CD8+ stem cell-like memory T cell subset is associated with disease progression in chronic hepatitis C virus infection

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Research Article

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Abstract

Background The dysfunction of memory $CD8^+$T cell can not be reverted by successful clearance of hepatitis C virus (HCV) after direct-acting antivirals (DAA) therapy, increasing the risk of reinfection with HCV. Stem cell-like memory T cells (Tscm) with superior capacities of long-lasting, self-renewing, and multipotency that contribute to the maintenance of immune function.

Methods We investigated the impact of HCV infection on $CD8^+$Tscm, and the possible role this compartment may has in disease progression, by using DAA-naïve HCV monoinfected and HIV/HCV coinfected cohorts. The distribution of memory $CD8^+$T cell subsets and the level of T cell immune activation were determined by flow cytometry. Associations between $CD8^+$Tscm and other memory T cell subsets, HCV viral load, as well as the level of T cell immune activation were analyzed.

Results We observed that the proportion of $CD8^+$Tscm increased in both HCV and HIV/HCV individuals. The proportion of $CD8^+$Tscm had a positive correlation with that of $CD8^+$Tcm, and a negative correlation with that of $CD8^+$Tem, representing the contribution of $CD8^+$Tscm in T cell homeostasis. In addition, higher frequency of $CD8^+$Tscm indicated lower HCV viral load and less T cell immune activation in HCV monoinfection, which suggested that $CD8^+$Tscm may associate with effective control of HCV replication.

Conclusions $CD8^+$Tscm may has protective immunity to HCV infection. Considering the characteristics of Tscm, our current study opens new opportunity for Tscm-based vaccine design and immunotherapy development to achieve HCV elimination.

Introduction

Over 70 million people worldwide are infected with hepatitis C virus (HCV), of which 20% will progress to live fibrosis/cirrhosis and ultimately to hepatocellular carcinoma (HCC)[1, 2]. In addition, due to the similar transmission routes sharing between human immunodeficiency virus type 1 (HIV-1) and HCV, such as sexual or vertical transmission, and intravenous drug use (IDU), about 6% HIV-1 infected individuals are coinfected with HCV[3]. In spite of the high HCV clearance rates in most individuals (~ 98%) offered by IFN-free direct-acting antivirals (DAA) therapy, DAA-resistance, and the partial restoration of adaptive immune functions following DAA-mediated HCV cure, especially the incomplete recovery of memory $CD8^+$T cell responses, will affect the treatment outcomes, and increase risks for HCV reinfection and other bacterial or viral infection after cure in HCV monoinfected and HIV/HCV coinfected individuals [4, 5]. Therefore, the continuing efforts are required to develop novel strategies to achieve HCV eradiation.

Adoptive cell therapies (ACT) have shown promising efficacy against viral infections, leukemias, and solid tumors[6–9]. Selection of transferred T cells with longevity and strong cytotoxicity are much more important for clinical applications. Efficient and persistent memory $CD8^+$T cell responses are essential for long-term defense against viral infection or malignancies[10, 11]. Memory T cell compartment can be divided into three subsets according to a developmental hierarchy: stem cell-like memory T cells (Tscm),

central memory T cells (Tcm), and effector memory T cells (Tem)[12–14]. Compared to Tcm and Tem, Tscm has superior capacities of long-lasting, quick emergence, self-renewing, and multipotency for generation of other memory T cells [15–17]. The $CD8^+$Tscm subset has more beneficial effects of antiviral or antitumor responses in clinic, as well as the immune reconstitution after hematopoietic stem cell transplantation (HSCT) [18–20]. Of note, functional HCV-specific $CD4^+$ and $CD8^+$Tscm can be elicited robustly followed by an adenoviral (Ad)/modified vaccinia Ankara (MVA) vector-based vaccination[21, 22]. In addition, yellow fever-specific $CD8^+$Tscm cells can stably maintain for at least 25 years for preventing infection post vaccination[23]. SARS-CoV-2-specific $CD8^+$Tscm in COVID-19 convalescent patients is sustained for 10 months[24]. Besides, our previous study has demonstrated that Tscm is responsible for immune reconstitution in HIV-1 chronically infected patients[25]. Therefore, $CD8^+$Tscm becomes a novel target for vaccine design against viral infection and T-cell based immunotherapy development for antiviral and antitumor therapy or immune reconstitution post transplantation.

Understanding of the changes of $CD8^+$Tscm compartment in HCV monoinfected and HIV/HCV coinfected subjects and its correlation with virological and immunological parameters in disease may offer insights on the further application of this subset in vaccine or immune therapy. We demonstrate for the first time that the proportion of $CD8^+$Tscm increases in both HCV monoinfection and HIV/HCV coinfection. Furthermore, the proportion of $CD8^+$Tscm is inversely correlated with HCV viral load or the level of T cell immune activation in HCV monoinfection, indicating the important role of $CD8^+$Tscm in the protection of disease progression in HCV infection.

Materials And Methods

Study cohorts

Thirty-six HIV-exposed seronegative individuals (HESN), 25 ART-treated HIV-1 monoinfected patients (HIV+), 28 untreated HCV chronically monoinfected patients (HCV+), and 18 HIV-virologically suppressed but HCV-untreated HIV/HCV coinfected patients (HIV/HCV) were enrolled from four cohorts established in Beijing Youan Hospital. (□) HESN was anti-HIV, anti-hepatitis B virus (HBV), and anti-HCV antibody negative individuals, which was recruited from the men who have sex with men (MSM) high risk behavior of HIV-1 infection screening cohort; (□) HIV+ patients with negative anti-HBV and anti-HCV antibodies were recruited from chronically HIV-infected antiretroviral therapy (ART)-treatment cohort. The median HIV-1 infection duration was 4 years, and patients was treated at least 2 years with an undetectable plasma HIV-1 RNA. HIV+ samples selected in this study was 96 weeks with ART. The ART regimen included AZT (zidovudine)/3TC (lamivudine)/NVP (nevirapine) (*n*=12) or TDF (tenofovir)/ 3TC/EFV (efavirenz) (*n*=13); (□) HCV+ patients with negative anti-HIV and anti-HBV antibodies were recruited from HCV-infected cohort. The median HCV infection duration was 3 years. HCV+ samples selected in this study were treatment-naïve timepoint; (□) HIV/HCV patients with negative anti-HBV antibody were recruited from HIV/HCV coinfected cohort. Patients in this cohort had been diagnosed with HIV-1 prior to HCV infection. The median HIV-1 infection and treatment duration was 5, and 3 years, respectively. The

ART regimen against HIV-1 included AZT/3TC/NVP (*n*=8) or D4T (Stavudine)/3TC/EFV (*n*=10). The median HCV infection duration was 4 years. Samples of this groups selected for this study were plasma HIV-1 RNA undetectable and HCV untreated. All subjects enrolled in this study had $CD4^+$T count more than 200 cells/μl. Detailed information of study subjects was listed in Table 1.

Peripheral blood mononuclear cells (PBMCs) from whole blood were isolated using a Ficoll-Hypaque gradient centrifugation method and stored in liquid nitrogen.

Ethics statement

This study and all the relevant experiments were approved by the Beijing Youan Hospital Research Ethics Committee (approval number: 2019-014), and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Immunophenotyping

Cryopreserved PBMCs were thawed in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) and were washed with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). The cells were surface stained with the following mouse anti-human mAbs at room temperature for 20 min in the dark: CD3-APC-cy7 (Clone SK7), CD4- FITC (Clone SK3), CD8-Percp-cy5.5 (Clone RPA-T8), CD45RA-PE-cy7 (Clone HI100), CCR7-APC (Clone G043H7), CD27-PE (Clone O323), CD95-BV421 (Clone DX2), CD38-PE (Clone HIT2), and HLA-DR- APC (Clone TU36). Relative isotype controls were also stained. All antibodies and isotype controls were purchased from Biolegend (San Diego, CA, USA). At least 50,000 lymphocytes were acquired with BD FACS Canto II flow cytometer (BD Biosciences, San Diego, CA, USA). Data were analyzed with Flowjo Software version 10 (Tree Star Inc., Ashland, OR, USA). Stem cell-like memory T cells (Tscm) were defined as $CD45RA^+CCR7^+CD27^+CD95^+$, central memory T cells (Tcm) were defined as $CD45RA^-CCR7^+$, and effector memory T cells (Tem) were defined as $CD45RA^-CCR7^-$.

Measurements of $CD4^+$ T cell numbers and plasma viral load

Routine $CD4^+$ T TruCount (BD Biosciences), plasma HIV-1 viral load (Abbott Molecular Inc., Des Plaines, IL, USA), and plasma HCV viral load (Roche Diagnostics, Mannheim, Germany) were measured according to the manufacturers' instructions. The detecting limitation of HIV-1 and HCV is 50 copies/ml, and 15 IU/ml, respectively.

Statistical analysis

Data in figures are expressed as means ± standard deviation (SD), whereas that were expressed as median with range in Table 1. All statistical analyses were performed using GraphPad Prism software v5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Comparisons between two groups was calculated using a Student's *t* test for data with normal distribution, and a nonparametric Mann-Whitney *U*-test were

used with data that were not normally distributed. Correlations were determined by the Spearman rank correlation test. A *p*-value less than 0.05 represented significance.

Results

Elevated $CD8^+$Tscm proportion in HCV monoinfected and HIV/HCV coinfected patients

To determine the role of $CD8^+$Tscm during HCV monoinfection or HIV/HCV coinfection, we firstly analyzed the proportion of this compartment in untreated HCV + and HIV-virologically suppressed with viral load of < 50 copies/ml but HCV-untreated HIV/HCV coinfected individuals. HESN and ART-experienced HIV + patients with viral load of < 50 copies/ml were used as controls. The gating strategy for $CD8^+$Tscm analysis was shown in Fig. 1a. $CD8^+$T subpopulation was defined as $CD3^+CD8^+$, and were further divided into three memory compartments according to CD45RA, CCR7, CD27, and CD95 expression on the cell surface: central memory (cm, $CD45RA^-CCR7^+$), effector memory (em, $CD45RA^-CCR7^-$), and stem cell-like memory (scm, $CD45RA^+CCR7^+CD27^+CD95^+$). As shown in Fig. 1b and c, compared to HESN, the proportion of $CD8^+$Tscm in both HCV + and HIV/HCV significantly increased (all $p < 0.001$), however, there was no difference of this $CD8^+$T compartment between HCV + and HIV/HCV ($p = 0.715$). HIV + had the lowest $CD8^+$Tscm frequency among four groups (all $p < 0.001$). HCV + had the highest $CD8^+$Tcm and the lowest $CD8^+$Tem frequencies among four groups (Fig. 1b).

$CD8^+$Tscm involved in T memory cell maintenance during HCV infection

The frequency of $CD8^+$Tscm had a positive correlation with that of $CD8^+$Tcm in HIV+ ($r = 0.640$, $p < 0.001$) and HCV+ ($r = 0.425$, $p = 0.024$), and a similar correlation was found when considering that in HIV/HCV ($r = 0.445$, $p = 0.086$) (Fig. 2a). Oppositely, negative correlation between the frequency of $CD8^+$Tscm and that of $CD8^+$Tem was observed in HESN (r=-0.630, $p < 0.001$), HIV+ (r=-0.538, $p = 0.005$), and HCV+ (r=-0.447, $p = 0.016$), and a similar trend in HIV/HCV (r=-0.418, $p = 0.084$) (Fig. 2b). These findings indicated that, as the initial stage of memory T cell differentiation, Tscm compartment may support the Tcm development, which subsequently further differentiated into Tem in HCV infection.

Association between $CD8^+$Tscm and HCV clinical parameters

Next, we investigated the possible influence of $CD8^+$Tscm on the virological progression in HCV + and HIV/HCV infected patients. There was no significant difference in plasma HCV viral load between HCV+ (median:5.33 $\log_{10}$IU/ml) and HIV/HCV (median:5.92 $\log_{10}$IU/ml) (Table 1). Reverse relationships were observed between plasma HCV viral load and the proportion of $CD8^+$Tscm, $CD8^+$Tcm, respectively, in HCV+ ($CD8^+$Tscm: r=-0.563, $p = 0.002$; $CD8^+$Tcm: r=-0.401, $p = 0.034$), but not in HIV/HCV ($CD8^+$Tscm: $r = 0.133$, $p = 0.599$; $CD8^+$Tcm: r=-0.256, $p = 0.338$) (Fig. 3a and b). In addition, the proportion of $CD8^+$Tem was not related to HCV viral load in both HCV+ ($r = 0.163$, $p = 0.407$) and HIV/HCV (r=-0.280, $p = 0.261$) (Fig. 3c).

Since immune activation was an independent predictor for HIV-1 disease progression, as well as an key factor to drive liver injury and fibrosis in HCV infected patients[26], we consequently analyzed the possible connection between $CD8^+$Tscm and the level of immune activation that were represented by the frequency of CD38 and HLA-DR coexpression on $CD8^+$T cell surface in HCV + and HIV/HCV. As shown in Fig. 4a, HCV + had the highest level of $CD38^+HLA\text{-}DR^+CD8^+$T cell among four groups (all $p \leq 0.001$). Moreover, compared to HESN and HIV+, the frequency of $CD38^+HLA\text{-}DR^+CD8^+$T cell in HIV/HCV was increased (all $p \leq 0.001$). Of note, the level of immune activation in HIV + was still higher than HESN, although virological suppression after successful ART in HIV + individuals ($p < 0.001$). There was a trend of positive relationship between the frequency of $CD38^+HLA\text{-}DR^+CD8^+$T cell and plasma HCV viral load in HCV+ ($r = 0.347$, $p = 0.070$), but not in HIV/HCV ($r = 0.107$, $p = 0.705$) (Fig. 4b), indicating that HCV replication may drive the elevated the level of immune activation in HCV infected patients. Correlation analysis revealed that within HCV+, the frequency of $CD38^+HLA\text{-}DR^+CD8^+$T cell was reversely related to that of $CD8^+$Tscm (r=-0.387, $p = 0.041$) (Fig. 5a), and $CD8^+$Tcm (r=-0.379, $p = 0.046$) (Fig. 5b). Similar relationship was not found when considering same parameters in HESN, HIV+, and HIV/HCV. Besides, there was no association between the frequency of $CD38^+HLA\text{-}DR^+CD8^+$T cell and that of $CD8^+$Tem within all groups, with the exception of a positive correlation in HESN ($r = 0.509$, $p = 0.002$) (Fig. 5c). We also analyzed the association between the memory $CD8^+$T cell compartments and the level of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) in HCV + and HIV/HCV groups. However, no significant correlation but a negative trend was observed between ALT or AST and the proportion of $CD8^+$Tscm, $CD8^+$Tcm, as well as $CD8^+$Tem in both HCV + and HIV/HCV groups (data not shown).

Discussion

We here observed the significant increase of the frequency of $CD8^+$Tscm in both HCV + and HIV/HCV individuals. However, association between higher level of $CD8^+$Tscm compartment and lower level of HCV RNA or that of T cell immune activation was only found in HCV+.

The contribution of HCV-specific $CD8^+$T cells in the control of ongoing viral replication and consequent liver disease upon HCV infection has been extensively studied [4, 27, 28]. After successful HCV clearance, HCV-specific $CD8^+$T cells shift toward a memory phenotype with the ability to readily expanded upon reinfection, making contribution to the rapid and effective protection from reinfection[29, 30]. Persist viral stimulation drives the dysfunction of memory $CD8^+$T cells through up-regulation of co-inhibitory molecules, metabolic derangements, proteostasis dysregulation, and chromatin silencing on $CD8^+$T cells[5]. Although administration of DAA results in a high rate of HCV cure and sustained virological responses (SVR), the memory $CD8^+$T cell dysfunction is irreversible in HCV and HIV/HCV patients, leading to the poor spontaneous control of HCV reinfection or other infections[31–34].

$CD8^+$Tscm compartment has been identified as the precursor of other memory T cell subsets, supporting the long-time T cell memory in the postantigen phase and lifelong protection by yellow-fever

vaccination[16, 23]. Consistent with results found in HIV-1[18], we observed that the $CD8^+$Tscm subset was positively correlated with $CD8^+$Tcm and negatively correlated with $CD8^+$Tem in frequency in both HIV + and HCV + groups, HIV/HCV group also had the similar trend (Fig. 2), indicating that the differentiation of $CD8^+$Tscm could be a key factor to maintain the homeostasis of memory T cell development in HIV or HCV monoinfection, as well as coinfection of HIV and HCV.

To date, however, the dynamics of $CD8^+$Tscm during HCV infection and the role of $CD8^+$Tscm on the control of HCV infection is not well analyzed. In HIV-1 infected individuals, the proportion and number of HIV-1-specific $CD8^+$Tscm were associated with $CD4^+$T cell number and HIV-1 viral load on ART[35]. Low frequency of $CD8^+$Tscm and lack of polyfunctional $CD8^+$Tscm were associated with severe disease progression in chronic chagasic patients[36]. CD19-CAR-modified $CD8^+$Tscm strategy showed enhanced polyfunctionality and long-lasting antitumor responses against human B-cell malignancies[20]. These findings provide us evidences that $CD8^+$Tscm has the function of antiviral and antitumor activity. In our study, negative correlation between $CD8^+$Tscm and HCV viral load was observed in HCV monoinfected individuals, indicating that $CD8^+$Tscm may has the suppressive capacity to HCV replication (Fig. 3).

It is well known that HIV-1 and HCV infection induce T cell immune activation due to the persist viral exposure[26, 37–39]. We observed a negative correlation between the proportion of $CD8^+$Tscm and the level of $CD8^+$T cell immune activation in HCV monoinfection (Fig. 5). We hypothesized that $CD8^+$Tscm inhibits T cell immune activation through controlling HCV replication, since the positive correlation trend observed between HCV viral load and the level of immune activation in HCV monoinfection (Fig. 4b). Of note, similar association between $CD8^+$Tscm and HCV viral load or the immune activation was not found in HIV/HCV coinfected patients, probably due to the influence of administration of ART for HIV-1 treatment and immune suppression induced by HIV-1. Furthermore, the immune activation was not decreased to normal level in HIV+, which is likely caused by the persist residual virus replication despite ongoing ART in HIV-1 infected individuals[40]. ALT and AST are two key indicators related to liver injury. However, we just observed a negative relationship trend between $CD8^+$Tscm and these two indicators, perhaps due to the less samples used in our study.

This study had several limitations. Firstly, the sample size is relatively small, more samples should be recruited to confirm our results. Secondly, we didn't analyze the relationship between $CD8^+$Tscm and the degree of live fibrosis, which is an important disease progressive parameter in HCV infection, since lacking of relative documents. Thirdly, due to the insufficient cells, we just got the correlation analysis result between $CD8^+$Tscm and HCV viral load from this observational study, which cannot demonstrate directly the role of $CD8^+$Tscm in the control of HCV replication. The capacity of HCV-specific $CD8^+$Tscm to HCV control should be further studied *in vitro* in future.

Conclusions

In summary, our study describes the change of $CD8^+$Tscm in HCV infection and identifies $CD8^+$Tscm as a controller to HCV replication. Considering the unique features of Tscm, our findings may have implications in the case of $CD8^+$Tscm as a potent target for T-cell based vaccine design and immunotherapy for HCV cure.

Abbreviations

ACT (adoptive cell therapy), Ad (adenoviral), ALT (alanine aminotransferase), ART (antiretroviral therapy), AST (aspartate aminotransferase), COVID-19 (coronavirus disease 2019), DAA (direct-acting antivirals), HBV (hepatitis B virus), HCC (hepatocellular carcinoma), HCV (hepatitis C virus), HESN (HIV-exposed seronegative individuals), HIV-1 (human immunodeficiency virus type 1), HSCT (hematopoietic stem cell transplantation), IDU (intravenous drug use), IFN (interferon), MSM (men who have sex with men), MVA (modified vaccinia Ankara), *n* (number), PBMC (peripheral blood mononuclear cell), RNA (ribonucleic acid), SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), Tcm (central memory T cell), Tem (effector memory T cell), Tscm (stem cell-like memory T cell).

Declarations

Data availability

The data for this study are available by contacting the corresponding author upon reasonable request.

Animal Research (Ethics)

Not applicable.

Consent to Participate (Ethics)

This study and all the relevant experiments were approved by the Beijing Youan Hospital Research Ethics Committee (approval number: 2019-014), and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Consent to Publish (Ethics)

All authors read and approved the final manuscript.

Clinical Trials Registration

Not applicable.

Author contribution

X.L., BB.S., Y.G., and T.Z. conceived the study, X.L., BB.S., and W.W. performed experiments, X.L., BB.S. analyzed data, X.L., BB.S. wrote the manuscript, B.S., H.W., Y.G., and T.Z. revised the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Table

Table 1 Clinical data for study subjects

Baseline Characteristics	*HIV-exposed seronegative individuals (HESN)*	*HIV-1 monoinfection (HIV+)*	*HCV monoinfection (HCV+)*	*HIV-1/HCV coinfection (HIV/HCV)*
Subjects (*n*)	36	25	28	18
Median age, yrs (range)	36 (19-52)	35 (19-59)	55 (26-80)	37 (20-51)
Sex (male/female)	36/0	25/0	12/16	10/8
Median $CD4^+$T cell count, cells/μl (range)	884 (497-1425)	575 (296-1319)	–	352 (201-886)
Duration of HIV-1 infection, yrs (range)	–	4 (3-7)	–	5 (1-14)
Duration of HIV-1 treatment, yrs (range)	–	2	–	3 (1-6)
Median serum HIV-1 RNA, copies/ml (range)	–	< 50	–	< 50
Duration of HCV infection, yrs (range)	–	–	3 (3-6)	4 (1-13)
Median serum HCV RNA, log10 IU/ml (range)	–	–	5.33 (1.45-7.66)	5.92 (3.65-6.98)
HCV genotype, n				
1b	–	–	17	6
2a	–	–	9	1
others	–	–	2	0
unknown	–	–	0	11
Median ALT, μM (range)	–	30.2 (14-57)	54.7 (5.3-416.1)	35.5 (12-145)
Median AST, μM (range)	–	26.8 (18-51)	48.4 (12.4-206.3)	40.3 (21-106)

–, data not available

Figures

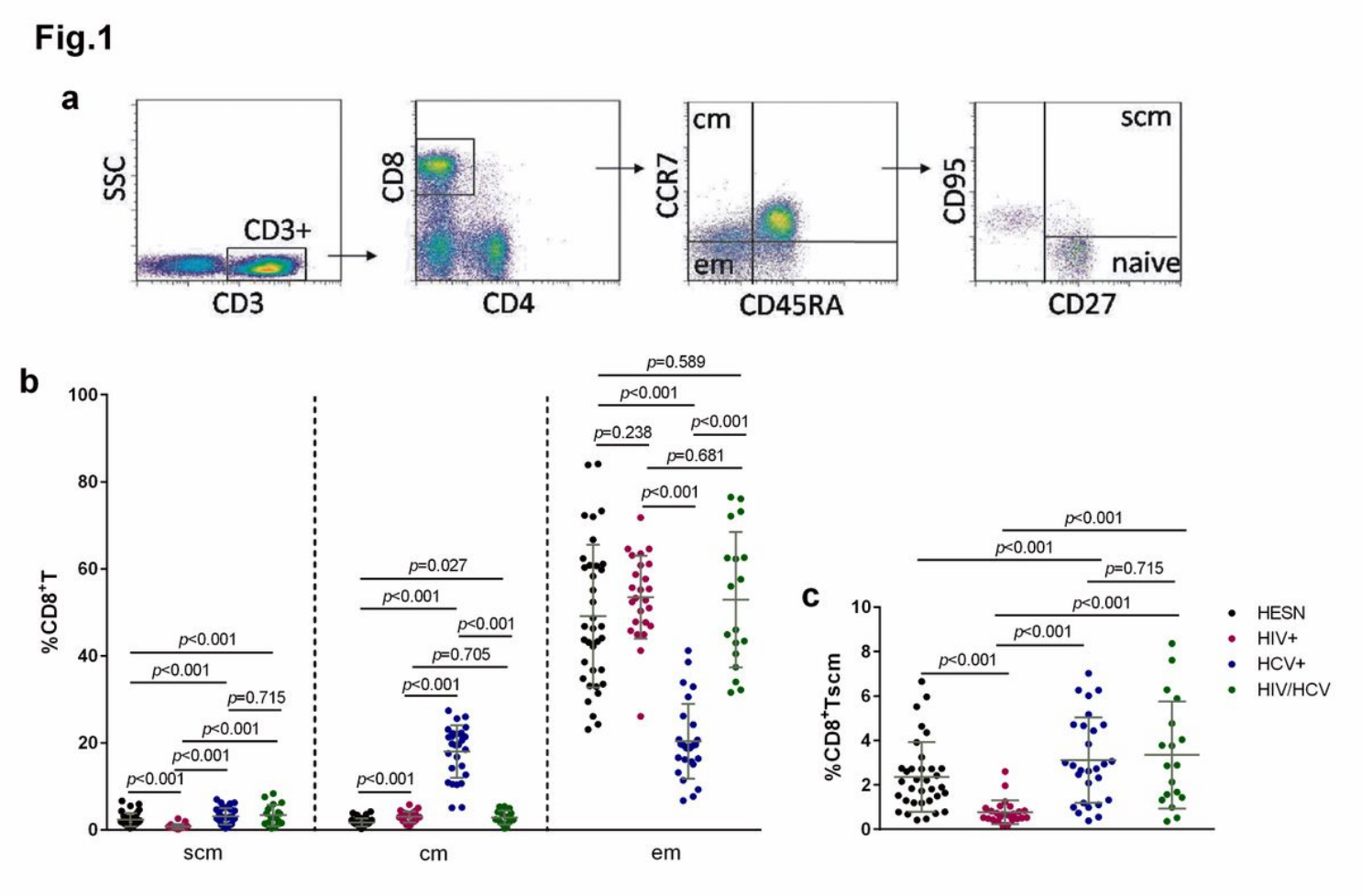

Figure 1

Distribution of memory CD8$^+$T cells in HESN, HIV+, HCV+, and HIV/HCV. a The gating strategy for flow cytometric analysis of memory B cells in PBMCs: stem cell-like memory T cells (Tscm, CD45RA$^+$CCR7$^+$CD27$^+$CD95$^+$), central memory T cells (Tcm, CD45RA$^-$CCR7$^+$), and effector memory T cells (Tem, CD45RA$^-$CCR7$^-$). **b** Comparison of memory CD8$^+$T cell subset in HESN (*n*=36), HIV+(*n*=25), HCV+ (*n*=28), and HIV/HCV (*n*=18). **c** Enlarged scatter plot of CD8$^+$Tscm subset in four groups.

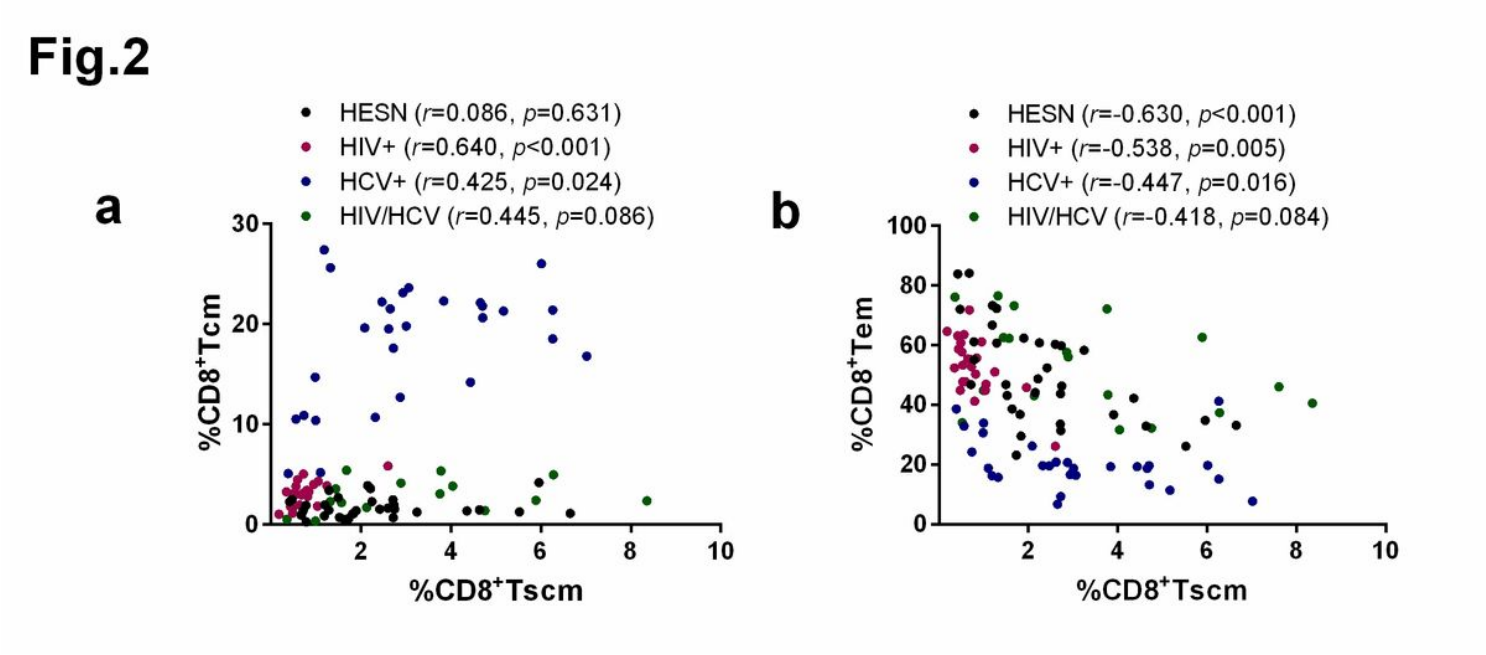

Figure 2

CD8$^+$Tscm is the progenitor for other memory CD8$^+$T cells. Correlation analysis between the frequency of CD8$^+$Tscm and that of CD8$^+$Tcm (**a**) or CD8$^+$Tem (**b**) in HESN, HIV+, HCV+ and HIV/HCV.

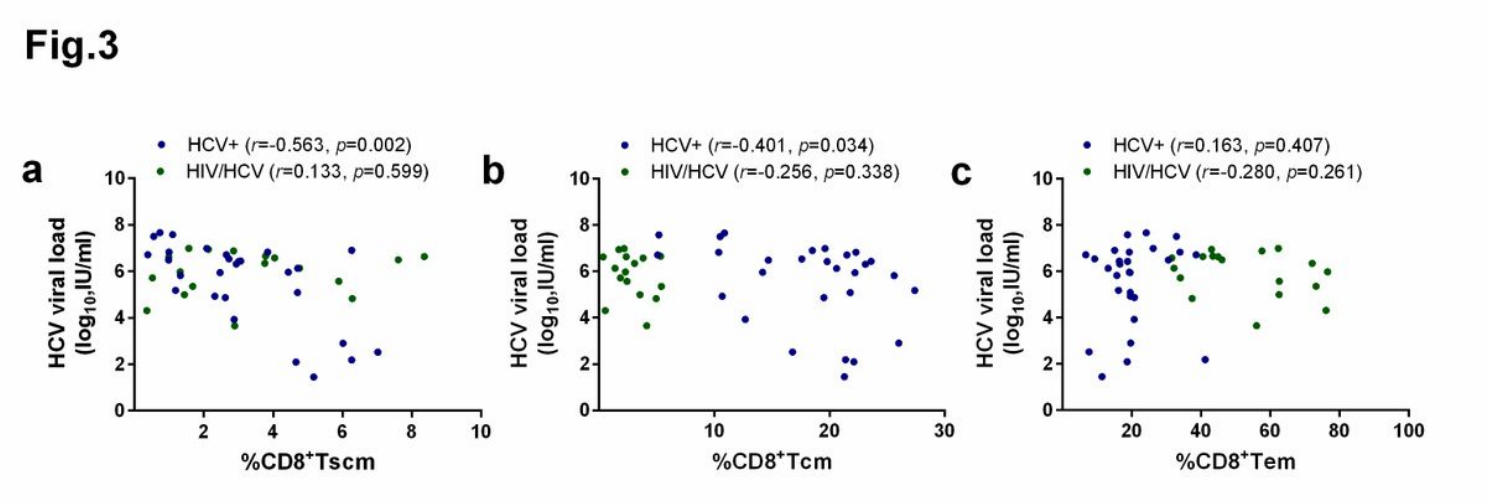

Figure 3

CD8$^+$Tscm is correlated with low HCV viral load in HCV monoinfection. Correlation analysis between plasma HCV viral load and the frequencies of CD8$^+$Tscm(**a**), CD8$^+$Tcm (**b**), and CD8$^+$Tem (**c**) in HESN, HIV+, HCV+ and HIV/HCV.

Fig.4

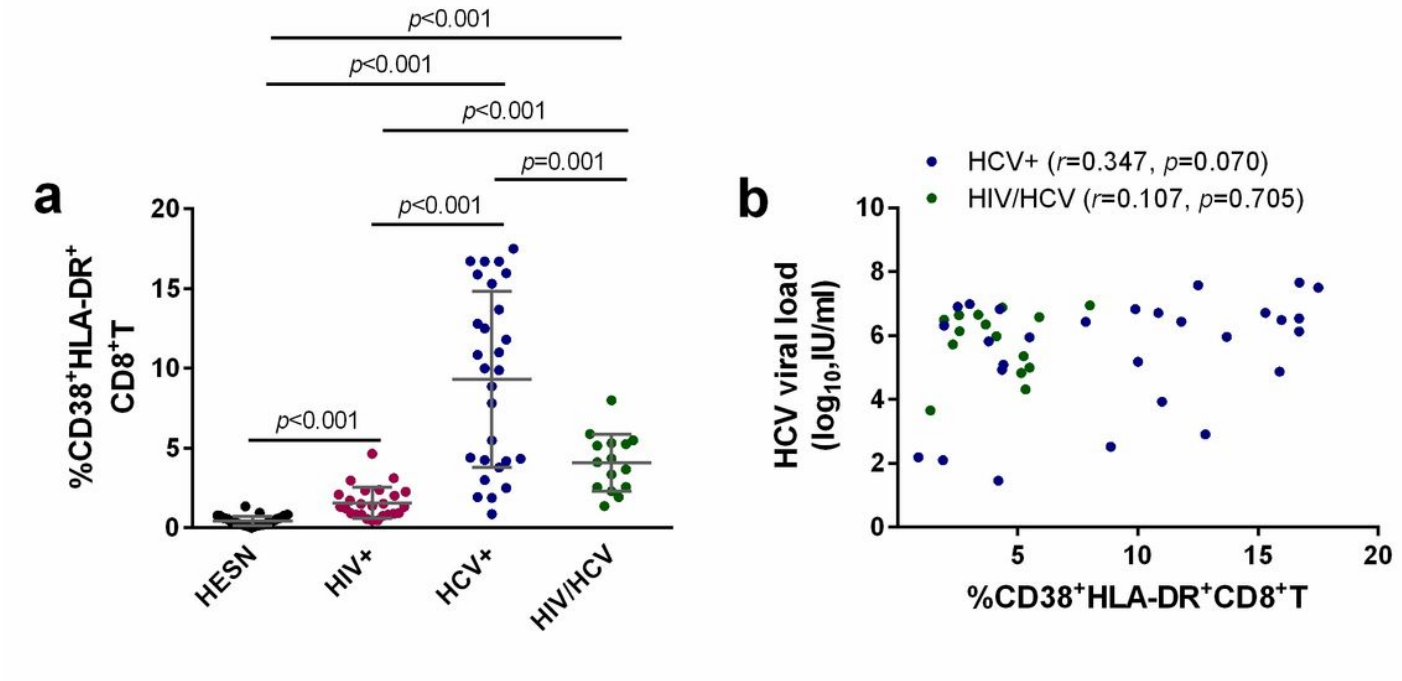

Figure 4

Elevated T cell immune activation in HCV monoinfected and HIV/HCV coinfected individuals. a Comparison of the proportion of $CD38^+HLA\text{-}DR^+CD8^+T$ cell in HESN, HIV+, HCV+ and HIV/HCV. **b** Correlation analysis between the proportion of $CD38^+HLA\text{-}DR^+CD8^+T$ cell and plasma HCV viral load in HCV+, and HIV/HCV.

Fig.5

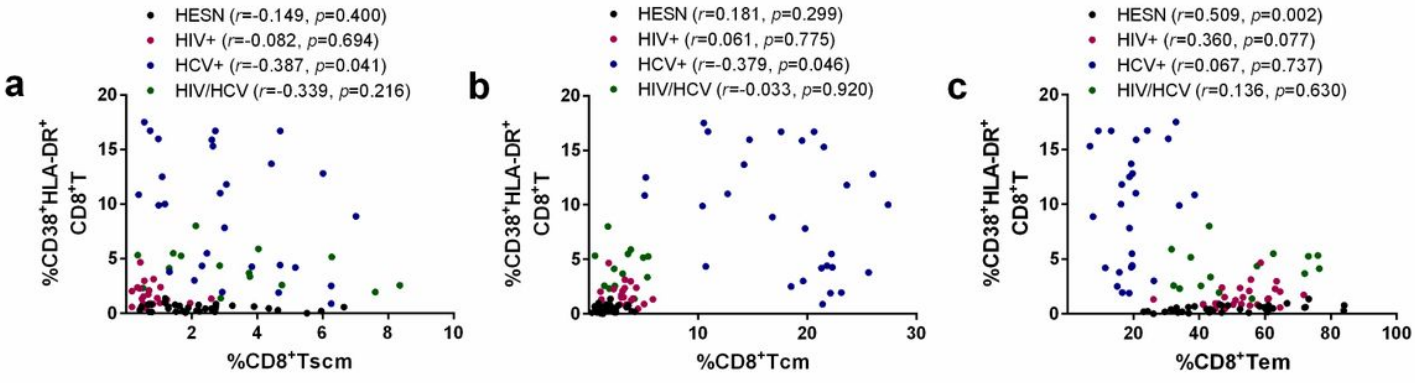

Figure 5

$CD8^+$Tscm is correlated with low level of T cell immune activation in HCV monoinfection. Correlation analysis between the proportion of $CD38^+HLA\text{-}DR^+CD8^+$T cell and the frequencies of $CD8^+$Tscm(**a**), $CD8^+$Tcm (**b**), and $CD8^+$Tem (**c**) in HESN, HIV+, HCV+ and HIV/HCV.