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CD3+CD56+ Natural Killer-like T cells in HIV+ patients with acute hepatitis C

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Background

Coinfection with the hepatitis C virus (HCV) in HIV+ patients has become a major problem. In Western Europe and the U.S.A approximately 30% of HIV+ patients are positive for HCV. Despite their impaired immune system, about 20% of HCV co-infected patients are able to spontaneously eliminate HCV. Previously we showed that natural killer cells are able to inhibit the HCV replication in the early stage of infection favoring spontaneous clearance. CD3+CD56+ NK-like T cells represent a T cell subpopulation that also expresses natural killer cell receptors and shares properties from both cell types. In this study, we investigated phenotype and function of CD3+CD56+ NK-like T cells in acute hepatitis C in HIV-positive individuals.

Methods

36 HIV+ patients with acute hepatitis C were enrolled into this study, including 13 patients with self-limited course of infection and 23 patients that subsequently developed chronic hepatitis C. Furthermore, 8 HIV+ mono-infected patients as well 12 HIV-/HCV- healthy individuals were analyzed. CD3+CD56+ NK-like T cells were characterized phenotypically by FACS analysis. IFN-γ secretion, degranulation (CD107a) and inhibition of HCV replication was studied using HCV replicon containing HuH7$_{A2}$ cells as targets.

Table 1. Patient Characteristics

	HIV(+) / aHCV		HIV(+)	HIV(-)/HCV(-)
	akut -> spontan ausgeheilt	akut -> chronisch		
Number	13	23	8	12
Male sex [a]	13 (100%)	23 (100%)	6 (75%)	7 (58%)
Age (years) [c]	39 (31-50)	41.22 (27-49)	51 (35-66)	30.4 (21-54)
Risk Factors				
MSM [a][b]	11 (85%)	21 (91%)	6 (75%)	-
Others/Unknown [a]	2 (15%)	2 (9%)	2 (25%)	-
Clinical data				
ALT U/L [c]	721 (23 - 3208)	629 (21 - 3644)	26 (16 - 40)	n.a. [d]
AST U/L [c]	459 (16 - 1638)	345 (24 - 2006)	20 (16 - 24)	n.a. [d]
HIV-Status				
HIV RNA negative (under HAART)	11 (85%)	16 (70%)	8 (100%)	-
CD4 Zellen /µl [c]	670 (358 - 904)	627 (300 - 1377)	737 (421 - 1199)	n.a. [d]
CD8 Zellen /µl [c]	1221 (407 - 1794)	1105 (220 - 2775)	1128 (336 - 1940)	n.a. [d]
HCV-Status				
HCV RNA (x 10^{-6} IU/mL) [c]	14,7 (<0.1 -69)	11.2 (<0.1 - 69)	-	-
HCV-Genotypes:				
Genotype 1[a]	8 (62%)	15 (65%)	-	-
Genotype 2[a]	0 (0 %)	0 (0 %)	-	-
Genotype 3[a]	0 (0 %)	0 (0 %)	-	-
Genotype 4[a]	0 (0 %)	7 (31 %)	-	-
Undetermined Genotype [a]	5 (38%)	1 (4 %)	-	-

[a] Number of cases (number/total in %)
[b] MSM; men who have sex with men
[c] Mean (range)
[d] na – not analyzed

Results

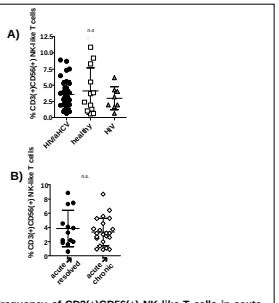


Figure 1. Frequency of CD3(+)CD56(+) NK-like T cells in acute HCV infection
Analyzing the frequency of circulating NK-like T cells we did not observe any significant differences between the study groups (**Figure. 1A**). Furthermore, when patients with acute hepatitis C were analyzed separately, no statistically significant differences were observed between patients that were able to clear HCV infection spontaneously and those who subsequently developed chronic hepatitis C (**Figure. 1B**).

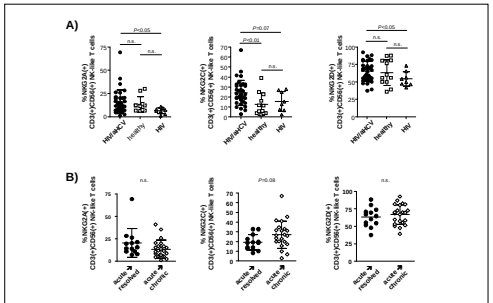


Figure 2. Expression of C-Type Lectin receptors on CD3(+)CD56(+) NK-like T cells
Phenotypic characterization of NK-like T cells revealed acute hepatitis C to be associated with increased expression of the NK-like cell receptors NKG2A, NKG2C and NKG2D compared to the control groups (**Figure 2A**). However, expression of these receptors was not associated with outcome of acute hepatitis C (**Figure 2B**).

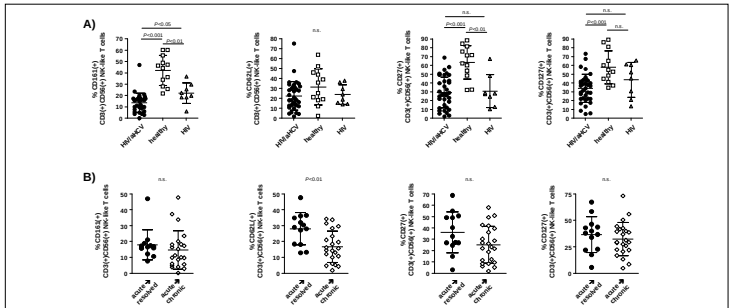


Figure 3. Expression of maturation markers on CD3(+)CD56(+) NK-like T cells
Regarding surface expression of the maturation markers CD161, CD62L, CD27 and CD127 on CD3(+)CD56(+) NK-like T cells we found HIV(+) patients in the acute phase of infection to display a significantly lower frequency of NK-like T cells positive for CD161, CD27, and CD127, respectively than healthy individuals. However, only expression of CD161 expression differed significantly between HIV mono-infected patients and HIV patients with acute hepatitis C (**Figure 3A**).
Interestingly, we found that in the group of HIV(+) individuals with acute hepatitis C a high expression of CD62L was associated with spontaneous clearance of HCV. No such differences were found with respect to surface expression of the other tested surface molecules (**Figure 3B**).

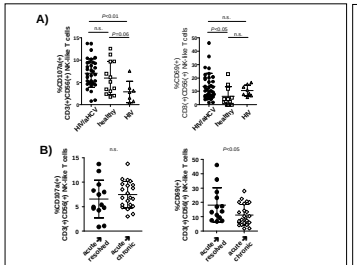


Figure 4. Activation level of CD3(+)CD56(+) NK-like T cells in the acute phase of HCV infection
For the analysis of the activation level of CD3(+)CD56(+) NK-like T cells we compared the spontaneous Degranulation (CD107a) as well as CD69 expression between the study groups. We observed a significant higher spontaneous degranulation (CD107a) by HIV+ patients with acute HCV infection than in HIV+ mono-infected controls and a significant higher expression of CD69 compared with healthy individuals (**Figure 4A**). In the group of HIV patients with acute hepatitis C, a high CD69 expression was associated with spontaneous clearance of acute hepatitis C (**Figure 4B**).

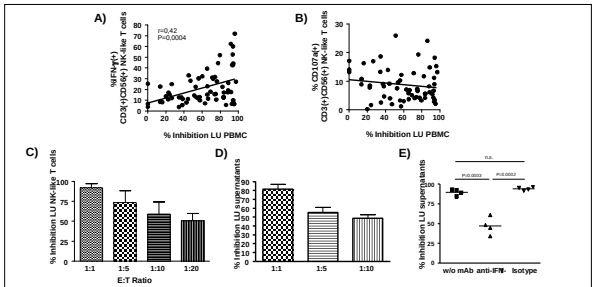


Figure 5. CD3(+)CD56(+) NK-like T cells inhibit viral replication via IFN-γ
After we noted a positive correlation between NK-like T cell-secreted IFN-γ and inhibition of viral replication in PBMCs (**Figure 5A**) we analyzed the ability of isolated CD3(+)CD56(+) NK-like T cells to block HCV replication. Following co-incubation of isolated NK-like T cells (n=7) with HuH7$_{A2}$HCV replicon cells we showed that NK-like T cells were able to block HCV replication even at a low E:T ratio of 1:20 (**Figure 5C**). Next we analyzed whether blocking of hcv replication is cell-cell contact dependent. After incubation of HuH7$_{A2}$ HCV replicon cells with supernatants of the previous co-incubation, we also observed a high capacity to block viral replication, confirming soluble factors to play an important role (**Figure 5D**). In addition, we observed that co-incubation with an IFN-γ-specific antibody significantly reduced anti-HCV activity of NK-like T cells, which further supports a role for IFN-γ in NK-like T cell-mediated anti-HCV activity (**Figure 5E**).

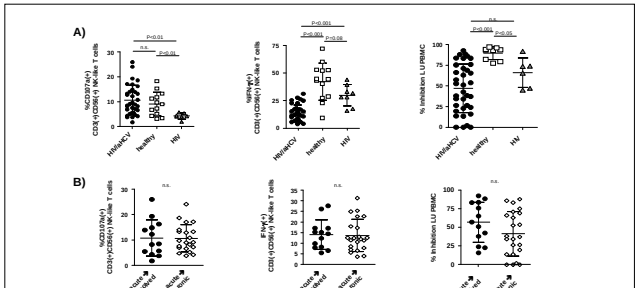


Figure 6. Impaired IFN-γ capacity on CD3(+)CD56(+) NK-like T cells from HIV+ patients in acute HCV infection
Functional analysis of CD3(+)CD56(+) NK-like T cells showed acute hepatitis C to be associated with higher frequency of degranulating NK-like T cells compared with HIV+ patients. In conflict, IL-12/IL-15 induced IFN-γ secretion was significantly lower in NK-like T cells from HIV-positive patients acutely infected with HCV in comparison with HIV+ mono-infected patients and healthy individuals. In addition HIV+ patients with acute HCV infection showed the lowest ability to block HCV replication *in vitro* (**Figure 6A**).
In the group of HIV+ patients with acute HCV infection no significant differences were found between patients that subsequently cleared the virus and patients that developed chronic hepatitis C (**Figure 6B**).

Conclusion

Taken together, our data indicate that CD3(+)CD56(+) NK-like T cells are able to block HCV replication in an IFN-γ dependent manner but are functionally impaired in HIV+ patients with acute hepatitis C.

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