, causing progressive immune dysfunction [(4)].On the other hand, DC 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 [(5)].

SUBJECTS AND METHODS:

Two groups of blood bags were selected from the blood bank according to: history taking, the presence of HCV antibodies by ELISA and HCV RNA by PCR.

1- **Twenty Blood bags from HCV Infected donors** (double positive PCR and ELISA HCV antibody) (Group I).

2- **Twenty Blood Bags from HCV negative healthy donors** (double negative PCR and ELISA HCV antibody) (Group II).

The study groups were subjected to the following:

1) Peripheral blood mononuclear cells (PBMCs) separation and isolation by density gradient centrifugation method.
2) Immature DCs generation and pulsing by core, NS3/NS4 and NS5 HCV peptides.
3) Isolation of NK cells by NK Cell Isolation Kit ***(Miltenyi Biotec, Germany).***
4) Co-culture of the NK cells with DCs pulsed with HCV peptides.
5) Flowcytometric characterization of cellular surface markers (CD16, CD69, NKG2D, NKP46, NKp30 and KLRG1) expression on peripheral blood NK cells ($CD3^{-}CD56^{+}$)

Received: 14/08/2016
Accepted: 25/08/2016
DOI : 10.12816/0033769

using Guava® easyCyte HT ***(Millipore, France).***

6) Determination of NK/ DCs viability after co-culture by Annexin staining.

Statistical Methodology:

The data were coded, entered and analyzed using Statistical Package for Special Sciences **(SPSS)** software computer program version ***"V. 23.0, IBM Corp., USA, 2015)***. Statistical tests used in the study are:

1) Description of **quantitative** non parametric data was carried out by using: median %.
2) The multiple comparison **(Mann Whitney Test)** was also followed to investigate the possible statistical significance between each 2 groups.

Significance level (P) was expressed as follows:

- ***P***> 0.05 is non-significant ***(NS).***
- ***P***< 0.05 is significant ***(S).***
- ***P***<0.01 is highly significant ***(HS).***

RESULTS:

The effect of co-culture of NK (HCV $^{+}$ and healthy) with dendritic cells (HCV^{+} and healthy) were compared to each other to detect the changes in NK characteristics (KLRG1, NKP30, NKG2D, NKP46, CD16+ and CD69+) (Table 1)

a. On comparing the effect of (healthy NK and HCV DCs) co-culture on NK cells characteristics to (HCV NK and HCV DC) co-culture, the following was found: a statistically significant down regulation in the expression of KLRG1 by NS3-4 &NS5 peptide. Also expression of NKP30 was statistically significant down regulated NS5 peptides. On the other hand, there was a statistically significant up-regulation in expression of NKP46 and NKG2D in response to core peptide. Meanwhile, CD69 showed a statistically significant down-regulation in core peptide pool and a significant up-regulation in NS3-NS4 peptide pool. The effect of replacing HCV+ DC with healthy DC was mainly an upregulation in the expression of NKP46 and NKG2D by all three peptides. There was upregulation of KLRG1, NKP30 by core and NS5. There was no significant difference in CD16 and CD69 (core and NS3-4) with an increase in the expression of CD16 only by NS5 peptides.
b. On comparing NK cell response in HCV NK and healthy DCs co-culture to HCV NK and HCV DC co-culture, the following was found: a statistically significant upregulation in the expression of NKP46 and NKG2D by all three peptides and a significant upregulation of KLRG1, NKP30 expression by core and NS5 peptide pools. On the other hand, CD16 and CD69 showed only a statistically significant increase in their expression by NS5 peptide.
c. On comparing the effect of culture of healthy NK and HCV DCs co-culture to HCV NK and healthy DC on NK cells characteristics, the following was found: The presence of healthy DCs resulted in a statistical significant increase in all parameters by all peptides except CD69 in response to core peptides

Apoptosis in Different culture conditions

Immature DCs, but not mature DCs, are killed by NK cells which appear to be dependent on NKp30. Annexin was used to detect the cytolytic function of NK cells in the different culture conditions. This was done by comparing the percentage of apoptotic cells and living cells in cultures with HCV+NK and those with healthy NK. Chronic HCV NK cells showed significantly higher apoptosis of DC by NS3-4 and NS5 with no difference by core. This is due to the higher frequency of KLRG1 and NKP30 in NK cells of NS3-4 and NS5 in chronic HCV cases than those with healthy NK and the lack of significant difference in the expression of NKP30 by core peptides by both HCV+NK and healthy NK. At the same time co-culture of HCV+NK and and healthy NK with HCV+ iM DC resulted in a degree of maturation of DC in response to the three peptides. In healthy NK culture core peptides produced a higher degree of maturation with significantly higher expression of HLA-DR+ and HLA-DR+ CD 86+while NS3-4 only produced increased HLA-DR+CD86+and NS5 peptides produced only increased HLA-DR+. Thus more immature DC together with increased NKP30+ result in higher apoptosis in NS3-4 and NS5 peptide pools.

On the other hand there was an increased percentage of living cells in the healthy NK culture conditions in both core and NS3-4 pools. (Table 2)

DISCUSSION:

In the current study, we aimed to investigate the NK cells activation and inhibition

status, and their role in interaction with DCs, utilizing different combinations between NK cells and DCs in the presence of HCV peptides in a ratio of 5NK:1DCs.

The status of NK cells was assessed through studying the activation markers, CD16, CD69, NKG2D, NKp46, NKp30 and the inhibitory marker KLRG-1.

Our experiment showed that HCV NK cells and HCV DCs (E1) and healthy NK and HCV DCs (E2) co-cultures had varying degrees of upregulation of both NK cell activation and inhibitory markers in the three peptide pools but the number of upregulated NK cell markers was more in HCV NK cells and HCV DCs (E1) co-culture. This could be in part attributable to the effect of HCV on NK cells since in both co-cultures DCs were HCV affected.

Indeed, the mechanisms underlying DCs dysfunction during HCV infection are not fully understood. The core, NS3, NS4, and NS5 proteins can impair DCs function by diminishing the HLA and costimulatory molecule expression, reducing cytokine production, inhibiting TLR signaling, and decreasing allostimulatory activity[6].

Moreover, circulating pDCs in chronic HCVinfected patients exhibit: diminished HLA-DR expression, a markedly reduced capacity to secrete IFN-α and, consequently, decreased anti-viral potency. mDCs in chronic HCV infection secrete significantly lower levels of IL-12 and increased concentrations of IL-10, which tends to skew the immune response toward tolerance and a reduced ability to induce T-cell proliferation and Th1 polarization[7].

On the other hand, in terms of alterations in NK receptor expression during HCV infection, there are few consistent findings. Expression of natural cytotoxicity receptors (NCR; NKp30 and NKp46) or NKG2D, as activating receptors, are reported as either upregulated [8, 9, 10] or downregulated on peripheral blood NK cells depending on the study [11, 12].

Moreover, no change in expression levels of NKG2D, NKp46, or CD16 on NK cells exposed to HCV infected cells was observed and the inhibition of in-vivo NK functions did correspond with reduced surface expression of the natural cytotoxicity receptor NKp30 [13].

The discrepancy between studies could be referred to the heterogeneity of patients studied regarding number and selection methods, viral genotype or duration of the disease.

However, in HCV NK cells and healthy DCs co-culture (E3) a higher frequency of upregulation of all NK cell markers in all peptide pools was observed. This finding not only could be explained by the presence of HCV infection, but also could be the result of the interaction between NK cells and healthy DCs. Healthy DC lead to a significant upregulation of the most two important NK cells markers KLRG1 and NKp30.

NK cell cytotoxicity can be enhanced by DCs dependent mechanisms, including DC-derived soluble factors (IL-12, IL-2, and IL-15) as well as contact-dependent factors like NKG2D, NKp46, and NKp30. The NK cell–activating mechanism used by DCs has been shown to relate to the stimuli used in DC maturation [14].

In our study, HCV NK cells and HCV DCs co-culture (E1) showed significantly higher apoptosis of both cells due to higher frequency of upregulation of NK cell markers in all HCV peptide pools. Apoptosis of DCs may be due to the higher frequency NKp30 in HCV NK cells than those with healthy NK. Thus more immature DCs due to HCV together with increased NKp30 result in higher apoptosis in different HCV peptide pools with culture ratio 5NK:1DCs.

This explanation could be supported by what was previously reported :a low ratio of NK cells to immature DCs (1:5) promotes DCs maturation, whereas a higher ratio of NK cells to immature DCs (5:1) can result in NK cell-mediated killing of DCs in a process referred to as “DC editing”. NK cells can kill immature DCs and NKp30 on NK cells plays an important role in this process. This crosstalk between DCs and NK cells plays an important role in regulating the antiviral immune response [15]. Regarding the importance of NKp30 in DCs NK cells crosstalk, both mature as well as immature DCs express ligands for NKp30 which is involved in DCs maturation or killing and NK cells have the capacity to eliminate autologous and allogeneic immature DCs. NKp30 has been shown to play a major role in immature DCs lysis which become susceptible to NK cell mediated cytotoxicity because of low expression of HLA-E irrespective of expression of other HLA molecules. In contrast, mature DCs

express higher amounts of HLA-E and are, therefore, kept untouched [14]. Thus, it can be hypothesized that by elimination of immature DCs, NK cells ensure the activation of adaptive immune responses by preventing inadequately matured DCs from interacting with T cells.

On the other hand, apoptosis of NK cells that has been found in our study was more in HCV NK and HCV DCs co-culture (E1) than healthy NK cells and HCV DCs co-culture (E2) this could be due to the increased KLRG1 level of expression on HCV NK cells. It was also found that despite of the concomitant increase in NK cells activation markers, KLRG1 increase was with higher frequency.

KLRG1 is one of several inhibitory killer cell lectin-like receptors expressed by NK cells and T lymphocytes, mainly $CD8^+$ effector/memory cells that can secrete cytokines but have poor proliferative capacity [16].

HCV seems to up-regulate KLRG1. Importantly, the expression levels of KLRG1 is inversely associated with the capacity of NK cells to proliferate and to produce IFN-γ but positively associated with apoptosis of NK cells in response to inflammatory cytokine stimulation [17].

REFERENCES:

1-Rizzello V, Bonaccorsi I, Dongarrà ML, Fink LN and Ferlazzo G (2011): Role of Natural Killer and Dendritic Cell Crosstalk in Immunomodulation by Commensal Bacteria Probiotics. J Biomed Biotechnol., 2011(473097):1-10.

2-Walzer T, Dalod M, Robbins SH, Zitvogel L and Vivier E. (2005): Natural-killer cells and dendritic cells: "l'union fait la force Blood, 106(7):2252-8.

3-Lodoen MB and Lanier LL (2005): Viral modulation of NK cell immunity. Nat Rev Microbiol., 3(1):59-69.

4-Alter G, Kavanagh D, Rihn S, Luteijn R, Brooks D, Oldstone M, van Lunzen J and Altfeld M (2010): IL-10 induces aberrant deletion of dendritic cells by natural killer cells in the context of HIV infection. J Clin Invest., 120(6):1905-13.

5-Andrews DM, Estcourt MJ, Andoniou CE, Wikstrom ME, Khong A, Voigt V, Fleming P, Tabarias H, Hill GR, van der Most RG, Scalzo AA, Smyth MJ and Degli-Esposti MA (2010): Innate immunity defines the capacity of antiviral T cells to limit persistent infection. J Exp Med., 207(6):1333-43.

6- Krishnadas DK, Ahn JS, Han J, Kumar R and Agrawal B (2010): Immunomodulation by hepatitis C virus-derived proteins: targeting human dendritic cells by multiple mechanisms. Int. Immunol.,22(6):491-502.

7- Losikoff PT, Self AA and Gregory SH (2012): Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C. Virulence ,3(7):610-20.

8- De Maria A, Fogli M, Mazza S, Basso M, Picciotto A, Costa P, Congia S, Mingari MC and Moretta L (2007): Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients. Eur J Immunol.,37(2):445-55.

9- Oliviero B, Varchetta S, Paudice E, Michelone G, Zaramella M, Mavilio D, De Filippi F, Bruno S and Mondelli MU (2009): Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. Gastroenterology,137(3):1151- 1160.

10- Ahlenstiel G, Titerence RH, Koh C, Edlich B, Feld JJ, Rotman Y, Ghany MG, Hoofnagle JH, Liang TJ, Heller T and Rehermann B (2010): Natural killer cells are polarized toward cytotoxicity in chronic hepatitis C in an interferon-alfa-dependent manner. Gastroenterology,138(1):325-35.

11- Alter G, Jost S, Rihn S, Reyor LL, Nolan BE, Ghebremichael M, Bosch R, Altfeld M and Lauer GM (2011): Reduced frequencies of $NKp30^+NKp46^+$, $CD161^+$, and $NKG2D^+$ NK cells in acute HCV infection may predict viral clearance. J Hepatol.;55(2):278-88.

12- Nattermann J (2011): NK cells in acute hepatitis C. J Hepatol.,55(2):265-7.

13- Holder KA, Stapleton SN, Gallant ME, Russell RS and Grant MD (2013): Hepatitis C virus-infected cells downregulate NKp30 and inhibit ex vivo NK cell functions. J Immunol.,191(6):3308-18.

14- Van Elssen CH, Oth T, Germeraad WT, Bos GM and Vanderlocht J (2014): Natural killer cells: the secret weapon in dendritic cell vaccination strategies. Clin Cancer Res.,20(5):1095-103.

15- Sehgal M, Khan ZK, Talal AH and Jain P (2013): Dendritic Cells in HIV-1 and HCV Infection: Can They Help Win the Battle? Virology (Auckl),4 (1):1-25.

16- Kared H, Martelli S, Ng TP, Pender SL and Larbi A (2016): CD57 in human natural killer cells and T-lymphocytes. Cancer Immunol Immunother.,65(4):441-52.

17- Wang JM, Cheng YQ, Shi L, Ying RS, Wu XY, Li GY, Moorman JP and Yao ZQ (2013): KLRG1 negatively regulates natural killer cell functions through the Akt pathway in individuals with chronic hepatitis C virus infection. J Virol.,87(21):11626-36.

Table 1: Comparison of Different Culture Conditions on NK Characteristics (Mann Whitney Test)

Parameter	HCV NK+HCV DC group 1	HCV DC + HEALTHY NK (group2)			HCV NK + HEALTHY DC (group3)			Group 2 & 3	
Core peptide	Median%	Median %	Z	P1	Median %	z	P2	z	P3
NK KLRG1 +	20.0	18.0	.977	.329	**33.5**	3.414	**.001**	2.160	**.031**
NK NKP3 +	7.5	15.0	1.303	.193	**28.5**	3.416	**.001**	3.240	**.001**
NK NKG2D +	5.0	**8.0**	2.010	**.044**	**49.0**	3.414	**.001**	3.325	**.001**
NK NKP46 +	0.5	**4.0**	2.958	**.003**	**18.0**	3.464	**.001**	3.240	**.001**
NK CD16+	13.0	9.0	1.643	.100	19.0	1.758	.079	3.320	**.001**
NK CD69+	8.5	**7.0**	2.010	**.044**	7.5	.854	.393	.569	.569
NS3 –NS4 peptide									
NK KLRG1 +	11.0	**5.0**	2.301	**.021**	32.0	1.707	.088	3.325	**.001**
NK NKP3 +	9.5	4.0	.670	.503	27.5	1.707	.088	3.325	**.001**
NK NKG2D +	7.5	8.0	.645	.519	**36.0**	3.464	**.001**	3.325	**.001**
NK NKP46 +	1.5	0	1.691	.091	**14.5**	3.414	**.001**	3.325	**.001**
NK CD16+	11.5	12.0	.986	.324	16.5	1.319	.187	2.770	**.006**
NK CD69+	4.5	**7.0**	1.972	**.049**	11.5	1.732	.083	3.325	**.001**
NS5 peptide									
NK KLRG1 +	17.5	**8.0**	3.612.	**.000**	**43.5**	3.031	**.002**	3.325	**.001**
NK NKP3 +	11.0	**4.0**	2.805	**.023**	**29.0**	2.165	**.030**	3.240	**.001**
NK NKG2D +	5.0	6.0	.977	.329	**31.5**	3.414	**.001**	3.240	**.001**
NK NKP46 +	2.5	1.0	1.327	.184	**16.0**	3.464	**.001**	3.240	**.001**
NK CD16+	12.5	10.0	.000	1.00	**19.0**	2.721	**.007**	2.160	**.031**
NK CD69+	7.5	5.0	1.628	.103	**10.5**	2.165	**.030**	2.160	**.031**

P1: group 1 in comparison to group 2
P2 : group 1 in comparison to group 3
P3: group 2 in comparison to group 3

Table 2: Apoptosis in Different culture conditions (Mann Whitney Test)

Parameter	**HCV DC + HCV NK**	**HCV DC + HEALTHY NK**		
Core peptides	Median%	Median %	z	P
Annexin Positive	**12**	6.8	3.621	**.000**
CD56+ Annexin +	**4.8**	2.8	3.621	**.000**
CD14- Annexin +	**2.1**	1.7	2.012	**.044**
Living Cells	83.3	**92.0**	3.621	**.000**
Living CD 56+	66.0	**76.0**	3.674	**.000**
Living CD 14-	81.0	**87.0**	3.674	**.000**
NS3-NS4 peptides				
Annexin +	**7.2**	4.1	3.621	**.000**
CD56+ Annexin +	**3.3**	1.9	3.621	**.000**
CD14- Annexin+	**2.5**	1.03	3.621	**.000**
Living Cells	88.0	**95.0**	3.724	**.000**
Living CD 56+	67.0	**73.0**	3.621	**.000**
Living CD 14-	**85.0**	82.0	2.012	**.044**
NS5 peptides				
Annexin Positive	5.2	2.9	1.207	.227
CD56+ Annexin +	**2.4**	1.1	2.012	**.044**
CD14- Annexin +	**1.4**	.600	2.014	**.041**
Living Cells	92.0	96.0	1.658	.097
Living CD 56+	67.0	72.0	.408	.683
Living CD 14-	77.0	86.0	1.207	.227