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# Dendritic Cells in Hepatitis C Virus Infection

**Mona Mohamed Rafik, Nahla Mohamed Zakaria,**
**Hala Ghareeb, Mohamed, Nesrine Aly Mohamed, Ghada Maged Mohsen**
Clinical pathology department, Ain Shams University.

## ABSTRACT

**Background:** The global prevalence of chronic hepatitis C is estimated at 2.8%**.** There is markedly higher prevalence in the Middle East about 14.7% in Egypt**.** Dendritic cells (DCs) are one of the major Antigen presenting cells in the body. They bridge innate and adaptive immunity and impact priming of HCV-specific immune responses. The current study was aimed to investigate the DC activation status, and their role in interaction with natural killer (NK) cells utilizing different setups with healthy NK and $HCV^+$ DC, $HCV^+$ NK and healthy DC, healthy DC and healthy NK and finally $HCV^+$ NK and $HCV^+$ DC in the presence of HCV peptides and a ratio of 5 NK: 1DC.

**Results:** DC-NK interaction in chronic HCV infection is mainly affected by the affection of DCs by HCV leading to a maturation defect (decreased expression of HLA DR, CD 86 and CD 83). Healthy NK cells were able to stimulate the maturation of DCs particularly with core peptide whereas NS3-4 had no effect. When DCs were healthy, all peptides were able to produce significant maturation of DCs even when co-cultured with $HCV^+$ NK cells. Co-cultured $HCV^+$ NK cells and $HCV^+$ DCs showed significantly higher apoptosis of both cells. This could be attributed to the immature moDCs more with chronic HCV infection due to the fact that immature DCs typically under express HLA-class I molecules that would protect from NK-mediated lysis.

**Conclusion:** Cross-talk between DCs and NK cells plays an important role in the induction of both the innate and adaptive immune systems. HCV infection was found to impair the maturation of DCs. Thus consequently affecting its antigen presentation and T cell allostimulatory capacity and rendering them more liable to NK mediated lysis which could explain the persistence of infection and chronicity.

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

## INTRODUCTION:

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, interferon γ (INFγ) and cytotoxic activity of NK cells. Reciprocally NK cells either enhance the maturation of DCs or markedly augment their capacity to produce proinflammatory cytokines and to stimulate T-cell responses [(1)], or it could enhance immature DCs apoptosis, depending on the activation status of both players [(2)]**.** NK cells can also kill immature DCs. Thus, NK cells regulate DC homeostasis [(3)]**.** The NK cell activating receptor NKp30 appears to play a central role in DC maturation or apoptosis induced by NK cells [(4)]**.**

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 [(5)]**.**

## MATERIALS 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).



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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) Co-culture of (activated and naïve) NK with pulsed DCs.

4) Flow cytometric detection of DC markers using monoclonal antibodies against CD14, CD11c, HLA-DR, CD83, CD86, and annexin using Guava® easyCyte HT ***(Millipore, France).***

*The study was done after approval of the ethical board of Ain Shams University, and an informed written consent was taken from each participant in the study.*

**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

After co-culture of DCs ($HCV^+$ and healthy) with NK ($HCV^+$ and healthy), flow-cytometric analysis of the DCs was done to compare to the control culture to detect the effect of the co culture conditions on the DC characteristics (table 1):

a- When both NK and iDCs were obtained from HCV double positive blood bags. Core peptide pool produced an ***increase in CD 83$^+$, CD86$^+$, HLA DR$^+$CD83$^+$*** and NS5 pool produced an increase of ***CD83$^+$ and HLA$^-$DR***. NS3-4 peptides produced no significant change from the control culture.

b- When healthy NK cells (obtained from healthy blood bags) were co-cultured with $HCV^+$ iDCs (obtained from HCV double positive blood bags) after being pulsed with peptides. Significant upregulation of ***CD83$^+$,CD86$^+$, HLA-DR$^+$, and HLA-DR$^+$ CD83$^+$***occured in the core peptide culture, and NS5 with the exception of HLA-DR. NS3-4 peptides produced a decrease in ***HLA-DR$^+$CD86$^-$*** cells only.

c- When healthy iDCs were pulsed with HCV peptides then cocultured with $HCV^+$ NK cells (obtained from double positive blood bags) and all three peptide pools resulted in an increased expression of all DC markers with maturation.

## DISCUSSION

In the current study, we aimed to investigate the DC activation status, and their role in interaction with NK cells utilizing different setups with healthy NK and $HCV^+$ DC, $HCV^+$ NK and healthy DC, healthy DC and healthy NK and finally $HCV^+$ NK and $HCV^+$ DC in the presence of HCV peptides and a ratio of 5 NK: 1DC.

The status of DCs was assessed through studying the surface markers, HLA DR, CD11c, CD14, CD83 and CD86.

The control culture was achieved by co culturing healthy DCs not pulsed with peptides with healthy NK cells. DCs showed reduced expression of HLA DR, CD 86 and CD 83, i.e. immature phenotype of DCs, owing to the lack of stimulus for their activation.

In comparison to the control culture, co-culturing ***HCV$^+$ DCs*** with ***healthy NK*** gave significant increase in CD83, CD86 and HLA-DR expression with the core and NS5 peptides (with no effect on HLA DR in case of NS5) . NS3-NS4 showed no significant difference. i.e. healthy NK cells stimulate the maturation of $HCV^+$ DCs mainly with core peptide but not with NS3-NS4 peptide.

Our experiment also showed that co culturing ***healthy DCs*** pulsed with core, NS3-NS4 and NS5 peptides with ***HCV$^+$ NK cells***, showed significant increase in DCs count, HLA $DR^+$, CD $83^+$ and $CD86^+$ expression compared to the control culture, giving the mature DC phenotype. I.e. healthy DCs didn't show any maturation arrest in comparison to $HCV^+$ DCs.

Co- culture of ***HCV $^{+}$DCs*** with ***HCV $^{+}$NK*** in the presence of core peptide showed significant increase in CD83 and CD 86, no significant change after pulsing with NS3-NS4, and significant increase in HLA-DR and CD83 with NS5 peptide in comparison to the healthy control. Our results indicate that HCV affection of DCs make them more liable to maturation arrest and decreased expression of co stimulatory molecules even when co cultured with healthy NK cells with the peptide having the most effect on DCs maturation status being the core peptide followed by NS5 while NS3-NS4 has no effect.

In accordance with our study it was found that the presence of HCV genomic RNA in moDCs alters their immunostimulatory capacity and that the ability of DCs to stimulate T cells can be disrupted by down regulation of MHC I and co-stimulatory molecules, which could suggest an initial mechanism that can lead to a chronic state ***(1)*****.** moDCs have also been demonstrated to be impaired in HCV-infected patients, with reduced IL12 production, reduced allostimulatory activity and increased IL10 production ***(7)*****.**

In contrast other studies mentioned that DCs in chronic HCV infected patients were neither functionally nor phenotypically impaired, exhibited normal activity and expressed typical maturation markers and CD83, CD86 and HLA-DR levels similar to those expressed by healthy patients ***(5)*****.**

A variety of factors contribute to these discrepant results including: (a) the limited number of patients enrolled in studies; (b) patient related factors such as age, gender, co-infection and alcohol use; (c) duration of infection, liver inflammation and disease progression; (d) tissue source of DCs analyzed (i.e., peripheral blood vs. liver) and (e) whether the data originate from freshly isolated DCs or monocyte-derived DCs obtained from chronic HCV-infected patients ***(5)*****.**

## REFERENCES

**1- Wehner R, Dietze K, Bachmann M and Schmitz M (2011):** The Bidirectional Crosstalk between Human Dendritic Cells and Natural Killer Cells. J Innate Immun., 3(3): 258–263.

**2- 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., 473097: 1-10.

3- **Lee SW, Park H, Nayoung K, and Hong S (2013):** Natural Killer Dendritic Cells Enhance Immune Responses Elicited by α- Galactosylceramide-Stimulated Natural Killer T Cells. Biomed Res Int., 2013: 460706.

4- **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.

5- **Losikoff PT, Self AA and Gregory S (2012):** Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C. Landes Bioscience., Virulence, 3:7, 610–620.

6- **Eksioglu EA, Bess JR, Zhu H, Xu Y, Dong H, Elyar J, Nelson D and Liu C (2010):** Hepatitis C Virus Modulates Human Monocyte-Derived Dendritic Cells. J Viral Hepat., 17(11): 757–769.

7- **Crosignani A, Riva A, and Della Bella S (2016): A**nalysis of peripheral blood dendritic cells as a non-invasive tool in the follow-up of patients with chronic hepatitis C. World J Gastroenterol., 28; 22(4): 1393–1404.

***Table (1)*:** The effect of different co- culture conditions on DC characteristics in comparison to the control culture (Mann Whitney test):

| Parameter | Control Culture | $HCV^+$ NK+$HCV^+$ DC | | | HEALTHY NK+ $HCV^+$ DC | | | $HCV^+$ NK + HEALTHY DC | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Median % | Median% | z | P | Median % | z | P | Median | z | P |
| **Core peptide** | | | | | | | | | | |
| **CD14- CD11c +** | 22.0 | 28.0 | ..676 | .499 | 30.0 | 1.231 | .218 | **71.0** | 3.695 | **.000**** |
| **CD14- CD11C+ CD83 -** | 76.0 | **30.5** | 4.245 | **.000**** | **60.0** | 2.257 | **.024*** | **15.0** | 3.710 | **.000**** |
| **CD14-CD11C+ CD83+** | 24.0 | **69.5** | 4.245 | **.000**** | **40.0** | 2.257 | **.024*** | **85.0** | 3.710 | **.000**** |
| **CD14- CD11C+ CD86 -** | 77.0 | **52.5** | 2.047 | **.041*** | **39.0** | 2.667 | **.008**** | **30.0** | 3.180 | **.001**** |
| **CD14-CD11C+ CD86+** | 23.0 | **47.5** | 2.047 | **.041*** | **61.0** | 2.667 | **.008**** | **70.0** | 3.180 | **.001**** |
| **CD14-CD11c+HLADR+** | 28.0 | 20.5 | .342 | .732 | **45.0** | 2.701 | **.007**** | **69.5** | 3.174 | **.000**** |
| **CD4-CD11c + HLADR+ CD83 -** | 74.0 | **42.5** | 2.028 | **.043*** | **54.0** | 2.659 | **.008**** | **12.5** | 3.695 | **.000**** |
| **CD14-CD11c + HLADR+ CD83 +** | 26.0 | **57.5** | 2.028 | **.043*** | **46.0** | 2.659 | **.008**** | **87.5** | 3.695 | **.000**** |
| **CD14-CD11c + HLADR + CD 86-** | 75.0 | 75.5 | .678 | .498 | 30.0 | 1.847 | .065 | **12.5** | 3.710 | **.000**** |
| **CD14- CD11c+ HLADR+ CD 86 +** | 25.0 | 24.5 | .678 | .498 | 70.0 | 1.847 | .065 | **84.0** | 3.460 | **.001**** |
| **NS3-NS4 peptide** | | | | | | | | | | |
| **CD14- CD11c +** | 22.0 | 28.0 | 1.355 | .175 | 32.0 | 1.023 | .355 | **74.5** | 3.965 | **.000**** |
| **CD14- CD11C+ CD83 -** | 76.0 | 65,5 | 1.355 | .175 | 71.0 | 1.647 | .100 | **38.0** | 2.650 | **.008**** |
| **CD14-CD11C+ CD83+** | 24.0 | 34.5 | 1.355 | .175 | 29.0 | 1.647 | .100 | **62.0** | 2.650 | **.008**** |
| **CD14- CD11C+ CD86 -** | 77.0 | 71.5 | .339 | .735 | 65.0 | .616 | .538 | **34.0** | 3.180 | **.001**** |
| **CD14- CD11C+CD86+** | 23.0 | 21.5 | .682 | .495 | 35.0 | 1.026 | .305 | **66.0** | 3.180 | **.001**** |
| **CD14-CD11c+HLADR+** | 28.0 | 25.5 | .686 | .493 | 29.0 | .417 | .677 | **76.0** | 3.774 | **.000**** |
| **CD14- CD11c + HLADR+ CD83 -** | 74.0 | 76.0 | .338 | .735 | 65.0 | 1.432 | .152 | **21.5** | 3.695 | **.000**** |
| **CD14-CD11c HLADR+ CD83+** | 26.0 | 24.0 | .338 | .735 | 35.0 | 1.432 | .152 | **78.5** | 3.695 | **.000**** |
| **CD14-CD11c+ HLADR+ CD 86 -** | 75.0 | 70.0 | .509 | .610 | **39.0** | 2.257 | **.024*** | **37.0** | 2.650 | **.008**** |
| **CD14- CD11c+ HLADR+ CD 86 +** | 25.0 | 30.0 | .509 | .610 | 61.0 | 1.847 | .065 | **63.0** | 2.650 | **.008**** |
| **NS5 peptides** | | | | | | | | | | |
| **CD14- CD11c +** | 22.0 | **40.0** | 2.028 | **.043** | 35.0 | 1.642 | .101 | **57.5** | 3.167 | **.002**** |
| **CD14- CD11C+ CD83-** | 76.0 | **40.0** | 2.033 | **.042** | **41.0** | 2.881 | **.004**** | **26.5** | 3.710 | **.000**** |
| **CD14-CD11C+ CD83+** | 24.0 | **60.0** | 2.033 | **.042** | **59.0** | 2.881 | **.004**** | **73.5** | 3.710 | **.000**** |
| **CD14- CD11C+ CD86-** | 77.0 | 69.0 | .339 | .735 | **55.0** | 2.667 | **.008**** | **28.0** | .3.180 | **.001**** |
| **CD14-CD11C+ CD86+** | 23.0 | 31.0 | .339 | .735 | **45.0** | 2.667 | **.008**** | **72.0** | 3.180 | **.001**** |
| **CD14 CD11c + HLADR+** | 28.0 | **46.0** | 3.099 | **.002** | 19.0 | 1.454 | .146 | **69.0** | 3.774 | **.000**** |
| **CD14- CD11c + HLADR+ CD83-** | 74.0 | 58.5 | 1.525 | **.127** | **60.0** | 2.462 | **.014*** | **21.0** | 3.695 | .000****** |
| **CD14-CD11c + HLADR+ CD83 +** | 26.0 | 43.5 | 1.525 | **.127** | **40.0** | 2.462 | **.014*** | **79.0** | 3.695 | **.000**** |
| **CD14-CD11c + HLADR + CD 86-** | 75.0 | 68.0 | .509 | .610 | 55.0. | 1.436 | .151 | **33.5** | 2.650 | **.008**** |
| **CD14- CD11c+ HLADR+ CD 86 +** | 25.0 | 32.0 | .509 | .610 | 45.0. | 1.436 | .151 | **66.5** | 2.650 | **.008**** |

P < 0.05: Significant difference (*), P <0.01: Highly significant difference (**).