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ORIGINAL ARTICLE

Direct-acting antiviral therapy enhances total CD4+ and CD8+ T-cells responses, but does not alter T-cells activation among HCV mono-infected, and HCV/HIV-1 co-infected patients

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KEYWORDS
Hepatitis C virus;
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Direct-acting antiviral;
T-cell;
Immune activation;
Intracellular cytokine assay

Summary
Aim: Chronic immune activation and poor T-cell immune response are strongly associated with disease progression and pathogenesis of both hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-1 infections. Little is known about the impact of anti-HCV Interferon (IFN)-free direct-acting antiviral (DAA) therapy on the systemic T-cells activation and patterns of peripheral T-cells producing pro-inflammatory cytokines.
Patients and methods: Forty-five subjects including 18 HCV mono-infected, 17 HCV/HIV-1 co-infected patients under antiretroviral therapy (ART), and 10 healthy controls (HCs) were recruited. Blood samples were collected at baseline (T0) and 12 weeks after the end of DAA therapy (T1). Cell phenotypes (CD3, CD4, CD8), activation markers (CD38 and HLA-DR), and frequency of IFN-γ, interleukin (IL)-17, and IL-22 producing CD4+ and CD8+ T-cells were measured by flow cytometry. Plasma levels of related cytokines were also measured by enzyme-linked immunosorbent assay (ELISA).

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Results: Both HCV, and HCV/HIV-1 patients before and after therapy, showed significant higher percentages of any T-cell subset expressing CD38 and/or HLA-DR compared to HCs. No differences were observed in T-cells activation at T1 compared to T0 in patient groups, and when HCV patients were compared to HCV/HIV-1 group ($P > 0.05$). After therapy, the potential of total circulating T helper (Th) and T cytotoxic (Tc) cells producing IFN-γ, IL-17, and IL-22 were increased. Plasma level of IFN-γ at baseline was showed difference compared to HCs, and significantly reduced after therapy ($P < 0.05$).
Conclusion: Total T-cells immune response enhances after therapy, however, the state of immune activation may remain elevated for a longtime after the end of treatment and contribute to post-Sustained Virologic Response (SVR) consequences.

Abbreviations

HCV	hepatitis C virus
HIV-1	human immunodeficiency virus-1
DAA	direct-acting antiviral
ART	antiretroviral therapy
T0	time 0
T1	time 1
IFN	interferon
IL	interleukin
Th	T helper
Tc	T cytotoxic
AIDS	acquired immunodeficiency syndrome
HLA	human leukocyte antigen
SVR	sustained virologic response
EDTA	ethyleneiaminetetraacetic acid
PBMCs	peripheral blood mononuclear cells
HCs	healthy controls
ALT	alanine aminotransferase
ELISA	enzyme-linked immunosorbent assay
APC	allophycocyanin
FITC	fluorescein isothiocyanate
PE	phycoerythrin
FMO	fluorescence minus one
FBS	fetal bovine serum
PMA	phorbol myristyl acetate
GT	genotype
NA	not applicable

Introduction

Immune activation plays a key role in the pathogenesis of both hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-1 infections. Although HIV-1 patients demonstrate decreased levels of inflammation and a corresponding rise in CD4 counts, chronic immune activation is not completely extinguished by antiretroviral therapy (ART), and may contribute to long-term negative consequences such as non-AIDS-related conditions, especially end stage liver disease, cardiovascular disease, and malignancies [1,2]. Furthermore, chronic HCV infection may also lead to chronic immune activation and contribute with HIV-1 in maintaining a state of chronic inflammation, leads to progressive T-cell exhaustion and results in global immune dysregulation [3,4].

On the other hand, T-cell response to HCV is generally poor in chronic infection, and even exacerbated in HCV/HIV-1 co-infection due to the HIV-mediated loss of CD4+ T-cells and impairment of the function of HCV-specific CD8+ T cells [5—7]. Several studies have highlighted the key roles of interferon-gamma (IFN-γ), interleukin (IL)-17, and IL-22 producing T helper (Th1, Th17, and Th22) cells in HCV infection and liver fibrosis, however, the role of T cytotoxic (Tc1, Tc17, and Tc22) cells and related cytokines in these issues is yet to be elucidated [8—11]. It has been suggested that the recovery of HCV-specific T-cell responses during antiviral therapy similar to those observed in spontaneous resolvers of HCV infection may contribute to the resolution of chronic infection [12,13]. Nowadays,IFN-free direct-acting antiviral (DAA) therapy can efficiently clear HCV infection [14], however, it is poorly understood whether DAAs without therapeutic immune stimulation are able to restore HCV-specific T-cell response. In the present study, we evaluated the frequency of CD38 and/or HLA-DR expression, defined patterns of IFN-γ, IL-17, and IL-22 production by peripheral CD4+ and CD8+ T cells, and measured plasma levels of related cytokines among HCV mono-infected, and HCV/HIV-1 co-infected patients before (T0), after the end of DAA therapy at the time of sustained virologic response (SVR)-12 weeks (T1) and compared them to healthy controls (HCs).

Materials and methods

Study population

From February 2015 to November 2016, 45 subjects were recruited at the Division of Infectious Diseases, Policlinico Umberto I, Rome, Italy, and divided into 3 groups matched for age and sex, including 18 HCV mono-infected patients, 17 HCV/HIV-1 co-infected patients under successful ART with less than 37 HIV-1 RNA copies/mL at baseline, and 10 HCs. Patients with other diseases possibly interfering with their immune system (i.e., auto-immune disease, liver disease from non-viral causes, malignity, or any other

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