International Journal of Infectious Diseases 45 (2016) 5–12

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Dynamic changes in CD45RA^{-}Foxp3^{high} regulatory T-cells in chronic hepatitis C patients during antiviral therapy

Zhiqin Li [a,b,1], Yu Ping [b,c,1], Zujiang Yu [a], Meng Wang [b], Dongli Yue [b], Zhen Zhang [b], Jianbin Li [d], Bin Zhang [b,e], Xuezhong Shi [f,**], Yi Zhang [b,c,g,*]

[a] Department of Infectious Disease, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[b] Biotherapy Center, the First Affiliated Hospital of Zhengzhou University, No. 1 Jianshe Road, Zhengzhou 450052, Henan, China
[c] School of Life Sciences, Zhengzhou University, Zhengzhou, Henan, China
[d] Henan Red Cross Blood Center, Zhengzhou, Henan, China
[e] Department of Medicine–Division of Hematology/Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
[f] Department of Epidemiology and Biostatistics, College of Public Health, Zhengzhou University, No. 100 Kexue Avenue, Zhengzhou 450000, Henan, China
[g] Department of Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 27 January 2016
Accepted 6 February 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Treg cells
HCV antiviral therapy
IFN-α
Ribavirin

SUMMARY

Objectives: CD4^{+}Foxp3^{+} regulatory T-cells (Treg) are known to accumulate under certain pathological conditions. This study was conducted to evaluate the characteristics of and dynamic changes in Treg cells in chronic hepatitis C (CHC) patients during antiviral therapy.
Methods: One hundred and forty-five subjects were enrolled in this study, including 105 CHC patients and 40 healthy donors. The phenotypes and functions of Treg cells were analyzed by flow cytometry.
Results: A significant elevation in Treg cells was observed in the peripheral blood of CHC patients compared with healthy donors. Interestingly, compared with non-suppressive Treg (non-Treg) and resting Treg (rTreg) cells, activated Treg (aTreg) cells expressed higher levels of ectonucleotidase, CD39, and CD73. After treatment with interferon alpha (IFN-α) and ribavirin (RBV) in vitro, the frequencies of total Treg cells and aTreg cells in peripheral blood mononuclear cells (PBMC), as well as the levels of transforming growth factor beta (TGF-β) secreted by aTreg and non-Treg cells, were significantly decreased. Importantly, it was found that levels of aTreg cells in patients with a sustained virological response (SVR) were lower than in relapsed patients, suggesting that a high frequency of aTreg cells might be associated with a poor clinical outcome in HCV infection.
Conclusion: These results demonstrate a decreasing trend in aTreg cells, which express higher levels of CD39, CD73, and TGF-β, in SVR patients during antiviral therapy.

1. Introduction

Hepatitis C virus (HCV) is one of the causes of chronic liver disease, and HCV infection results in the development of chronic liver diseases such as cirrhosis and hepatocellular carcinoma.[1,2] One of the reasons for the high rates of chronic HCV infection is immunosuppression, which may be specifically mediated by the regulatory T-cells (Treg cells).[3,4] The accumulation of Treg cells plays a pivotal role in suppressing the antiviral effector T-cells that are essential for viral clearance.[5,6]

Recently, a growing body of evidence has indicated that Treg cells may contribute critically to the induction of immune tolerance and affect the proliferation, differentiation, and cytokine secretion of HCV-specific lymphocytes.[7–11] Some research groups have reported that the depletion of Treg cells increases the frequency of autoimmune reactions and enhances the development of autoimmune disease.[12–15] Treg cells show several changes in number and function during the course of many diseases, such as those caused by HCV, HIV, and herpes simplex, and autoimmune diabetes, etc.[14–16] Some studies have reported that CD45RA could

* Corresponding author. Tel.: +86-371-66295320; fax: +86-371-66295625.
** Corresponding author. Tel.: +86-371-67781728.
E-mail addresses: xzshi@zzu.edu.cn (X. Shi), yizhang@zzu.edu.cn (Y. Zhang).
[1] Zhiqin Li and Yu Ping contributed equally to this article.

http://dx.doi.org/10.1016/j.ijid.2016.02.006

Table 1
Characteristics of healthy donors and patients enrolled in this study[a]

Characteristics	Healthy donors (n = 40, F/M 25/15)	HCV-infected patients (n = 105, F/M 60/45)
Age, years	46 (40–59)	47 (40–60)
ALT level, IU/ml	Negative data	36 (22–50)
AST level, IU/ml	Negative data	36 (36–48)
ALP, IU/ml	Negative data	59 (55–91)
Albumin level, g/l	Negative data	39.7 (35.60–47.20)
Albumin to globulin, g/l	Negative data	1.37 (1.18–1.54)
HCV genotype, 1b/2a	Negative data	62/43
HCV RNA, $\log_{10}$ IU/ml	Negative data	6.10 (5.70–6.16)

HCV, hepatitis C virus; F, female; M, male; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.
[a] Results are presented as the median (range).

be used to differentiate resting Treg (rTreg) cells, non-suppressive Treg (non-Treg) cells, and activated Treg (aTreg) cells.[17,18] $CD4^+CD45RA^+$ forkhead box P3 (Foxp3)^{low} Treg cells – antigen-experienced cells – are named rTreg cells. $CD4^+CD45RA^-Foxp3^{high}$ Treg cells, named aTreg cells, have strongly suppressive function and proliferating ability in vivo. $CD4^+CD45RA^-Foxp3^{low}$ Treg cells are non-Treg cells, which have the function of cytokine secretion.[19,20]

The combination therapy of interferon alpha (IFN-α) and ribavirin (RBV) has shown positive outcomes in more than 80% of patients with acute HCV infections and in 50% of patients with chronic HCV infections. To date, it is unclear how Treg cells change in chronic hepatitis C (CHC) patients during IFN-α and RBV therapy. To further explore this issue, the present study was performed to determine whether or not Treg cells take part in the immune microenvironment of CHC patients during antiviral therapy. The expression of inhibitory markers and cytokines, such as CD39, CD73, and transforming growth factor beta (TGF-β), were analyzed in three subsets of Treg cells. It was found that $CD4^+Foxp3^+$ Treg cells presented a decreasing trend in patients with a sustained virological response (SVR) during antiviral therapy. Consequently, the data demonstrated that patients could have a beneficial immune microenvironment after treatment with IFN-α and RBV.

2. Patients and methods

2.1. Ethics statement

The study was approved by the First Affiliated Hospital of Zhengzhou University. The samples were collected after written informed consent was obtained. The whole consent procedure was

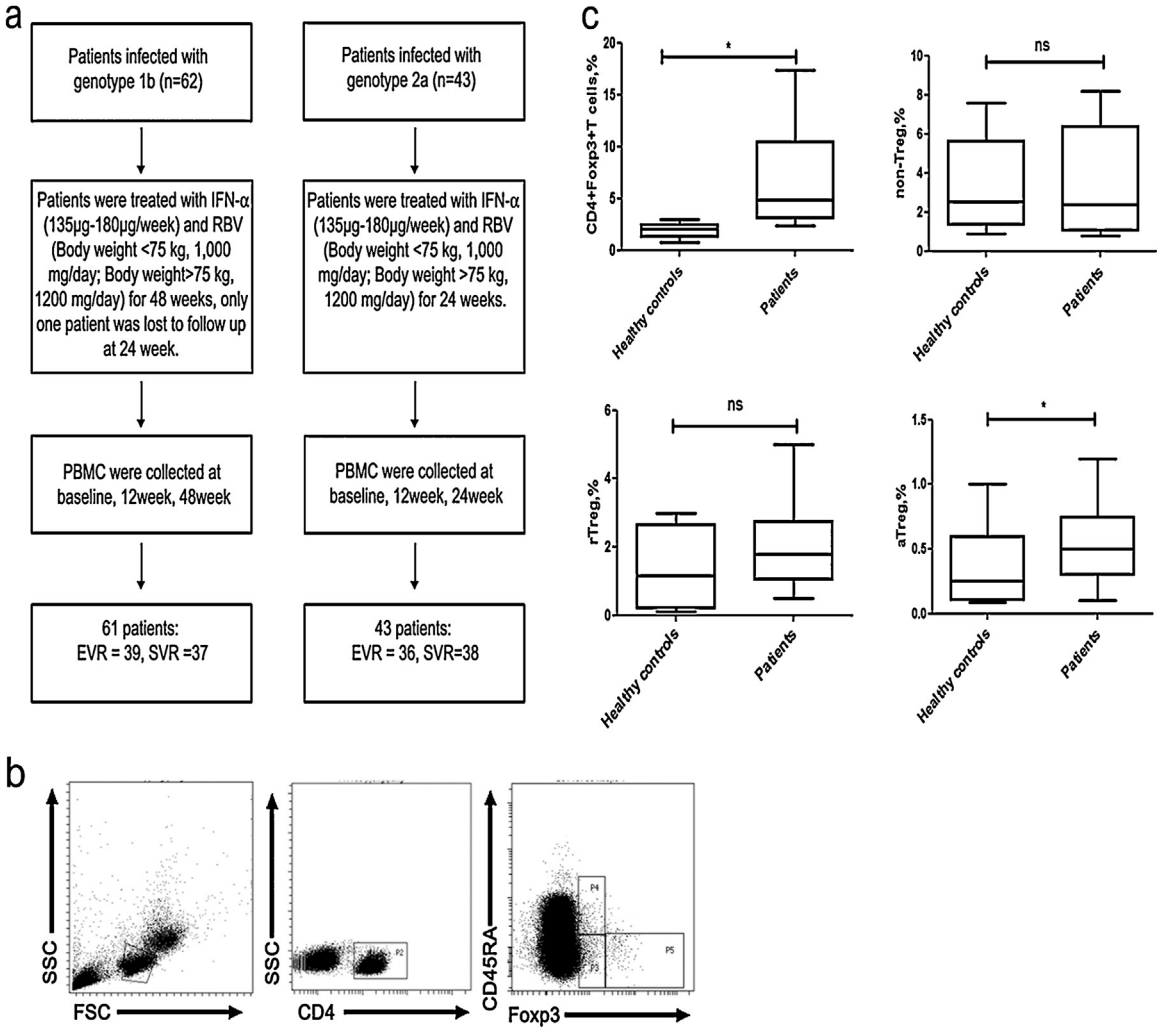

Figure 1. CHC patients had higher amounts of Treg cells, especially aTreg. (A) Study design: patients infected with genotypes 1b and 2a HCV were treated with IFN-α and RBV for 48 weeks and 24 weeks, respectively. (B) Representative dot plots of the percentages of Treg cells and three subsets in peripheral $CD4^+$ T-cells in healthy donors and chronic HCV-infected patients. (C) The percentages of $CD4^+Foxp3^+$ Treg cells and aTreg cells were decreased in patients compared with healthy controls. These data were measured by flow cytometry and are presented as percentages. Values are the mean of two independent experiments ± stand error of the mean. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (CHC, chronic hepatitis C; HCV, hepatitis C virus; IFN-α, interferon alpha; RBV, ribavirin.).