

# Interferon- $\alpha$ -induced CD100 on naïve CD8<sup>+</sup> T cells enhances antiviral responses to hepatitis C infection through CD72 signal transduction

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## Abstract

**Objectives:** We investigated the effects of CD100 on naïve CD8<sup>+</sup> T cells during hepatitis C virus (HCV) infection after interferon- $\alpha$  (IFN- $\alpha$ ) therapy to clarify the mechanism underlying the effect of IFN- $\alpha$  in enhancing the antiviral response.

**Methods:** The CD100 molecules on subsets of CD8<sup>+</sup> T cells were analysed with flow cytometry. The effects of CD100-overexpressing naïve CD8<sup>+</sup> T cells were determined with ELISAs and an MTT cytotoxicity assay. The role of CD100–CD72 signal transduction was analysed with a neutralization and transwell assays.

**Results:** HCV infection reduced CD100 expression on CD8<sup>+</sup> T cells, whereas IFN- $\alpha$  treatment significantly increased CD100 expression on naïve CD8<sup>+</sup> T cells. The increased CD100 interacted with the CD72 receptor and enhanced PBMC cytokine secretion (IFN- $\gamma$  and tumour necrosis factor- $\alpha$ ) and cytotoxicity.

**Conclusions:** IFN- $\alpha$ -induced CD100 on naïve CD8<sup>+</sup> T cells promotes PBMC cytokine secretion and cytotoxicity through CD100–CD72 signalling during HCV infection.

## Keywords

IFN- $\alpha$  therapy, hepatitis C, CD100, naïve CD8<sup>+</sup> T cell

Date received: 30 May 2016; accepted: 5 October 2016

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## Introduction

Hepatitis C virus (HCV) infection is one of the major causes of liver injury worldwide, resulting in both cirrhosis and carcinoma.<sup>1</sup> Considerable evidence indicates that during HCV infection, CD8<sup>+</sup> T lymphocytes are important effectors in eliminating HCV infection.<sup>2,3</sup> These cells recognize infected hepatocytes and induce cellular apoptosis and the secretion of cytokines to promote viral clearance. Chronic hepatitis C (CHC) infection is characterized by impaired HCV-specific CD8<sup>+</sup> cytotoxic T cells. Most CHC patients display low levels of HCV-specific cytotoxic T lymphocytes (CTLs), regardless of the HCV viral load, in the early stages of antiviral treatment.<sup>4,5</sup> These cells are exhausted in the first step of HCV infection, which is characterized by the expression of negative regulators, such as PD-1, and impaired cell function,<sup>6,7</sup> ultimately resulting in the induction of apoptosis or improper cellular ageing.<sup>8</sup>

Two main types of treatment are currently available to CHC patients: interferon- $\alpha$  (IFN- $\alpha$ )-based treatments and IFN-free regimens.<sup>9</sup> In both cases, the treatment goal is a sustained viral response (SVR), defined as undetectable HCV RNA in the serum 6 months after the end of therapy. IFN- $\alpha$  directly activates interferon-stimulated genes (ISGs) to promote antiviral activity.<sup>10,11</sup> IFN- $\alpha$  also affects both the innate and adaptive immune responses in indirect ways, such as in the expansion of activated CD8<sup>+</sup> T cells,<sup>12-15</sup> antigen presentation, the enhanced maturation of dendritic cells, and the activation of B-cell responses.<sup>16,17</sup> IFN- $\alpha$  therapy can rescue long-lived HCV-specific CTL responses.<sup>18,19</sup> For example, in IFN- $\alpha$ -induced SVR patients, the frequencies and cytotoxicity of core-protein- and NS3 (non-structural protein 3)-specific CTLs were restored, and the frequency of peripheral HCV-specific CTLs correlated with SVR development.<sup>20-22</sup> However, the effects of IFN- $\alpha$  on naïve CD8<sup>+</sup> T cells have not been comprehensively studied.

IFN- $\alpha$  regulates the immune responses through many mechanisms. Among the inhibitory and activating receptors that are affected by IFN- $\alpha$ , CD100 has recently been investigated.<sup>23</sup> CD100, also known as SEMA4D, belongs to the family of immune semaphorins that is expressed on immune cells, and CD100 is abundantly expressed on resting T cells, NK cells, and antigen-presenting cells.<sup>24-26</sup> As a transmembrane glycoprotein, it is digested to its soluble form, sCD100, by specific matrix metalloproteinases.<sup>27</sup> CD100 or sCD100 functions through its receptors, which include CD72, plexin B1, and plexin B2. Interactions between CD100 and CD72 have been shown to positively regulate the immune response by affecting the immunoreceptor tyrosine-based inhibitory motifs (ITIM) of CD72.<sup>28,29</sup> The interaction of CD100 and plexin B is also well known for its roles in lymphocyte activation and proliferation,<sup>30,31</sup> skin healing,<sup>32</sup> invasive tumour growth, and organogenesis.<sup>33-35</sup>

To determine how the CD100 molecules on CD8<sup>+</sup> T cells vary during HCV infection and to examine the effects of IFN- $\alpha$  on the CD100-mediated antiviral response, the CD100 molecules on subsets of CD8<sup>+</sup> T cells was studied. The proportion of naïve CD8<sup>+</sup> T cell subsets increased in the peripheral blood of HCV patients, whereas the frequency of CD100 molecules decreased on both effector T-cell subsets and the naïve subset during HCV infection. Interestingly, IFN- $\alpha$  treatment significantly increased the expression of CD100 on naïve CD8<sup>+</sup> T cells. Moreover, CD100-overexpressing naïve CD8<sup>+</sup> T cells stimulated peripheral blood mononuclear cell (PBMC) cytokine secretion (IFN- $\gamma$  and TNF- $\alpha$ ) and cytotoxicity. The upregulation of IFN- $\gamma$  and TNF- $\alpha$  secretion was dependent on cell-cell interactions, which raised the possibility that CD100 on naïve CD8<sup>+</sup> T cells promotes an antiviral response through the CD100-CD72 signal transduction pathway. The effects of CD100-

**Table 1.** Clinical characteristics of the study subjects. Subjects included 20 HCV-naïve patients (HCV), seven non-responders (NR), 20 early virological response patients (EVR), 20 sustained virological response patients (SVR), and 20 healthy controls (HC). HCV RNA titres, and alanine transaminase (ALT) and HCV genotypes were measured clinically. Co-infected patients with hepatitis B, hepatitis D, or HIV were excluded.

	HCV (n = 20)	NR (n = 7)	EVR (n = 20)	SVR (n = 20)	HC (n = 20)
Age (years, mean $\pm$ SEM)	39.12 $\pm$ 4.65	45.43 $\pm$ 1.78	35.86 $\pm$ 2.76	40.31 $\pm$ 2.87	29.7 $\pm$ 3.16
Sex, female/male	10/10	4/3	10/10	10/10	10/10
ALT, IU/ml (mean $\pm$ SEM)	90.36 $\pm$ 17.9	58.71 $\pm$ 6.04	31.47 $\pm$ 5.12	22.18 $\pm$ 4.89	N/A
log(HCV RNA) (mean $\pm$ SEM)	6.56 $\pm$ 0.24	7.5 $\pm$ 0.27	<2.0	< 2.0	N/A
HCV genotype 2a/1b	14/6	1/6	14/6	N/A	N/A

SEM: standard error of mean; N/A: not applicable.

overexpressing naïve CD8<sup>+</sup> T cells on the enhanced cytokine secretion of PBMCs was disrupted by an anti-CD72 antibody, suggesting that CD100–CD72 signal activation mediates the enhancement of the PBMC antiviral responses. In summary, IFN- $\alpha$  treatment during HCV infection upregulates CD100 expression on naïve CD8<sup>+</sup> T cells and promotes the immune-cell responses via CD100–CD72 signal transduction.

## Materials and methods

### Study population

The study subjects included 20 healthy controls, 20 HCV-naïve patients, seven non-responders (NRs), 20 early virological response (EVR) patients, and 20 sustained virological response (SVR) patients. This information is summarized in Table 1. The HCV RNA titres were measured with a quantitative polymerase chain reaction assay (Qiagen, Shenzhen, China). Twenty age- and sex-matched healthy controls were included in the study. Patients co-infected with hepatitis B virus, hepatitis D virus, or human immunodeficiency virus (HIV) were excluded. Naïve patients were positive for anti-HCV antibodies and HCV RNA (anti-HCV+/HCV-RNA+) with no antiviral treatment. Patients with a

reduction in HCV RNA of <2log after at least 12 weeks of treatment were regarded as NR. EVR patients had undetectable serum HCV RNA at week 12, and SVR patients had undetectable HCV RNA after treatment for at least 6 months. The NR, SVR, and EVR patients were all treated with PEGylated IFN- $\alpha$ -2a (PEGASYS RBV; Roche, Branchburg, NJ, USA) and ribavirin. All experiments were approved by the Research and Ethical Committee of Tangdu Hospital, Fourth Military Medical University, Xi'an, China. Informed consent was obtained from all donors.

### Flow cytometry

PBMCs were isolated with Ficoll–Hypaque density centrifugation reagent Histopaque-1077 (Sigma, St Louis, MO, USA), according to the manufacturer's protocol. The isolated cells were stained with fluorescently labelled antibodies (1  $\mu$ g/ml) for 1 h at room temperature and then fixed with 2% paraformaldehyde (eBioscience, San Diego, CA, USA). The samples were assayed with flow cytometry (FACSAria II; BD Bioscience, Franklin Lakes, NJ, USA). The antibodies used included anti-hCD3–allophycocyanin (APC), anti-hCD3–peridinin chlorophyll

protein complex/Cy5.5<sup>®</sup> (PerCP/Cy5.5), anti-hCD8-APC/Cy7, anti-hgranzyme B-fluorescein isothiocyanate (FITC), and anti-hCD72-FITC (BD Biosciences); anti-hCD8-phycoerythrin (PE)/Cy7, anti-hCD45RA-PE/Cy7, anti-hCD100-APC, anti-hCD100-FITC, anti-hIFN- $\gamma$ -PE, anti-hperforin-PerCP/Cy5.5<sup>®</sup>, and anti-hTNF- $\alpha$ -PE (BioLegend, San Diego, CA, USA); anti-hCCR7-PerCP, anti-hCD100-PE, anti-hplexin B1-PE, and anti-hplexin B2-PerCP (R&D Systems, Minneapolis, MN, USA); and anti-hCD28 (Invitrogen, Carlsbad, CA, USA). The data were analysed with the FlowJo 7.6 software (Tree Star, Inc., Ashland, OR, USA).

### Cell culture and HCV transfection

Naïve CD8<sup>+</sup> T cells from the healthy controls and patients were isolated with a Naïve CD8<sup>+</sup> T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were washed with RPMI 1640 and then used for co-culture, stimulation, and a flow-cytometric analysis. Naïve CD8<sup>+</sup> T cells, PBMCs, and/or Huh 7.5 cells (American Type Culture Collection, Manassas, VA, USA) were co-cultured with Dulbecco's modified Eagle's medium (Mediatech Inc., Manassas, VA, USA) containing 2% foetal bovine serum (Life Technologies, Grand Island, NY, USA), 0.05 mM  $\beta$ -mercaptoethanol, 2 M L-glutamine (Mediatech Inc.), and 100  $\mu$ g/ml penicillin-streptomycin. JFH/Huh 7.5 cells were produced by transfecting cells with the FL-J6/JFH chimeric strain of HCV. Briefly, HCV FL-J6/JFH mRNA was transcribed with a TranscriptAid T7 High Yield Transcription Kit (Fermentas, Vilnius, Lithuania), as previously described.<sup>31</sup> Huh7.5 cells were transfected with the transcribed mRNA using DMR1E-C Reagent (Invitrogen). The transfected Huh7.5 cells were collected after 24 h for further experiments. The transfection efficiency was verified with an anti-NS5

antibody (BD Bioscience), as shown in Supplementary Figure 1(a).

### ELISA analyses

The secretion of IFN- $\gamma$  and TNF- $\alpha$  was evaluated with the Human IFN- $\gamma$  Platinum ELISA and Human TNF-alpha Platinum ELISA kits, respectively (eBioscience), according to the manufacturer's protocols. Briefly, diluted standard proteins and samples were added to each well, the plates were incubated at 4°C overnight, and antibodies were added after the samples were blocked. The substrate solutions were added and the reactions were stopped with 25  $\mu$ l of 2 M H<sub>2</sub>SO<sub>4</sub>. The results were determined with a microplate reader (BioTek; Shanghai, China). The IFN- $\gamma$  and TNF- $\alpha$  concentrations were calculated from standard curves using CurveExpert 1.3.

### Cytotoxicity assay

PBMCs were stimulated with CD100-over-expressing naïve CD8<sup>+</sup> T cells one day before their co-incubation with HCV-infected Huh7.5 cells for 2 days. PBMC cytotoxicity was detected with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based *In Vitro* Toxicology Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). Briefly, an amount of reconstituted MTT equal to 10% of the culture medium volume was added to each culture well and incubated for 2 h. The culture medium was removed from each well, and the resulting formazan crystals were dissolved in MTT Solubilization Solution. The samples were measured with a microplate reader (BioTek) at a wavelength of 570 nm.

### Statistical analysis

Statistical analyses were performed with GraphPad Prism version 5.0 (GraphPad Software, San Diego, California, USA)

and IBM SPSS Statistics 21.0 (IBM Corp., New York City, NY, USA). The Mann–Whitney *U* test or one-way ANOVA was used to compare different groups, and a paired *t* test or the Wilcoxon matched-pairs test was used to compare paired variables, depending on the data distribution. \**P* < 0.05 was considered significant.

## Results

### *Proportion of naïve CD8<sup>+</sup> T cells is increased in HCV-infected patients*

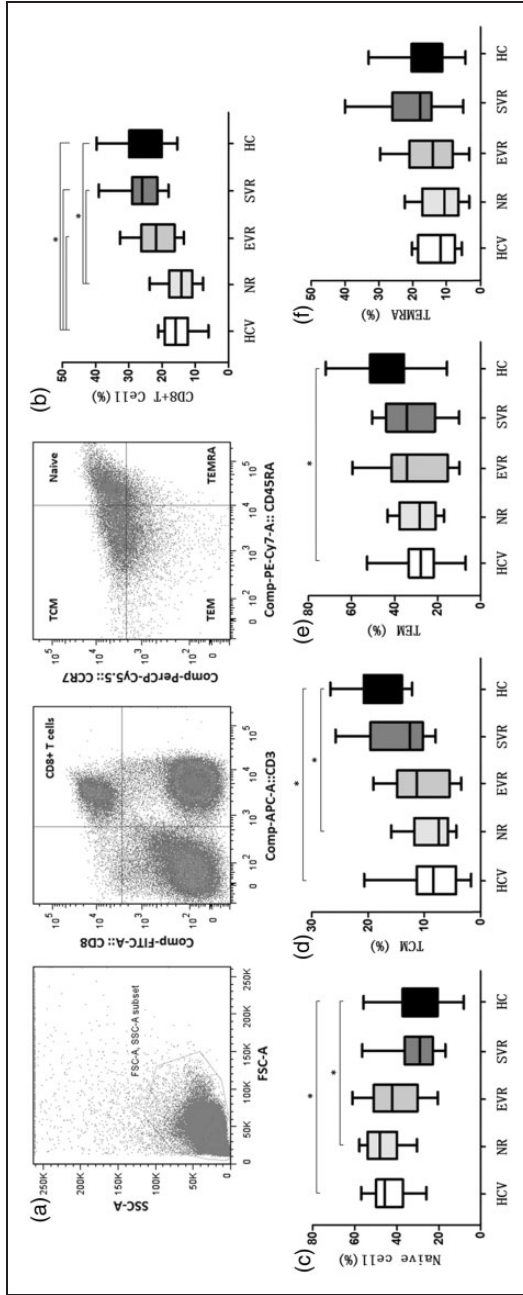
To evaluate the effects of HCV infection on CD8<sup>+</sup> T cells, the cell frequencies were assessed with flow cytometry. The total number of CD8<sup>+</sup> T cells was lower in the HCV patient group than in the healthy controls or IFN- $\alpha$ -treated EVR and SVR patients. To further investigate the changes in the CD8<sup>+</sup> T-cell subsets, the CD8<sup>+</sup> T cells were divided into naïve T cells (CD45RA<sup>+</sup>CCR7<sup>+</sup>), central memory T cells (TCM, CD45RA<sup>-</sup>CCR7<sup>+</sup>), effector memory T cells (TEM, CD45RA<sup>-</sup>CCR7<sup>-</sup>), and terminally differentiated effector memory T cells (TEMRA, CD45RA<sup>+</sup>CCR7<sup>-</sup>), based on staining for CCR7 and CD45RA.<sup>36,37</sup> The naïve CD8<sup>+</sup> T-cell subset was significantly larger in the chronically HCV-infected patients than in the SVR patients or healthy controls, whereas the TCM and TEM subsets were usually reduced during HCV infection (Figure 1).

Among the CD8<sup>+</sup> T-cell subsets, TEM and TCM are thought to be functional immune cells, whereas the naïve cells are regarded as a potential cell pool for immune development. The TEM subset provides immediate effector functions for antiviral responses, whereas the TCM subset acts as a self-renewing source for the total pool of memory cells.<sup>37</sup> Therefore, reductions in TCM and TEM in the circulation reflect the impairment of the adaptive immune responses during HCV infection.

### *IFN- $\alpha$ -based treatment upregulates CD100<sup>+</sup> naïve CD8<sup>+</sup> T cells during HCV infection*

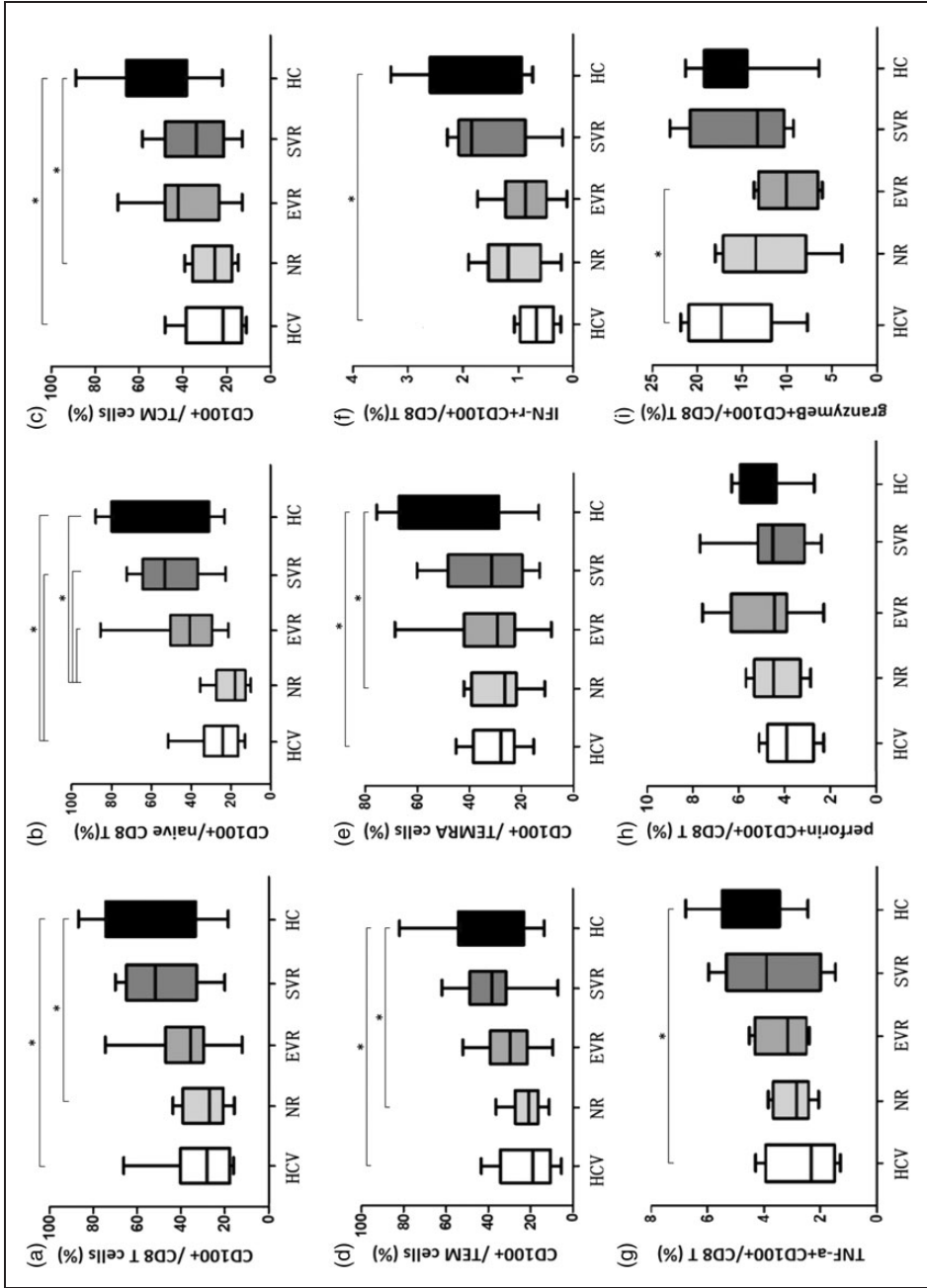
CD100 is an important positive regulator of the immune system.<sup>28,29</sup> In this experiment, CD100 expression on the CD8<sup>+</sup> T-cell subsets was investigated. CD100<sup>+</sup>CD8<sup>+</sup> T cells generally declined during HCV infection compared with those in the IFN- $\alpha$ -treated EVR/SVR patients and healthy controls. Similarly, CD100 expression on the naïve, TCM, TEM, and TEMRA T-cell subsets also decreased to some degree. Interestingly, CD100<sup>+</sup> naïve CD8<sup>+</sup> T cells were significantly elevated in the IFN- $\alpha$ -treated SVR patients. However, in the NR patients, the frequency of CD100<sup>+</sup> naïve CD8<sup>+</sup> T cells was comparatively low (Figure 2(a)–(e)). Naïve T cells are thought to be less functional in direct antiviral responses, but the upregulation of CD100 expression on these cells potentially promotes their antiviral functions by increasing their capacity for signal activation.

It has been reported that as a receptor, CD100 transduces signals through the extracellular regulated kinase (ERK) signalling pathway.<sup>32,38,39</sup> Therefore, the upregulation of CD100 could directly promote CD8<sup>+</sup> T-cell functions by activating CD100–ERK signalling. To test this hypothesis, we analysed the correlation between CD100 and functional cytokines, such as IFN- $\gamma$ , TNF- $\alpha$ , perforin, and granzyme B, in CD8<sup>+</sup> T cells. IFN- $\gamma$  and TNF- $\alpha$  are considered important T-cell cytokines, whereas perforin and granzyme B are closely associated with cytotoxicity.<sup>16,19</sup> CD8<sup>+</sup> T cells were co-cultured with HCV-infected Huh7.5 cells (Huh 7.5 cells transfected with the HCV strain JFH-1 genome). Cytokine expression was measured with flow cytometry. Although differences in cytokine expression were detected in the CD100-expressing CD8<sup>+</sup> T cells of the HCV patients and healthy controls (Figure 2 (f)–(i)), no

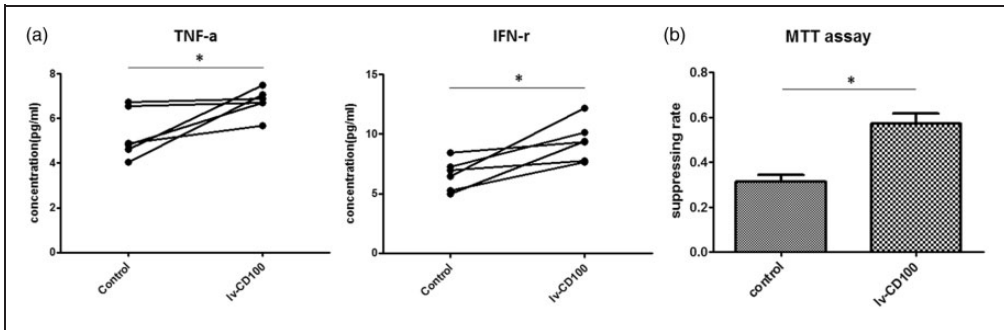


**Figure 1.** Proportions of CD8<sup>+</sup> T-cell subsets in HCV-infected patients. PBMCs from HCV patients (HCV), non-responders (NR), early virological response patients (EVR), sustained virological response patients (SVR), and healthy controls (HC) were analysed with flow cytometry. (a) Gating strategy for CD8<sup>+</sup> T-cell subsets from PBMCs in the flow-cytometric assay; (b) CD8<sup>+</sup> T-cell variations in the HCV, NR, EVR, SVR and HC groups. Frequencies of naive cells (c), TCM (d), TEM (e), and TEMRA (f) were also investigated as described.





**Figure 2.** Variations in CD8<sup>+</sup> T-cell subsets and cytokine secretion associated with CD100 expression. (a) CD100 levels on CD8<sup>+</sup> T cells were observed in HCV, NR, EVR, SVR, and HC groups; expression levels on naive CD8<sup>+</sup> T cells (b), TCM (c), TEM (d), and TEMRA (e) were investigated. IFN- $\gamma$  (f), TNF- $\alpha$  (g), perforin (h), and granzyme B (i) expression levels were analysed in the different groups of CD100<sup>+</sup>CD8<sup>+</sup> T cells.



**Figure 3.** CD100-overexpressing naïve CD8<sup>+</sup> T cells show increased PBMC IFN- $\gamma$ /TNF- $\alpha$  secretion and cytotoxicity.

(a) Naïve CD8<sup>+</sup> T cells (with or without CD100 overexpression) were co-cultured with PBMCs, and IFN- $\gamma$  and TNF- $\alpha$  secretion was examined in each group; (b) naïve CD8<sup>+</sup> T cells (with or without CD100 overexpression) and PBMCs were co-cultured before incubation with HCV-infected Huh7.5 cells. Cytotoxicity was measured with an MTT assay.

correlations were detected between cytokine secretion and CD100 expression after IFN- $\alpha$  treatment (Supplementary Figure 1(b)), suggesting that the CD100 molecules on CD8<sup>+</sup> T cells have little direct effect on the functions of these cells.

### *CD100 on naïve CD8<sup>+</sup> T cells upregulates IFN- $\gamma$ and TNF- $\alpha$ secretion from PBMCs*

We then investigated whether CD100 expression on naïve cells influences the immune responses through cell–cell interactions associated with CD100 receptors. A CD100-overexpressing lentiviral vector, Lv-hCD100, was constructed. Naïve CD8<sup>+</sup> T cells from healthy donors were isolated and transfected with the vector, and the cells were then co-cultured with the total PBMCs from each individual donor. Cytokine expression, including that of IFN- $\gamma$  and TNF- $\alpha$ , was examined in each group. As expected, the CD100-overexpressing naïve CD8<sup>+</sup> T cells significantly stimulated the secretion of IFN- $\gamma$  and TNF- $\alpha$  from PBMCs (Figure 3(a)).

To further investigate the promotion of cytotoxicity by the CD100 on naïve CD8<sup>+</sup> T

cells, PBMCs from healthy donors were first co-incubated with CD100-overexpressing naïve CD8<sup>+</sup> T cells and then with HCV-infected Huh7.5 cells (Huh 7.5 cells transfected with the HCV JFH-1 genome). Cytotoxicity was measured with an MTT assay. Stimulation by CD100-overexpressing naïve CD8<sup>+</sup> T cells enhanced the cytotoxicity of the Huh7.5 cells more strongly than did the blank control cells (Figure 3(b)). In summary, our results suggest that the upregulation of CD100 on naïve CD8<sup>+</sup> T cells promotes antiviral responses by promoting cytokine secretion and enhancing cytotoxicity.

### *CD100 induces IFN- $\gamma$ and TNF- $\alpha$ expression through its receptor CD72*

To exclude cell-interaction-independent effects among cells, naïve CD8<sup>+</sup> T cells, with or without CD100 overexpression, were co-cultured with PBMCs in a transwell apparatus, which blocked any direct inter-cellular interaction between the two cell types. Under these conditions, CD100-upregulated naïve CD8<sup>+</sup> T cells lost their ability to enhance IFN- $\gamma$  and TNF- $\alpha$  secretion



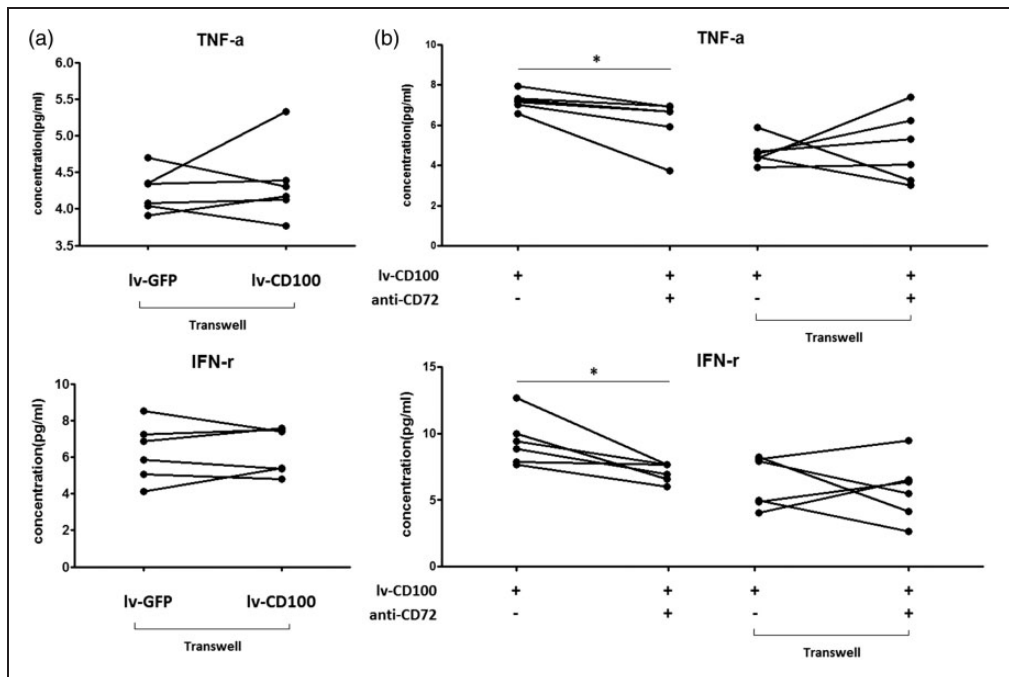
(Figure 4(a)), suggesting that their promotion of cytokine expression depends on direct cell–cell interactions.

Most immune cells express CD72,<sup>28</sup> which raises the possibility that the increased CD100 on naïve CD8<sup>+</sup> T cells functions through the downstream activation of the CD72 signal. If this hypothesis is correct, an anti-CD72 neutralizing antibody should disrupt the signal activated through CD100–CD72. Naïve CD8<sup>+</sup> T cells from healthy donors were transfected with the lentiviral vector Lv-hCD100. The cells were co-cultured with isolated PBMCs from each individual donor, with or without the addition of an anti-CD72 antibody, and the IFN- $\gamma$  and TNF- $\alpha$  expression levels were measured with ELISAs. As expected, the anti-CD72 neutralizing antibody

significantly downregulated IFN- $\gamma$  and TNF- $\alpha$  expression in the context of direct cell–cell interactions, but not in the cells in the Transwell apparatus (Figure 4(b)), indicating that the upregulated CD100 on naïve CD8<sup>+</sup> T cells induces IFN- $\gamma$  and TNF- $\alpha$  expression through its receptor CD72.

## Discussion

The traditional treatment for CHC is based on a combination of PEGylated IFN- $\alpha$  and ribavirin. With this regimen, SVR rates can reach approximately 70%–90%, depending on the infecting genotype(s), the disease baseline features, and the virological response patterns.<sup>40</sup> However, the traditional therapy is accompanied by several adverse effects, which are occasionally



**Figure 4.** CD100 induces IFN- $\gamma$  and TNF- $\alpha$  expression through its receptor CD72.

(a) Naïve CD8<sup>+</sup> T cells (with or without CD100 overexpression) were co-cultured with PBMCs in a Transwell apparatus, and TNF- $\alpha$  and IFN- $\gamma$  secretion was examined; (b) CD100-overexpressing naïve CD8<sup>+</sup> T cells and PBMCs were co-cultured under different conditions. TNF- $\alpha$  and IFN- $\gamma$  expression levels were examined with or without CD72 antibody neutralization.

serious. Therefore, efforts have been made to identify novel direct-acting antivirals (DAAs) that target the NS3/4A serine protease, NS5B polymerase, or other viral proteins. These new drugs include simeprevir, asunaprevir, and paritaprevir, which target NS3/4A, and sofosbuvir and dasabuvir, which target NS5B. The SVR rates for these new drugs can reach >90%.<sup>41</sup> However, the antiviral mechanisms of IFN- $\alpha$  and DAAs differ, in that IFN- $\alpha$  is a general activation drug that upregulates the immune responses, whereas DAAs target virus-specific processes. IFN- $\alpha$  therapy is a potent model of immune network regulation.

As a positive activator, CD100 plays important roles in both the humoral and cellular immune responses.<sup>25</sup> CD100 is involved in the T-cell responses during HIV infection<sup>42</sup> and in B-cell activities during chronic HCV infection.<sup>23</sup> However, it is still unclear whether CD100 on the naïve CD8<sup>+</sup> T-cell subset plays a role during IFN- $\alpha$ -based anti-HCV therapy. Here, we analysed the frequency and phenotype of the CD8<sup>+</sup> T cells associated with IFN- $\alpha$ -based treatment in HCV patients.

It is well known that HCV infection is associated with disturbances of the activation and polyclonal proliferation of T lymphocytes. The host's adaptive immune responses largely determine whether the virus is spontaneously eradicated or persists. HCV-specific CD4<sup>+</sup> T cells are exhausted and deleted in most persistently infected HCV patients, which in turn induces CD8<sup>+</sup> cell impairment.<sup>43</sup> However, according to our results, the subset of naïve CD8<sup>+</sup> T cells increases significantly in chronically HCV-infected patients, representing an important potential pool of antiviral activity. This upregulated naïve CD8<sup>+</sup> T cell pool may also be a potential drug target during HCV infection. This suggests a situation in which naïve CD8<sup>+</sup> T cells acquire dominance among the CD8<sup>+</sup> T-cell subsets, but exert only weak antiviral responses. Interestingly,

our results indicate that an IFN- $\alpha$ -based treatment could increase the expression of CD100 on naïve CD8<sup>+</sup> T cells, promoting the functions of these cells.

We have also shown that the increased CD100 on naïve CD8<sup>+</sup> T cells enhanced cytokine secretion and cytotoxicity through cell-cell interactions among immune cells, which was disrupted by an anti-CD72 neutralizing antibody, indicating that the IFN- $\alpha$ -induced CD100 on naïve CD8<sup>+</sup> T cells promotes immune-cell responses through CD100-CD72 signal transduction. The cytoplasmic domain of CD72 contains two ITIMs, which are associated with the inactivation of many immune signal responses. CD100 ligation could block negative CD72 signalling, thus promoting the immune responses. CD72 is broadly expressed on immune cells, including B cells, dendritic cells, macrophages, mast cells, and other cell types.<sup>43</sup> Although specific CD72-expressing cells were not investigated in this study, the increased CD100 on naïve CD8<sup>+</sup> T cells probably interacts with these other cell types, inducing antiviral responses. This represents an important mechanism of the immune stimulation triggered by IFN- $\alpha$  treatment.

### **Acknowledgements**

The authors thank Dr Charles M. Rice for kindly providing reagents and the HCV plasmid. This study was supported by the National Natural Science Foundation of China (81300314 and 81273218).

### **Declaration of conflicting interest**

The authors declare that there are no conflicts of interest.

### **Funding**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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