$CD56^{Bright}$ NK Cell Expansion and Low Expression of Cytotoxic Molecules in $CD56^{Dim}$ NK Cells in HIV/HCV Coinfected Patients

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Research Article

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Abstract

Background

HIV/HCV coinfection is associated with a rapid progression to liver damage. Specifically, the dysregulation of NK cell populations is of special interest, as NK cells have been shown to effectively block HCV replication as well as an anti-fibrogenic activity. The NKp30 receptor has been linked to tumor cell lysis and has a crucial role during viral infections. In the present study, we determine the subpopulations of NK cells and the expression of NKp30 receptor and cytotoxicity molecules in patients with HIV/HCV.

Results

HIV/HCV coinfected patients present greater liver damage than the HIV control group, using the APRI and FIB-4 indices. NK cells frequency was decreased in the HIV/HCV group compared to the HIV group. $CD56^{brigth}$ cell frequency was increased and $CD56^{dim}$ was decreased in HIV/HCV patients. NKp30 expression was higher in $CD56^{brigth}$ subpopulation and lower in $CD56^{dim}$ subpopulation in the HIV/HCV group. However, the expression of the ζ chain ($p < 0.0001$), granzyme B, and perforin ($p = 0.0474$), were significantly decreased in $CD56^{dim}$ NKp30 + cells from HIV/HCV patients.

Conclusions

The expansion of the highly dysfunctional $CD56^{brigth}$ subpopulation and the low expression of cytotoxicity markers in $CD56^{dim}$ cells reflects an altered activity in NK cells of coinfected patients, which could contribute to the accelerated progression of liver damage.

Background

Human Immunodeficiency Virus (HIV) infection is a serious public health problem worldwide. According to UNAIDS/WHO data in 2020, it was estimated that 37.7 million people were carriers of the HIV, of which only 27.5 million were under antiretroviral treatment (1). On the other hand, it is estimated that in the world are 71 million people with chronic infection by the hepatitis C virus (HCV). HCV causes around 10,000 deaths per year, in 60% of the patients, the infection becomes chronic, of which 20% develop cirrhosis and hepatocarcinoma (2). The presence of HIV is considered comorbidity that has a negative impact on the natural history of HCV infection which leads the chronic HCV infection to evolve faster, generating accelerated fibrosis progression, being the liver damage, the main cause of death in coinfected patients (3, 4).

A percentage of patients with HIV manage to overcome acute HCV infection spontaneously, suggesting that even those infected with HIV can eliminate HCV through the immune system (5). Specifically, the

dysregulation of Natural Killer (NK) cells subpopulations is of special interest, as NK cells have been shown anti-fibrogenic activity and have the particularity of effectively block HCV replication (6, 7). Between 30 and 50% of the lymphocytes resident in the liver are NK cells, which gives them a relevant role in the immune response against HIV/HCV coinfection (8–11).

NK cells have been immunophenotyped as CD3-, CD2 +, CD16 +, CD56 +, CD14- and CD19- (12, 13). Human NK cells can be divided into two subsets based on the expression of and the adhesion molecule CD56 (14). NK cells with high expression ($CD56^{bright}$) which constitute 10% of NK cells in peripheral blood, producing large amounts of pro-inflammatory cytokines, chemokines and generating a better response to soluble factors and have low expression of CD16 as well as killer inhibitory receptor (KIR). In contrast, those with low expression ($CD56^{dim}$) constitute 90% of NK cells in peripheral blood, characterized by expressing perforin, having greater cytotoxic activity, responding better to ligand-receptor binding, and presenting mostly the CD16 as well as KIR + receptors (15–17).

NK cell activation depends on the fine balance between activation and inhibition signals. The natural cytotoxic receptors (NKp30, NKp46, and NKp44) are an important group of activation receptors, their expression is variable and recognize molecules induced by cellular stress (18). NKp30 receptor trigger NK cells cytotoxicity and has been linked to tumor cell lysis and response in viral infections. NKp30 extracellular domain is characterized by a single variable-type immunoglobulin (Ig) -like. The intracellular tail of NKp30 has no signaling motif, but its transmembrane domain is associated with an ITAM (Immunoreceptor Tyrosine-based Activation Motif) motif, as well as adapter proteins like CD3ζ (CD3 zeta chain) and FcRy (Fc Receptor gamma chain), which are necessary for signal transduction (19).

Cytotoxicity aids in limiting the progression of liver damage fibrosis, regulating stellar cell-derived myofibroblasts, which play a key role in this pathogenic process. They are also responsible for modulating liver damage by balancing the local production of pro and anti-inflammatory cytokines through their activating or inhibiting receptors (20). Therefore, the objective of this study was to determine the subpopulations of NK cells and the expression of NKp30 receptor and cytotoxicity molecules in patients with HIV/HCV coinfection.

Results

Demographic and clinical characteristics of patients with HIV+/HCV coinfection

A total of 79 patients, including 31 HIV mono-infected, 18 HCV mono-infected, and 30 HIV/HCV coinfected individuals were enrolled in the study. Age, weight, $CD4^+$ and $CD8^+$ T-cell counts, HIV-1 RNA viral load, as well as liver enzyme levels were retrieved from patients files. A detailed description of patient characteristics is given in Table 1. Despite statistically significant differences in relation to weight; nevertheless, none of the patients were overweight or obese. In relation to liver function tests, most of the variables present statistically significant differences, it should be noted that the HCV mono-infected

group was the one with the highest level of liver damage, which is reflected in the results of the APRI and FIB 4 indices. Regarding the clinical status of HIV, the mono-infected and coinfected had a virological control, for which they have low viral load and their CD4 count is within the adequate values to rule out AIDS.

Table 1
Patient characteristics.

Demographic characteristics				
Variable	**HIV**	**HCV**	**HIV/HCV**	P **value**
Number	31	18	30	
Age (years)	44 ± 9.5	42 ± 7	41 ± 9	0.6273*
Weight (Kg)	75 ± 8	81 ± 12	70 ± 10	**0.0011***
Liver function tests				
AST UI/L	23 (20–26)	58 (41–96)	32.5 (22–56)	**0.0007****
ALT U/L	34 (23–41)	59 (47–83)	34.5 (21.7–60.2)	**0.0174****
ALP UI/L	95.23 ± 32.48	99 ± 44.72	97.60 ± 21.81	0.2987*
GGT UI/L	52.0 (27–71)	40.5 (28.7–80.7)	69.5 (27.5–117.3)	0.7109**
Total Protein g/dL	7.06 ± 0.45	6.5 ± 0.43	7.5 ± 0.57	**0.0001***
Total bilirubin mg/dL	0.45 (0.37–0.61)	1.3 (0.5–2.3)	0.58 (0.45–0.88)	**0.0134****
Direct bilirubin mg/dL	0.10 (0.07–0.12)	0.51 (0.26–1.1)	0.16 (0.12–0.25)	**0.0001****
Indirect bilirubin mg/dL	0.36 (0.29–0.49)	0.99 (0.53–1.4)	0.41 (0.31–0.66)	**0.0045****
Albumin	4.42 ± 0.25	3.9 ± 0.75	4.27 ± 0.28	**0.0083***
HIV status				
CD4 (Cells/µL)	528.0 (409.0–711.0)	ND	419.0 (269.5–677.0)	**0.0417******
CD8 (Cells/µL)	778.1 ± 292.8	ND	983.7 ± 486.3	0.0522***
Viral load (copies/mL)	39.0 (20.0–40.0)	ND	20.0 (20.0–40.0)	0.3713******
CD4/CD8 ratio	0.75 ± 0.20	ND	0.55 ± 0.31	**0.0047*****
APRI and FIB-4 indices				
APRI	0.32 (0.27–0.40)	1.75 (0.95–4.53)	0.52 (0.30–0.98)	**0.0002****
FIB-4	0.89 (0.69–1.09)	3.14 (1.76–8.66)	1.28 (0.75–1.57)	**0.002****

Demographic characteristics
* Ordinary one-way ANOVA, ** Kruskal-Wallis, ***Unpaired t test, ****U de Mann Whitney. $p < 0.05$ statistically significant. The qualitative variables were expressed in frequency or proportions and the quantitative variables in mean and standard deviation or median percentiles (25 to 75).

Frequency of NK cells in peripheral blood in HIV/HCV coinfection

We analyzed NK cell frequency in PBMC´s, among our study groups. NK cells were immunophenotyped and identified as CD56 + CD3- cells. We observed that HCV mono-infected and HIV/HCV coinfected patients had the lowest frequency of circulating NK cells compared to the HIV group (Fig. 1a). A higher percentage of $CD56^{dim}$ cells was observed in comparison to $CD56^{bright}$ cells (Fig. 1b), an increased frequency of $CD56^{bright}$ NK cells was associated with HCV mono-infection and HIV/HCV coinfection in contrast to HIV monoinfected group patients, with statistically significant differences between monoinfected groups, whereas in $CD56^{dim}$ cells was observed an increased frequency in HIV monoinfection (Fig. 1b). The surface expression of other markers in NK cells such as CD16 was also analyzed. First, in $CD56^{bright}$ subpopulation an increase was observed in the HCV group. Nevertheless, a statistically significant decrease in the frequency of $CD56^{dim}$ NK cells that express CD16 was observed in the HCV group (Fig. 1c), whereas in $CD56^{bright}$ NK cells an increase in the frequency of cells $CD16^{-}$ was observed in the HCV group in comparison with the other two groups but in $CD56^{dim}$ subpopulation a statistically significant increased frequency was observed in the HCV group (Fig. 1d).

Nkp30 Expression In Hiv/hcv Coinfected Patients

The expression of NKp30 receptor was evaluated in the subpopulations $CD56^{dim}$ and $CD56^{bright}$ of NK cells. Frequency of total NK cells that were positive for NKp30 was lower in coinfected patients, however, no statistically significant differences were observed between the study groups. A statistically significant increase in the frequency NK cells positive to NKp30 was observed in the $CD56^{dim}$ subpopulation, this on HCV coinfected patients with statistically significant differences when compared with HIV/HVC coinfected group (Fig. 2a). Relative expression was also evaluated using the Mean Fluorescence Intensity (MFI), and we observed that HCV monoinfected patients showed higher NKp30 expression levels than HIV monoinfected patients, this in general NK populations as well as in $CD56^{bright}$ and $CD56^{dim}$ (Fig. 2b).

Intracellular markers of cytotoxic and signal transduction in NK cells subpopulations in HIV/HCV coinfected patients

A decrease in signal activation of NK cells has been characterized due to the dissociation of CD3ζ from NKp30 in a viral interaction. Therefore, the expression level of the zeta chain was evaluated in the

subpopulations of NK cells (Fig. 3a).

NK cells mediate cytotoxicity through the release of granules containing perforin and granzyme B. NK cells are important in the immune response to viral infection, therefore, the expression of perforin and granzyme B was evaluated in of NK cells subpopulations.

In general, we observed a tendency were HVC patients presented a higher frequency of NK cells that also express perforin and granzyme. Whereas NK cells positive to perforin, showed a statistically significance between HCV monoinfected and HIV/HCV coinfected groups, this was appreciated in general CD56 NK cells, as well as in $CD56^{bright}$ cells (Fig. 3b). Regarding NK cells positive to granzyme B, a statistically significance was observed between HCV monoinfected patients when compared with the other two groups, this in $CD56^{bright}$ NK cells (Fig. 3c).

Serum Cytokine Levels In Hiv/hcv Coinfection

Cytokines initiate the inflammatory response, define the magnitude and nature of the specific immune response, and increase the lytic potential of NK cells in early stages of viral infections, and they are the main means of intracellular communication before a microbial invasion.

Proinflammatory cytokines were measured and was observed that HIV/HCV coinfected patients have a higher level of IL-8, IL-10, IL-12, and IL-23 compared to the monoinfected group. IFN-α, IFN- , IL-6, IL-18, IL-33, and TNF-α did not present statistically significant differences between the study groups (Fig. 4).

Discussion

NK cells play an important role in the immune response against viral infections, they have direct antiviral effects and promote activation of dendritic cells during viral replication. However, alterations in the function of these cells caused for HIV infection, can affect the antiviral capacity. In this work, we shown that HIV/HCV coinfection is associated with an alteration in the subpopulations of NK cells, the natural cytotoxic receptor NKp30 as well as cytotoxic markers.

Comparison between the study groups regarding the clinical variants, indicates that patients coinfected with HIV/HCV have a lower number of $CD4^{+}$ cells, a high concentration of enzymes that indicate liver damage and probable fibrosis according to the APRI and FIB-4 indices. However, more tests are required to determine the extent of the liver damage, as abdominal ultrasound, CT scan or tissue sample (21).

In relation to NK cells, previous studies have reported that both monoinfections (HIV and HCV) are associated with significant NK cell alterations, mainly related to altered phenotype, especially a decrease in the subpopulation $CD56^{dim}$ causing a limited cytotoxic activity (4, 22–24). It has also been described that HIV/HCV coinfection causes a low frequency of circulating NK cells compared to monoinfection, because HIV inhibits the activation of DCs, which leads to a dysfunction of NKs cells, therefore the loss of

DCs-NKs interaction in the coinfection scenario generates a limited response of NK cells (5, 25–27). In our study, we found that HIV/HCV coinfected patients, as well as HCV monoinfected patients, have the lowest frequency of circulating cells compared to the HIV group, as previously described by Kaczmarek *et al.*, their results showed that monoinfected (HIV and HCV) and the coinfected group did not show significant differences in the frequency of circulating NK cells. In the analysis of $CD56^{dim}$ cytolytic effector cells, a lower distribution was found in coinfected patients, which would imply a decreased cytolytic NK cells response that could compromise effector functions.

Similarly, several studies reported alterations in frequency of the NK cell receptor expression in HIV and HCV monoinfections (4, 23, 28, 29). In the same way, it has been shown a superficial expression of the NKp30 receptor in the HIV/HCV coinfection (4). Regarding the expression of the NKp30 receptor, in our results in total NK cells and $CD56^{dim}$ subpopulation, it could be observed that it was lower in coinfected patients than in monoinfected patients. HIV infection has been associated with a significant decrease in NKp30 expression, whereas HCV infection has been associated with decreases in the expression of NCRs (28–30). In this context, the cytotoxic activity of NK cells could decrease even more in a coinfection scenario. Relative expression was measured between HIV and coinfected group, an increase in the number of NKp30 receptors per NK cell, as well as per $CD56^{bright}$ and $CD56^{dim}$ cells were found in the group of coinfected patients. Which means that fewer NKp30 + cells are found in the group of coinfected patients, but that each cell expresses more receptors compared to HIV patients.

On the other hand, dissociation of CD3ζ from NKp30 in viral infections, drive to a low intracellular signal transduction (31). Moreover, a direct antagonistic interaction between the human cytomegalovirus tegument protein, pp65 and NKp30 receptor reduce the cytotoxicity of NK cells by dissociating the adapter protein from the NKp30 ζ chain (32). Therefore, we evaluated CD3ζ expression in NKp30 positive cells, and finding a lower expression in HIV/HCV coinfection compared to the monoinfection groups. In general, our results show that there is a greater dissociation of NKp30 and CD3ζ in patients with both viral infections. Therefore, we inferred that there is an alteration in the proportion of CD3ζ and NKp30, which would imply a lower transduction of chemical signals within the cell and more compromise of effector functions.

Furthermore, cytotoxicity markers were evaluated in the subpopulations of NK cells, and it was observed that the coinfected patients express a lower amount of granzyme B and perforin compared to the monoinfected groups; indicating that these cells are less equip for performance the cytotoxic function, which can lead to a decrease in viral clearance and HSC elimination in HIV/HCV coinfection, supporting the mechanisms that can generate an accelerated progression of liver disease. This information invites further investigation in the setting of HIV/HCV coinfected patients.

In addition to this, cytokines play an important role in viral infections as they mediate communication between immune cells and promote cell growth, differentiation, and activation. Some cytokines including IL-1β, IL-6, IL8, TNF-α and IFN- are important activators of the acute phase response. And IL-1, IL-10, IL-12, IL-15, IL-18, and IFN-α and β, are involved in the innate immune response.

It has been described that coinfected patients (HIV/HCV) have higher basal levels of IL-8, IL-10, IL-12, and IL-23 in contrast to patients monoinfected with HIV and healthy people, which could be due to by an effect of persistent activation of the immune system (33–35). In our study, these cytokines presented statistically significant differences between the study groups, coinciding that the coinfection group presented a higher concentration. These interleukins have characteristics that could influence the infection process. For example, the literature reports that viruses such as HCV induce the expression of IL-8 by stimulating TLR2 (34), IL-12, in collaboration with IL-18, increases the cytolytic function of NK cells, stimulating the secretion of IFN- by T and NK cells and the development of Th1-type responses (36), IL-12 can be classified as a regulator of innate immunity, because macrophages activated by microbes secrete it to develop effector functions of NK cells. Finally, IL-23 is a cytokine produced mainly by antigen-presenting cells and plays an important role in a wide variety of viral infections, although the exact mechanisms are still unclear (35).

HIV/HCV coinfection pathogenesis is complex, the alteration of the immune response associated with cirrhosis may be reflected in the increased production and high circulating levels of proinflammatory cytokines, although currently neither the inflammatory profile nor the alterations in immune function between HIV and HCV co-infected patients with different levels of liver fibrosis have been well established. It should be clarified that we do not have a direct conclusion about the infection or the NK cells, but it allows us to have an overview of the inflammatory state of the coinfected patients.

Conclusion

Difficulties in understanding the role of cellular immune responses in the progression of HCV disease increase in the presence of HIV infection. The expansion of the highly dysfunctional $CD56^{brigth}$ subpopulation and the low expression of cytotoxicity markers in $CD56^{dim}$ cells reflects an altered activity in NK cells, this could suggest that viral synergy plays a relevant role in immune function in HIV+/HCV coinfected patients, which could contribute to the accelerated progression of liver damage.

Methods

Patients

Monoinfected (HIV or HCV) and coinfected man over 18 years and under 65 years (with a previous diagnosis between 5 - 10 years), with effective antiretroviral therapy for HIV (<40 copies / mL) for at least 6 months, assist for medical consultation to the “Hospital Civil de Guadalajara Fray Antonio Alcalde” and the Gastroenterology Unit of the “Centro Médico Nacional de Occidente of the Instituto Mexicano del Seguro Social”. The diagnosis was based on clinical data, serological and molecular tests.

Determination of liver damage

Liver damage degree was determined using non-invasive liver fibrosis prediction methods such as APRI and FIB-4. For the APRI index, we considered the following scale: < 0.5 absence of fibrosis (F1), between

0.5-1.5 indeterminate and > 1.5 fibrosis (F3). In the case of FIB-4, a value <1.45 has a negative predictive value of 90% for advanced liver fibrosis (F1). In contrast, a value >3.25 has 97% specificity and a 65% positive predictive value for advanced cirrhosis (F3).

Peripheral blood mononuclear cells (PBMCs) extraction

PBMCs were isolated from venous blood from mono-infected and co-infected patients (HIV and HVC) by Ficoll-Paque density gradient centrifugation. The cell pack was re-suspended in RPMI-1640 solution supplemented with 20% fetal bovine serum (FBS) and DMSO (Sigma-Aldrich, Burlington, MA). Finally, they were gradually frozen and cryopreserved liquid nitrogen gas phase until use.

Flow cytometry

Cells were stained with different combinations of the following fluorochrome-labeled antibodies, for surface marker APC/Cy7 anti-human CD3, PE/Cy7 anti-human CD56 (NCAM), PerCP/Cy5.5 anti-human CD16, PE anti-human CD337 [NKp30], then they were incubated for 45 minutes at 4°C and protected from light. Subsequently, cells were permeabilized with IntraPrep Permeabilization Reagent (Beckman Coulter, Brea, CA) Kit and incubated with the antibodies for intracellular labeling: FITC anti-CD247 (TCRζ, CD3ζ), APC anti-human Perforin, Alexa Fluor® 700 Mouse anti-Human Granzyme B, for 45 minutes and protected from light. To more accurately, determine the negative value of intracellular fluorochromes, fluorescence minus one was performed. The detection was processed in the Attune NxT Flow Cytometer equipment and data was analyzed with FlowJo software version 10 (Ashland, OR).

Multiplex cytokine profiling by flow cytometry

LEGENDplex™ bead-based multiplex assays (Biolegend, San Diego, CA) used to quantifying cytokines. All reagents must be at room temperature (20-25°C) before use. For measuring serum, we add 25 µL of Matrix B to the standard tubes, 25 µL Assay Buffer to sample tubes, 25 µL of each standard-to-standard tubes, 25 µL of each diluted serum sample to sample tubes, 25 µL of mixed beads to all tubes and 25 µL Detection Antibodies to all tubes. Then, we cover the entire rack with aluminum foil to protect the tubes from light and shake for 2 hours at room temperature, after that, all tubes are washed with 25 µL SA-PE and cover again with aluminum foil for 30 minutes at room temperature, later we centrifugate the tubes at 1000 x g for 5 minutes and remove the supernatant. We continue adding 200 µL of 1x Wash Buffer to all tubes, vortexing for 1 minute and centrifuge at 1000 x g for 5 minutes and removing the supernatant, finally we read samples on a flow cytometer.

Statistical analysis

Descriptive statistics of the variables were determined. For this, the qualitative variables were expressed in frequency or proportions and the quantitative variables in mean and standard deviation or median percentiles (25 to 75). For the Inferential statistics, first, we determined the data distribution for the subsequent analysis. Groups comparisons were performed using the Ordinary one-way ANOVA omnibus test coupled with the Tuckey post-hoc test when needed, or the non-parametric counterpart Kruskal-

Wallis H coupled with the Dunn's post-hoc test when needed. A p <0.05 was considered statistically significant for all analyzes. Data analyzes were performed using GraphPad Prism software v8.0 (San Diego, CA).

Abbreviations

NK
Natural Killer
HIV
human immunodeficiency virus
HCV
hepatitis C virus
UNAIDS/WHO
Joint United Nations Program on HIV/AIDS/World Health Organization
KIR
Killer inhibitory receptor
NCRs
Natural Cytotoxicity Receptors
ITAM
Immunoreceptor tyrosine-based activation Motif
MFI
mean fluorescence intensity
PBMC
Peripheral blood mononuclear cell
FBS
fetal bovine serum
DMSO
Dimethyl sulfoxide.

Declarations

Ethics approval and consent to participate

The study was conducted under the international ethical standards and principles for medical research in human beings, all methods were performed in accordance with the relevant guidelines and regulations (Declaration of Helsinki). All participants were told about the purpose of the study, procedures, benefits and potential risks. Informed consent was documented with signed consent forms before participating in the study. The study has been approval by the "Comité de Ética del Centro Universitario de Ciencias de la Salud" of the Universidad de Guadalajara (number approval CI-02020)

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

NVM, MTBT and STA conceived the original idea and contributed with data interpretation. LAGH and JFAV contributed with clinical approach and patients' recruitment, CLGI, PCR and GGS performance the experiments. CLGI and NVM wrote the manuscript. All authors discussed the results and contributed to the final version,

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Not applicable

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Figures

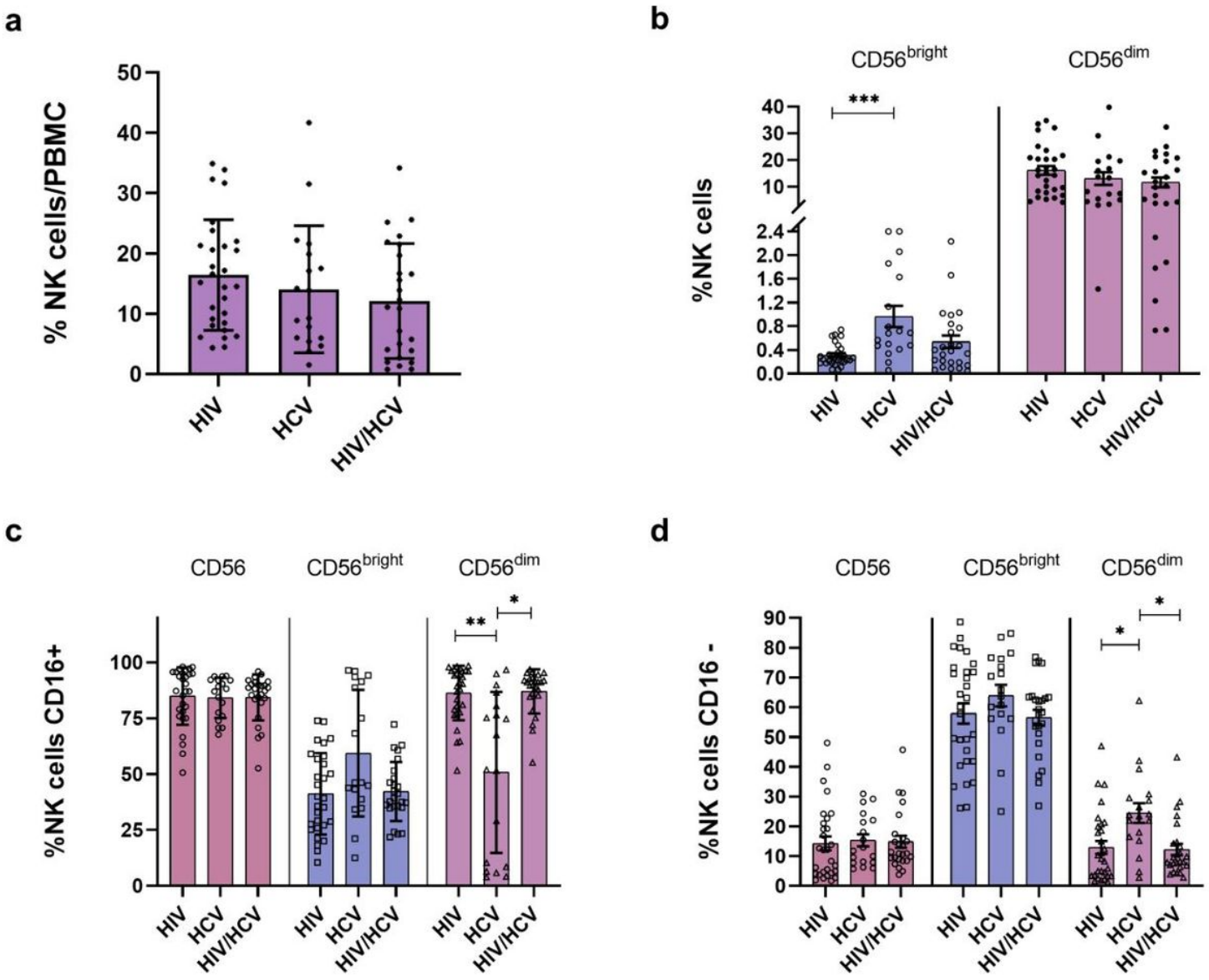

Figure 1

Distribution of circulating NK cells. a) Circulating NK cell frequency in peripheral blood mononuclear cells (PBMC) in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. **b)** Frequency distribution of $CD56^{dim}$ and $CD56^{bright}$ NK cells from total NK cells in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. **c)** Distribution of CD16+ NK cells in HIV mono-infected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. **d)** Distribution of CD16- NK cells in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. *p≤0.05, ** p≤0.01, ***p≤0.001.

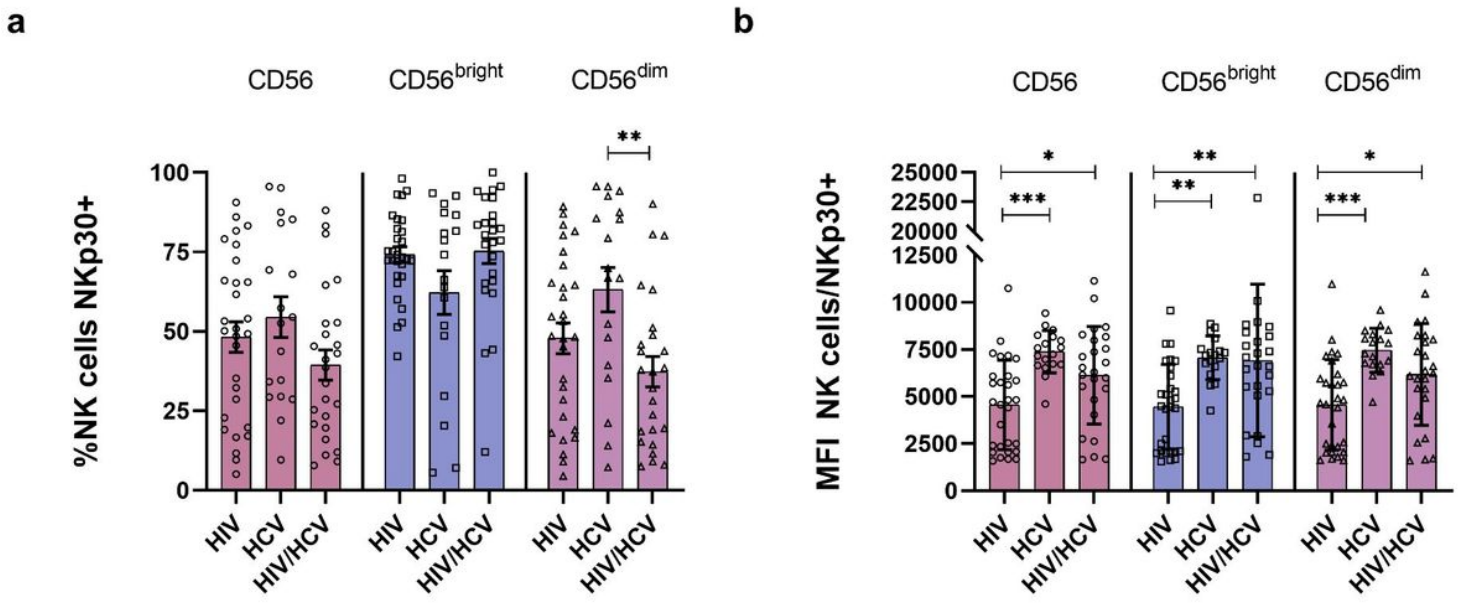

Figure 2

NKp30 expression in HIV/HCV coinfected patients. a) Expression of the natural cytotoxic receptor NKp30 in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. **b)** MFI in NK cell subpopulations in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. *p≤0.05, ** p≤0.01, ***p≤0.001.

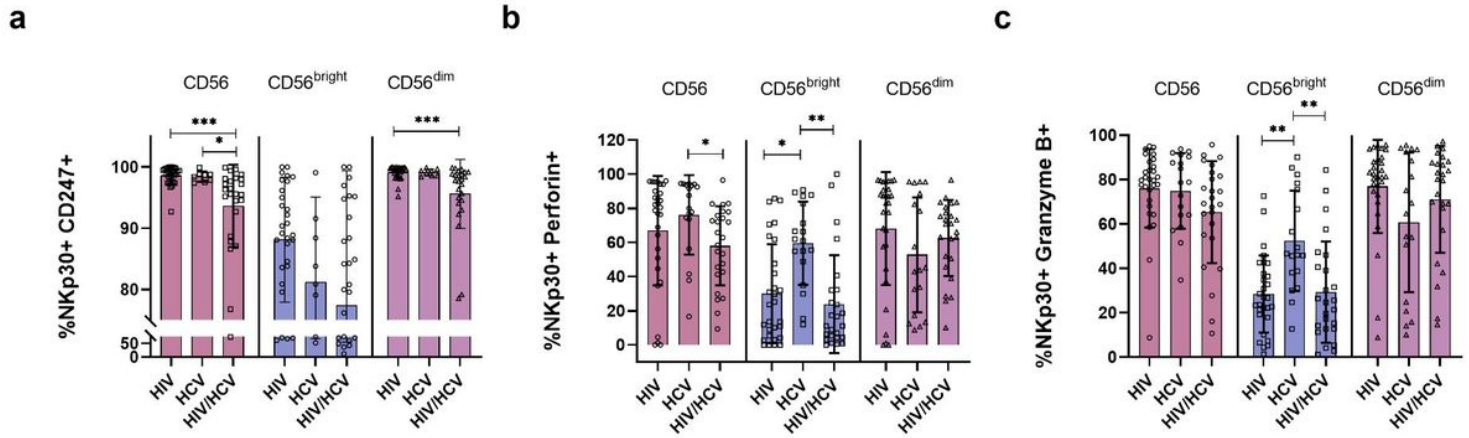

Figure 3

Zeta chain, perforin and granzyme b expression in NK cell subpopulations. a) Frequency of CD247+ (CD3ζ), NKp30+ cells in NK cell subpopulations in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients. **b)** Frequency of NK cell subpopulations positive to NKp30 and perforin in HIV monoinfected patients, HCV monoinfected patients, and HIV/HCV coinfected patients.

c) Frequency NK cell subpopulations positive to NKp30 and granzyme B in HIV monoinfected patients, HCV monoinfected patients, and HIV+/HCV co-infected patients. *p≤0.05, ** p≤0.01, ***p≤0.001.

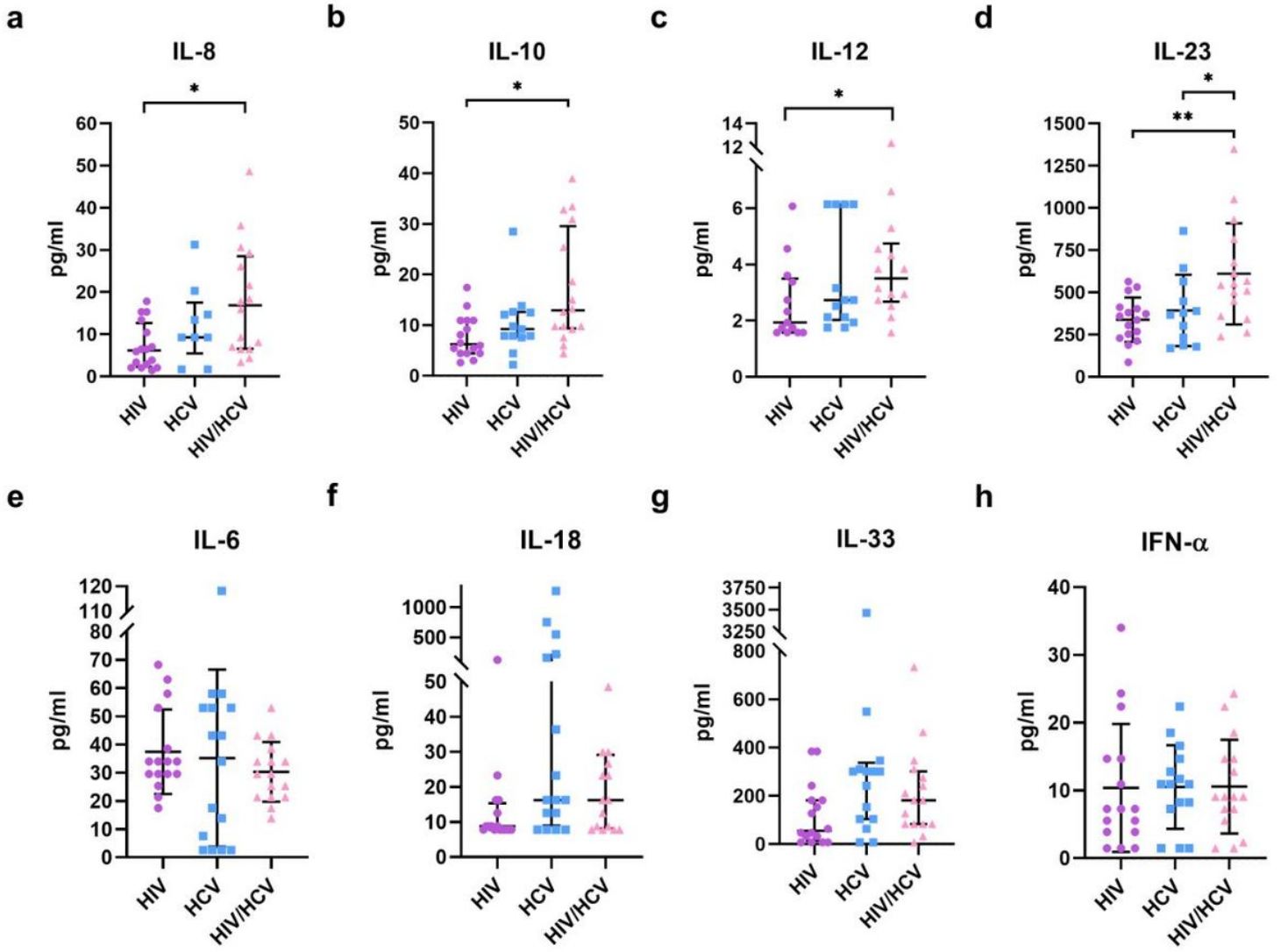

Figure 4

Serum cytokine concentration levels. Concentrations in pg/mL of **a)** IL-8. **b)** IL-10. **c)** IL-12. **d)** IL-23. **e)** IL-6. **f)** IL-18. **g)** IL-33. **h)** IFN-α. *p≤0.05, ** p≤0.01, ***p≤0.001.