International Scholars Journals

African Journal of Immunology Research ISSN 2756-3375 Vol. 11 (2), pp. 001-009, February, 2023. Available online at www.internationalscholarsjournals.org © International ScholarsJournals

Full Length Research Paper

Reduced IL-17A producing lymphocytes in chronic hepatitis C individuals treated with Sofosbuvir/Velpatasvir at CHU Campus of Lomé, Togo

Maléwé KOLOU[1,2], Gnatoulma KATAWA[3], Christèle NGUEPOU TCHOPBA[3], Tégmaba GNODJA[4], Marthe Oukoé AMESSOUDJI[3], Pélagie Edlom TCHADIE[3], Roland KOGOE[4], Aklesso BAGNY[4]

[1]Laboratoire de Biochimie / Centre Hospitalier Universitaire Sylvanus Olympio- Lomé, Togo.
[2]Laboratoire de Biologie Moléculaire et Immunologie (BIOLIM) / Faculté des Sciences de la Santé (FSS)- Université de Lomé, Togo.[3]Unité de Recherche en Immunologie et Immuno-modulation (UR2IM) /Laboratoire de Microbiologie et de Contrôle Qualité des Denrées Alimentaires (LAMICODA)/Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA)-Université de Lomé, Togo.[4]Service d'hépato-gastro-entérologie / Centre Hospitalier Universitaire Campus, Lomé-Togo.

Accepted 22 January, 2023

Abstract

Direct-acting antiviral (DAA) therapy is known to clear efficiently hepatitis C virus (HCV) infection. Inflammatory Th17 cells are increased in the blood and liver of chronic hepatitis C (CHC) patients and may be involved in the pathogenesis of CHC. Thus, the effect of DAAs on Th17 cells remains to be investigated. The present study was conducted in order to investigate immune response in former HCV positive individuals treated with Sofosbuvir/Velpatasvir. $CD4^+$ T cells from former HCV-infected subjects treated with Sofosbuvir/Velpatasvir and treatment-naïve chronic HCV-infected subjects were characterized by flow cytometry. Then, their functionality was assessed upon TCR activation in a cell culture system. Cytokines from plasma and cell culture supernatants were measured by ELISA sandwich method. We observed significantly reduced level of IL-17A-producing lymphocytes among Sofosbuvir/Velpatasvir treated individuals. In contrast, IL-17A level was significantly increased in treated compared to untreated group, upon TCR activation, as well as that of IFNγ and IL-5. Furthermore, reduced $CD4^+$IL-17A$^+$cells, $CD4^+$RORγt$^+$IL-17A$^+$cells and IL-17A were observed among treated individuals. This study provides that Sofosbuvir/Velpastasvir therapy reduced the Th17 inflammatory response responsible for the pathogenesis of HCV. IL-17A appears as a biomarker to control the efficacy of HCV treatment.

Keywords: Hepatitis C Virus, chronic hepatitis C, direct-acting antiviral, Sofosbuvir, Velpatasvir.

INTRODUCTION

Hepatitis C virus (HCV) infection is one of the main causes of morbidity and mortality worldwide (Brunner and Bruggmann 2021). World Health Organization (WHO) estimated that 58 million people have chronic HCV infection, with about 1.5 million new infections occurring annually. Around 290,000 deaths from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer), were registered in 2019 (WHO 2022).

The immune system of a chronically infected patient is severely

Corresponding author Email: malewekolou@hotmail.fr, mahkatawa@yahoo.fr

dysregulated (Urbanowicz, Zagożdżon et al. 2019). Several studies have described an upregulation of inflammatory biomarkers in serum of chronic hepatitis C patients, when compared with healthy individuals (Nishitsuji, Funami et al. 2013, Abdulkarim, Ahmed et al. 2016). Among the immune deregulations, those related to subtype Th17/interleukin-17 (IL-17) axis have been recognized as key immunopathological and prognostic elements in patients with HCV. Indeed, serum levels of IL-17 have been found significantly higher in chronic hepatitis C individuals compared to uninfected (Sousa, Oliveira et al. 2012, Askoura, Abbas et al. 2022).

Th17 population has recently been identified as a unique $CD4^+$ T-helper subset characterized by IL-17A production, as signature cytokine (Cui, Li et al. 2021). Furthermore, the cell function and development of Th17 cells are programmed by the retinoid orphan receptor gamma t (*RORγt*)/RORC2, a nuclear hormone receptor (Gege 2016, Capone and Volpe 2020). Those cells are important contributors to hepatic inflammation and liver cirrhosis. Indeed, Th17 cells are especially increased in the blood and liver of chronic hepatitis C (CHC) patients (Chang, Wang et al. 2012, Rios, Valva et al. 2017).

Revolutionary changes have been made in the treatment of HCV within the past 25 years, moving from a poorly tolerated oral and injectable drug combination to all oral, well-tolerated combination treatment options with virologic cures for over 90% of patients with chronic HCV (Gentile, Maraolo et al. 2015, Basyte-Bacevice and Kupcinskas 2020). Indeed, the introduction of Direct-Acting Antiviral Agents (DAAs) in 2014, has brought the treatment of chronic hepatitis C (CHC) into a new era. DAAs target specific nonstructural proteins of the virus, resulting in disruption of viral replication and infection (Mohamed, El-Toukhy et al. 2020). The WHO recommends treatment with pangenotypic DAAs for all adults, adolescents and children with chronic hepatitis C from the age of 3 years (WHO 2022). Thus the combination Sofosbuvir/Velpatasvir, the first pangenotypic DAAs, appeared to be safe and effective for the treatment of HCV genotypes 1, 2, 3, 4, 5, and 6 (Chahine, Sucher et al. 2017, Huang, Hsieh et al. 2021).

In Togo, we previously estimated the burden of HCV at 5.64% (Kolou, Nadjir et al. 2018). Also, DAAs have been found effective in Togolese patients, with a rate of sustained virological response above 90% (Lawson-Ananissoh, Bagny et al. 2019). However, the effect of DAAs on Th17 cells remains to be explored. The present study has been conducted in order to investigate immune response in former HCV positive individuals treated with the combination therapy Sofosbuvir/Velpatasvir.

MATERIALS AND METHODS

Study Population

From February to July 2021, 12 subjects were recruited at hepato-gastroenterology service of “Centre Hospitalier Universitaire Campus” (CHU Campus) in Lomé, Togo. A semi-structured questionnaire was used to obtain participants data. The data collected included demographic and socioeconomic parameters, screening associated signs and symptoms, risk factors and treatment received.

Patients included in this study were: 1) chronic HCV-infected patients (n=8); and 2) patients treated with sofosbuvir/velpatasvir and showing sustained virologic response (SVR) with an undetectable viral load less than one year (n=4). HCV-infection was defined by detectable serum anti-HCV antibodies using an electrochemiluminescence immunoassay and/or HCV RNA detected by qualitative reverse transcription-polymerase chain reaction (RT-PCR). No patients received anti-HCV agent treatment before entering the study. Concomitant hepatitis B virus (HBV), hepatitis D virus (HDV), human immunodeficient virus (HIV) infections, autoimmune liver disease and liver cirrhosis were excluded.

Sample collection and preparation

For each participant, whole blood samples were collected in ethylene diamine tetra acetic acid (EDTA) tubes. Then plasma obtained after 5 mn centrifugation at 2000 rpm was stored at -20°C for further analyses.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by the Ficoll density gradient centrifugation method as described by Tchopba *et al.* (Tchopba, Katawa et al. 2021). In brief, 20 mL of whole blood was diluted in 15 mL of Dulbecco’s phosphate buffered saline (DPBS) and carefully added to 15 mL of Pancoll separating solution (PAN-Biotech). After 20 min of centrifugation at 2,000 rpm, the white layer of PBMCs was collected and washed 3 times in Roswell Park Memorial Institute medium supplemented with gentamicin 50 µg/mL, penicillin-streptomycin 100 µg/mL, L-glutamine 2 mM/mL (RPMI+++), and fetal bovine serum (FBS) at 10% (PAN-Biotech). Then cells were suspended in culture medium (RPMI supplemented + 10%FBS) at the concentration of $2*10^5$ cells/µl, cells were counted and their viability assessed by Trypan blue exclusion method.

Phenotyping peripheral blood mononuclear cells

Cells were cultured in RPMI supplemented + 10% FBS, in 96-wells U-bottom plates (Greiner Bio-One, Kremsmunster, Autriche), as follow: 100 µl of PBMCs suspension per well were stimulated with 50 µl of 1X cell stimulation cocktail (phorbol 12-myristate 13-acetate [PMA], Ionomycin, of Brefeldin A and Monensin) (eBioscience), and incubated at 37°C in presence of 5% CO_2 for 6 h as described by Tchopba *et al.* (Tchopba, Katawa *et al.* 2021). Thereafter, cells were harvested and stained as described by Katawa *et al.* (Katawa, Layland et al. 2015). Brief, cells were stained with anti-human CD4-APC (clone A161A1) and incubated for 30 mins (4°C). After employing Fix-Perm reagent (eBioscience), cells were

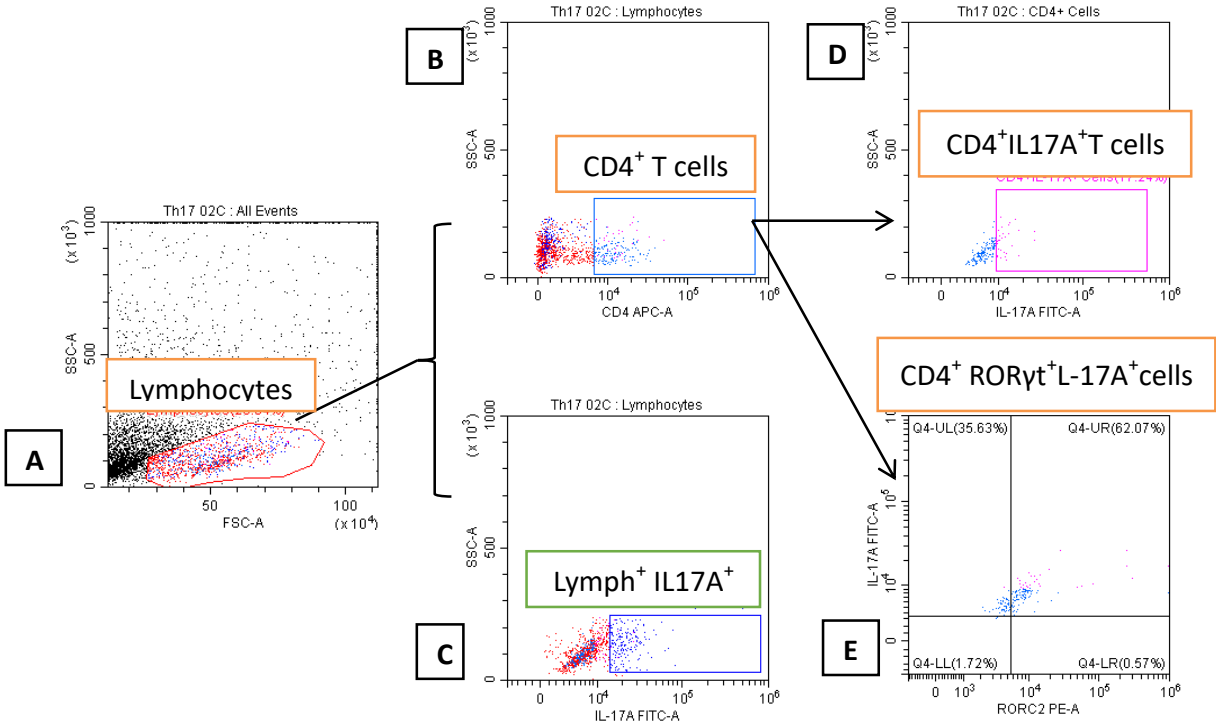

Figure 1: Gating strategy for assessing IL-17A producing cells: (A)Lymphocytes gate; (B) $CD4^+$lymphocytes (Th) gate among lymphocytes; (C) IL-$17A^+$ lymphocytes, (D) and (E) are respectively $CD4^+$IL-$17A^+$ Lymphocytes and $CD4^+$RORγ t^+IL-$17A^+$ lymphocytes.

blocked with Fc block (human TruStainFcX) and then incubated at 4°C for 30 mins with 1) Anti-human-RORγt-PE (clone AFKJS-9) and -IL-17A-FITC (clone BL168); 2) Anti-human T-bet-PE (clone 4B10) and -IFN-γ-FITC (clone 4S.B3); 3) Anti-human-GATA3-PE (clone 16E10A23) and -IL-4-FITC (clone MP4-25D2); 4) Anti-human-FoxP3-FITC (clone 206D) and -IL-10-PE (clone JES3-9D7). After further washing, cells were re-suspended in fix-perm buffer (eBioscience). Unstained and single stained cells were used as negative and positive controls respectively. Data were acquired on a flow cytometer (Cytoflex, Beckman Coulter, Brea, California, USA) and analyzed using CytExpert 2.1. Software (Beckman Coulter, Brea, California, USA). To avoid spectral overlap, fluorescence compensation was done using VersaComp Antibody CaptureBead Kit (Beckman Coulter). All reagents were obtained from BioLegend. The gating strategy is shown on Figure 1.

T-cell receptor (TCR) activation

In 96-well U-plates (Greiner Bio-One), $2*10^5$ PBMCs/well were left unstimulated or stimulated in duplicate with 0.5 µl of microbeads conjugated to monoclonal antibodies anti-CD3 and anti-CD28 (Dynabeads Human T-cells Activator CD3/CD28, Thermo Fisher Scientific, Watham, USA) in culture medium. Cells were then incubated at 37°C under 5% CO_2 for 24 hours. Then, supernatant was collected and stored at -20°C for further cytokines measurement.

Cytokines assays

Levels of cytokines, IL-6, TNFα, IFNγ, IL-4, IL-5, IL-10 and IL-17A were measured in plasma and cell cultures supernatants by ELISA sandwich technique, using ELISA kits (Affymetrixe Bioscience Inc., San Diego, CA, USA) following manufacturers instructions. Cytokines concentrations were measured at 450 nm on a HumaReader HS plate reader (Human Diagnostics Worldwide, Wiesbaden, France).

Statistical analysis

Statistical analysis were performed using GraphPad Prism version 5.02 (GraphPad Software Inc, La Jolla, USA).As we had small size (n<30), the Mann-Whitney U test was used to compare the 2 groups according to Sofosbuvir/Velpatasvir treatment (untreated vs treated). p-value< 0.05 was considered as significant.

Table 1: Characteristics of the study population.

Characteristics	Untreated HCV+(n = 08)	Treated subjects (n = 04)
Age (years) [min – max]	55,5 [37–76]	54 [41–67]
Sex ratio (M/F)	3	1
History of blood transfusion n (%)		
No	8 (100%)	3 (75%)
Yes	0 (0%)	1 (25%)
SOF /VEL therapy for 12 weeks	N/A	4 (100%)
HCV gnotype n (%)		
GT 2	N/A	4 (100%)

N/A: Not Applicable; GT 2: genotype 2; SOF: Sofosbuvir; VEL: Velpatasvir; M: Male; F: Female.

Ethics statement

This study was approved by the ethical board ("Comité de Bioéthique pour la Recherche en Santé", CBRS) of the Ministry of Health of Togo (N° 014/2021/CBRS). All participants were adults, and all of them provided written consent before collection of samples.

RESULTS

Study population characteristics

Participants to this study were 8 untreated HCV+ and 4 treated (former HCV+) patients. Their data are presented in Table 1.
Untreated HCV+ subjects had a mean age of 55.5 [37–76] years and 75% were male, while the treated were homogeneous according to the gender with a mean age of 54 [41–67] years. The majority of participants never received blood transfusions before their infection. All treated individuals were infected with HCV genotype 2 and were treated with SOFOVEL® (Sofosbuvir 400 mg + Velpatasvir 100 mg) -based treatment for 12weeks.

Reduced IL-17A-producing lymphocytes among treated individuals

Characterization of lymphocyte subsets revealed that IL17A-producing lymphocytes population was significantly low in treated subjects than untreated subjects (p=0.0364) (Fig. 2A). Moreover, a decreased $CD4^+IL\text{-}17A^+$cells, $CD4^+IFN\gamma^+$cells, and $CD4^+IL\text{-}10^+$cells was observed.; the same trend was observed on $CD4^+ROR\gamma t^+IL\text{-}17A^+$, $CD4^+Tbet^+IFN\gamma^+$ and $CD4^+FoxP3^+IL\text{-}10^+$, however, there was no statistically significant difference (Fig. 2). Furthermore, measurement of plasmatic cytokine showed no statistically significant difference on IL-17A, IFNγ, IL-6 and TNFα concentrations between the 2 groups (Fig. 3).

High expression of IL-17A upon TCR activation on the PBMCs of treated subjects

T lymphocytes functionality was studied by investigating their capacity to produce cytokines after activation of PBMCs with αCD3/CD28. It was observed, after stimulation of the TCR in both groups separately, a significant production of IL-17A, IFNγ and IL-10 (Fig.4). Moreover, comparing cytokines production between untreated and treated, we found a significant high level of IL-17A, IFNγ and IL-10 among treated than in treatment-naïve subjects (Table 2).

DISCUSSION

HCV infection is a human infection with a dichotomous outcome (viral clearance versus persistence) (Binder and Thimme 2020). The introduction of DAAs has revolutionized

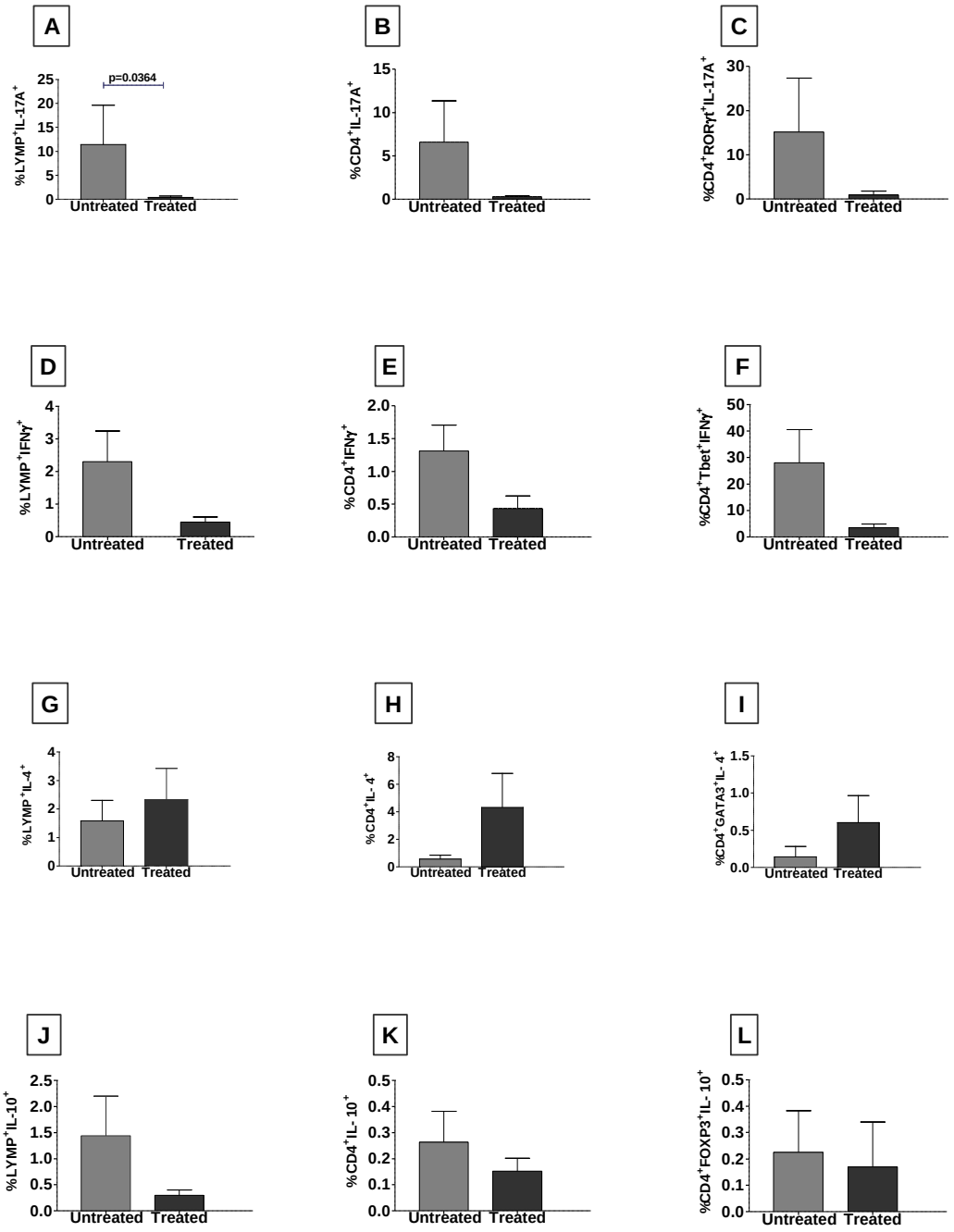

Figure 2: Lymphocytes profile. Peripheral blood mononuclear cells (PBMCs) of untreated (n=8) and treated (n=4) subjects were activated with cell stimulation cocktail, stained and characterized by flow cytometry to determine the frequencies of (A) Lymphocytes producing IL-17A, (B) $CD4^+IL\text{-}17A^+$, (C) $CD4^+ROR\gamma t^+IL\text{-}17A^+$, (D) Lymphocytes producing $IFN\gamma$, (E) $CD4^+IFN\gamma^+$, (F) $CD4^+Tbet^+IFN\gamma^+$, (G) Lymphocytes producing IL-4, (H) $CD4^+IL\text{-}4^+$, (I)$CD4^+GATA3^+IL\text{-}4^+$, (J) Lymphocytes producing IL-10, (K)$CD4^+IL\text{-}10^+$, (L)$CD4^+FoxP3^+IL\text{-}10^+$.Bars indicate the percentages of cells, expressed as median and IQR. A Mann-Whitney U test was performed to compare treated and untreated groups. $p<0.05$ is considered as significant.

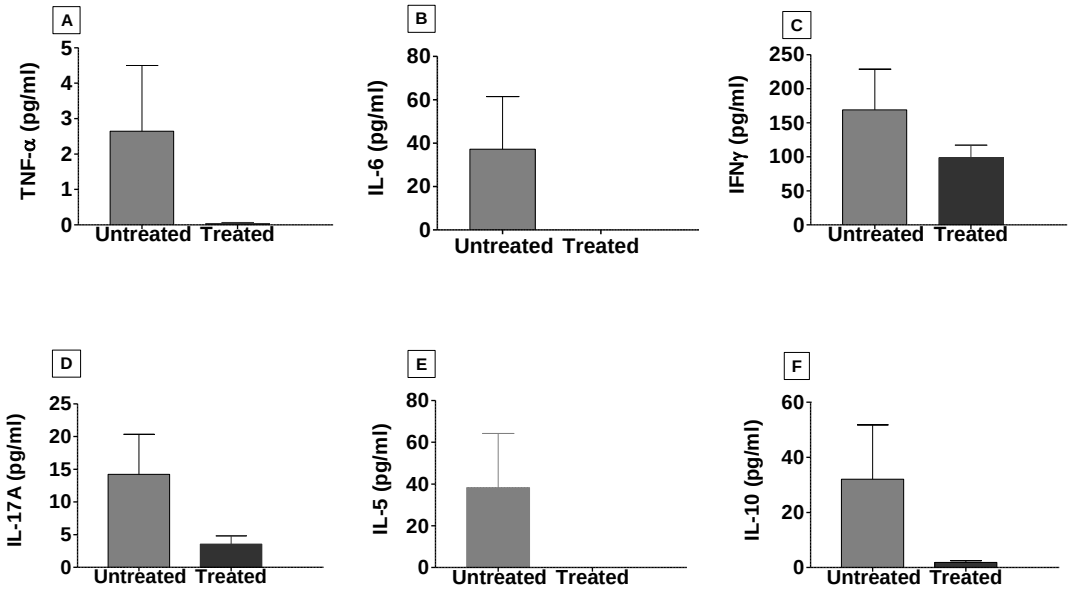

Figure 3: Systemic cytokines profile. Cytokines were measured in the plasma of untreated (n=8) and treated (n=4) subjects. Graphs show plasma levels of (A) TNF α, (B) IL-6, (C) IFN-γ, (D) IL-17A, (E) IL-5 and (F) IL-10 in untreated and treated individuals. Bars indicate the concentration of cytokines in each group. Data are expressed as median and IQR. A Mann-Whitney U test was performed to compare treated and untreated groups. $p<0.05$ is considered as significant.

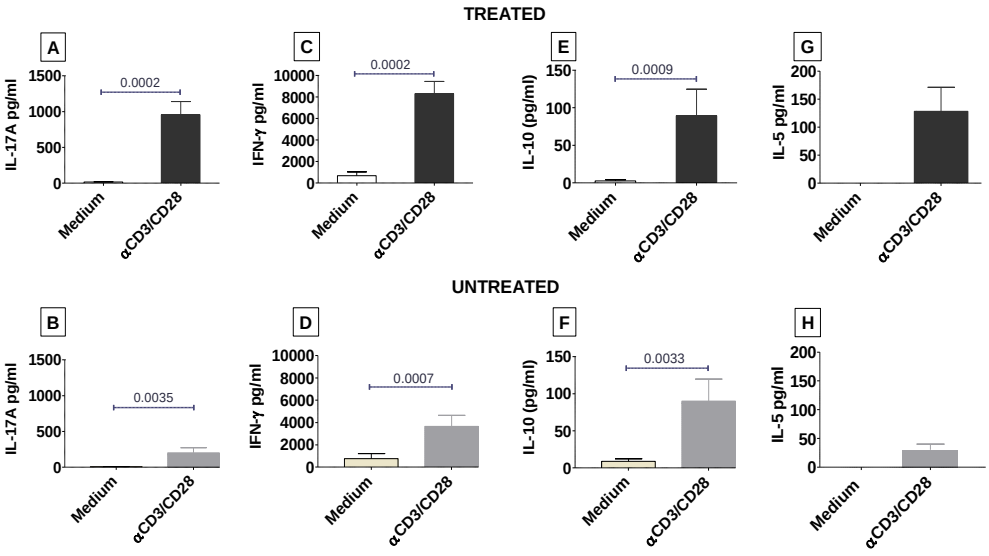

Fig. 4: **Cytokines profile in each group upon TCR activation.** PBMCs of untreated (n=8) and treated (n=4) subjects were stimulated with anti-CD3/anti-CD28 microbeads in a cell culture system for 24h. Then supernatants were collected and cytokines measured by ELISA sandwich method. Graphs show the levels of cytokines in each group (treated and untreated): (A) and (B) IL-17A; (C) and (D) IFN-γ, (E) and (F) IL-10, (G) and (H) IL-5 Bars indicate the concentration of cytokines in each group. Data are expressed as median and IQR. A Mann-Whitney U test was performed to compare treated and untreated groups. $p<0.05$ is considered as significant.

Table 2: **Comparison of cytokines concentration between untreated vs treated upon TCR activation**. Results are expressed as median [Q1 ;Q3]. Mann-Whitney U test was used to compare the data. p-value<0.05 is considered as significant.

	Cytokines concentration (pg/ml)		**p-value**
	Untreated	**Treated**	
IL-17A	50,83 [1,21; 468,8]	905,8 [470,2; 1503]	0,0096
IFNγ	1893 [780,8; 7453]	8460 [4897; 11380]	0,011
IL-5	8,52 [0,0; 56,43]	87,75 [35,93; 252,0]	0,0019
IL-10	42,14 [8,18; 118]	54,90 [17,61; 149,6]	0,48

the treatment of HCV. Indeed, the efficacy of Sofosbuvir/Velpatasvir on the clearance of HCV has been demonstrated, furthermore its effect on the Th17 immune response is elucidated in this study.

We characterized lymphocyte subpopulations and their functionality in chronic treatment-naïve HCV-infected patients and patients who received Sofosbuvir/Velpatasvir treatment, showing sustained virologic response (SVR) with an undetectable viral load less than one year. We found that all recovered patients have been infected with GT2, confirming that GT2 is the most frequent genotype in Togo as shown by Anyovi *et al.* (Anyovi, Soulier et al. 2021). In addition, the majority of individuals never received blood transfusion, thus the main route of transmission would not be related to blood transfusion. Hepatitis C is admittedly a bloodborne infection, but besides unscreened blood transfusions, the virus can be contracted through exposure to blood from unsafe injection practices, unsafe health care, injection drug use and sexual practices that lead to exposure to blood(WHO 2022).

Phenotyping lymphocytes, we found that the percentage of IL-17A-producing lymphocytes were significantly low in treated individuals. IL-17A being the signature cytokine of Th17 cells, this led us to search for the potential source of IL-17A by characterizing $CD4^+$T cells. As result, we observed a low percentage of $CD4^+IL\text{-}17A^+$ and $CD4^+ROR\gamma t^+IL\text{-}17A^+$ cell population. These results corroborate with those of El-Khier *et al.*, who reported a significant decrease of Th17 population in patients successfully treated with DAAs based on Sofosbuvir (Abou El-Khier, Elhammady et al. 2018). The non-significant low expression observed in our study may be due to the low sample size or could be due to host (genetics, immune response) and/or viral factors (genotype and viral load) that may interfere with the response after treatment.

Interestingly, data from systemic cytokine profile showed lower level of pro-inflammatory cytokines such as IL-17A, TNFα, IL-6 and IFNγ in treated individuals compared to untreated patients. This result is consistent with those reported by some authors (Saraiva, do Rosário et al. 2018, Khera, Du et al. 2021), demonstrating that treatment with DAAs normalize the altered signature of HCV infected and repair the inflammatory mediator balance. Thus Sofosbuvir/Velpatasvir therapy, while achieving virus clearance, restore immune inflammatory balance. We also observed a significantly higher production of cytokines such as IL-17A, IFNγ and IL-5 in treated subjects compared to untreated infected subjects after TCR activation. From a functional point of view, this suggests the existence of a cellular immune response after viral clearance (Thimme 2021). Indeed, the rapid clearance of HCV by therapy with Interferon (IFN)-free direct-acting antiviral (DAA) may result in a rapid clearance of HCV from infected patients which is sustained even after the end of treatment (Aregay, Owusu Sekyere et al. 2019).

This study had some limitations, one being that we were unable, due to budgetary constraints, to carry out the detection of HCV RNA in controls (treated subjects). In addition, the study groups used were relatively small; increasing sample size may allow to discern statistically significant differences.

CONCLUSION

Our study demonstrated that Sofosbuvir/Velpatasvir therapy reduces the level of IL-17A inflammatory response. Thus, IL-17A appears as a biomarker of treatment efficacy. Also, we showed the existence of a Th17 cellular response following activation of PBMCs of treated subjects. Further studies may investigate on memory and activation cell markers by increasing the sample size.

Authors Contributions:

G.K. and **K.M.** Conceived and designed the study, contributed reagents/materials.

B.A. and **R.K.** Carried out the clinical investigations, recruited patients.

G.T., A.O.M. and **P.E.T.** Recruited patients and performed the experiments.

G.K., C.N.T. and **K.M.** Analyzed and interpreted the data, prepared the draft of the manuscript and all authors participated in writing-review and editing.

ABBREVIATIONS:

IL- : Interleukin-

TNFα: Tumor Necrosis Factor

IFNγ Interferon gamma
ELISA: Enzyme linked immunosorbent assay
TCR: T-cell receptor

REFERENCES

Abdulkarim, A., A. Ahmed, S. Zahid, A. Khalid, A. Ayman and A.-h. Waleed (2016). "Assessment of pro-inflammatory cytokines in sera of patients with hepatitis C virus infection before and after anti-viral therapy." The Journal of Infection in Developing Countries**10**(10).

Abou El-Khier, N. T., D. Elhammady, M. M. Arafa, D. Shahin, E. Eladl, N. K. Abousamra, O. Sharaf-Eldeen, G. Shaker and M. E. Esmael (2018). "Th17 and IL-17 as Predictors of Hepatic Inflammation in Patients with Chronic Hepatitis C Virus Infection and Treated With Direct Antiviral Therapy." Egypt J Immunol**25**(2): 61-74.

Anyovi, F., A. Soulier, L. Poiteau, R. Soliman, S. D. Karou, J. Simpore, S. Chevaliez and G. Shiha (2021). "Genetic Variability of NS5B Region of Hepatitis C Virus in Togo." Medical Journal of Viral Hepatitis**5.3**(3): 8-13.

Aregay, A., S. Owusu Sekyere, K. Deterding, K. Port, J. Dietz, C. Berkowski, C. Sarrazin, M. P. Manns, M. Cornberg and H. Wedemeyer (2019). "Elimination of hepatitis C virus has limited impact on the functional and mitochondrial impairment of HCV-specific CD8+ T cell responses." Journal of Hepatology**71**(5): 889-899.

Askoura, M., H. A. Abbas, H. Al Sadoun, W. H. Abdulaal, A. S. Abu Lila, K. Almansour, F. Alshammari, E.-S. Khafagy, T. S. Ibrahim and W. A. H. Hegazy (2022). "Elevated Levels of IL-33, IL-17 and IL-25 Indicate the Progression from Chronicity to Hepatocellular Carcinoma in Hepatitis C Virus Patients." Pathogens**11**(1): 57.

Basyte-Bacevice, V. and J. Kupcinskas (2020). "Evolution and Revolution of Hepatitis C Management: From Non-A, Non-B Hepatitis Toward Global Elimination." Digestive Diseases**38**(2): 137-142.

Binder, B. and R. Thimme (2020). "CD4+ T cell responses in human viral infection: lessons from hepatitis C." J Clin Invest**130**(2): 595-597.

Brunner, N. and P. Bruggmann (2021). "Trends of the Global Hepatitis C Disease Burden: Strategies to Achieve Elimination." J Prev Med Public Health**54**(4): 251-258.

Capone, A. and E. Volpe (2020). "Transcriptional Regulators of T Helper 17 Cell Differentiation in Health and Autoimmune Diseases." Frontiers in Immunology**11**.

Chahine, E. B., A. J. Sucher and B. A. Hemstreet (2017). "Sofosbuvir/Velpatasvir: The First Pangenotypic Direct-Acting Antiviral Combination for Hepatitis C." Ann Pharmacother**51**(1): 44-53.

Chang, Q., Y. K. Wang, Q. Zhao, C. Z. Wang, Y. Z. Hu and B. Y. Wu (2012). "Th17 cells are increased with severity of liver inflammation in patients with chronic hepatitis C." J Gastroenterol Hepatol**27**(2): 273-278.

Cui, G., Z. Li, J. Florholmen and R. Goll (2021). "Dynamic stromal cellular reaction throughout human colorectal adenoma-carcinoma sequence: A role of TH17/IL-17A." Biomed Pharmacother**140**: 111761.

Gege, C. (2016). "Retinoid-related orphan receptor gamma t (ROR γ t) inhibitors from Vitae Pharmaceuticals (WO2015116904) and structure proposal for their Phase I candidate VTP-43742." Expert Opin Ther Pat**26**(6): 737-744.

Gentile, I., A. E. Maraolo, A. R. Buonomo, E. Zappulo and G. Borgia (2015). "The discovery of sofosbuvir: a revolution for therapy of chronic hepatitis C." Expert Opinion on Drug Discovery**10**(12): 1363-1377.

Huang, Y.-T., Y.-Y. Hsieh, W.-M. Chen, S.-Y. Tung, K.-L. Wei, C.-H. Shen, K.-C. Chang, C.-K. Lu, C.-W. Yen, S.-N. Lu, C.-H. Hung and T.-S. Chang (2021). "Sofosbuvir/velpatasvir is an effective treatment for patients with hepatitis C and advanced fibrosis or cirrhosis in a real-world setting in Taiwan." BMC Gastroenterology**21**(1): 259.

Katawa, G., L. E. Layland, A. Y. Debrah, C. Von Horn, L. Batsa, A. Kwarteng, S. Arriens, W. D. Taylor, S. Specht, A. Hoerauf and T. Adjobimey (2015). "Hyperreactive onchocerciasis is characterized by a combination of Th17-Th2 immune responses and reduced regulatory T cells." PLoS Negl Trop Dis**9**(1): e3414.

Khera, T., Y. Du, D. Todt, K. Deterding, B. Strunz, S. Hardtke, A. Aregay, K. Port, M. Hardtke-Wolenski, E. Steinmann, N. K. Björkström, M. P. Manns, J. Hengst, M. Cornberg, H. Wedemeyer and H. A. H. I. S. Group (2021). "Long-Lasting Imprint in the Soluble Inflammatory Milieu Despite Early Treatment of Acute Symptomatic Hepatitis C." The Journal of Infectious Diseases **226**(3): 441-452.

Kolou, M., L. Nadjir, F. Anyovi, G. Katawa, B. Abaltou and M. Salou (2018). "Seroprevalence des hépatites virales B et C au sein de la population générale de Lomé ."J. Rech. Sci. Univ. Lomé**20** (1).

Lawson-Ananissoh, L., A. Bagny, O. Bouglouga, L. Kaaga, R. Yakoubou, L. Kogoe and D. Redah (2019). "Effectiveness of Treatment of Chronic Viral Hepatitis C by Direct-Acting Antivirals in Togo." Open Journal of Gastroenterology**09**: 125-133.

Mohamed, A. A., N. E. R. El-Toukhy, E. M. Said, H. M. R. Gabal, H. AbdelAziz, W. Doss, H. El-Hanafi, H. H. El Deeb, S. Mahmoud, M. Elkadeem, H. S. Shalby and S. Abd-Elsalam (2020). "Hepatitis C Virus: Efficacy of New DAAs Regimens." Infect Disord Drug Targets**20**(2): 143-149.

Nishitsuji, H., K. Funami, Y. Shimizu, S. Ujino, K. Sugiyama, T. Seya, H. Takaku and K. Shimotohno (2013). "Hepatitis C virus infection induces inflammatory cytokines and

chemokines mediated by the cross talk between hepatocytes and stellate cells." J Virol**87**(14): 8169-8178.

Rios, D. A., P. Valva, P. C. Casciato, S. Frias, M. Soledad Caldirola, M. I. Gaillard, L. Bezrodnik, J. Bandi, O. Galdame, B. Ameigeiras, D. Krasniansky, C. Brodersen, E. Mullen, E. N. Matteo and M. V. Preciado (2017). "Chronic hepatitis C liver microenvironment: role of the Th17/Treg interplay related to fibrogenesis." Sci Rep**7**(1): 13283.

Saraiva, G. N., N. F. do Rosário, T. Medeiros and P. E. C. Leite (2018). "Restoring Inflammatory Mediator Balance after Sofosbuvir-Induced Viral Clearance in Patients with Chronic Hepatitis C." **2018**: 8578051.

Sousa, G. M., I. S. Oliveira, L. J. O. Andrade, M. L. B. Sousa-Atta, R. Paraná and A. M. Atta (2012). "Serum levels of Th17 associated cytokines in chronic hepatitis C virus infection." Cytokine**60**(1): 138-142.

Tchopba, C. N., G. Katawa, E. Padaro, P. E. Tchadié, S. D. Karou, A. Vovor and Y. Ameyapoh (2021). "Systemic T Cell Subsets and Cytokines in Patients With Homozygous Sickle Cell Disease and Asymptomatic Urinary Tract Infections in Togo." Ochsner Journal**21**(2): 163-172.

Thimme, R. (2021). "T cell immunity to hepatitis C virus: Lessons for a prophylactic vaccine." Journal of Hepatology**74**(1): 220-229.

Urbanowicz, A., R. Zagożdżon and M. Ciszek (2019). "Modulation of the Immune System in Chronic Hepatitis C and During Antiviral Interferon-Free Therapy." **67**(2): 79-88.

WHO (2022). Hepatitis C. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c. Accessed on 14th December 2022.