Apoptotic Epitope–Specific CD8+ T Cells and Interferon Signaling Intersect in Chronic Hepatitis C Virus Infection

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CD8+ T cells specific to caspase-cleaved antigens derived from apoptotic T cells represent a principal player in chronic immune activation. Here, we found that both apoptotic epitope–specific and hepatitis C virus (HCV)–specific CD8+ T cells were mostly confined within the effector memory (EM) or terminally differentiated EM CD45RA+ cell subsets expressing a dysfunctional T-helper 1–like signature program in chronic HCV infection. However, apoptotic epitope–specific CD8+ T cells produced tumor necrosis factor α and interleukin 2 at the intrahepatic level significantly more than HCV-specific CD8+ T cells, despite both populations expressing high levels of programmed death 1 receptor. Contextually, only apoptotic epitope–specific CD8+ T cells correlated with both interferon-stimulated gene levels in T cells and hepatic fibrosis score. Together, these data suggest that, compared with HCV-specific CD8+ T cells, apoptotic epitope–specific CD8+ T cells can better sustain chronic immune activation, owing to their capacity to produce tumor necrosis factor α, and exhibit greater resistance to inhibitory signals during chronic HCV infection.

Keywords. hepatitis C virus; apoptosis; CD8+ T cells; chronic immune activation.

The phenomenon of chronic immune activation (CIA) [1–3] is a hallmark of several chronic immune-mediated diseases and is attributed to polyclonal hyperactivation of B and T cells caused by both antigen-dependent stimuli (eg, self-antigens) and antigen-independent stimuli (eg, bystander activation) [1, 4]. Generally, CIA occurs subsequent to a primary immune response specific to persistent infections and, ultimately, amplifies chronic immunopathology [5, 6]. In human immunodeficiency virus (HIV) infection, CIA is closely linked to CD4+ T-cell apoptosis and depletion, sustains viral replication, and is associated with progression toward AIDS [1, 2, 4]. Recent experimental evidence has demonstrated that a direct causal link exists between disease progression in persistent viral infections and chronic interferon type I (IFN-I) signaling, CIA, and increased expression of negative immune regulatory molecules and that blockade of IFN-I signaling diminishes CIA and immune suppression, ultimately redirecting the immune environment to enable control of infection [7, 8].

We previously proposed that the enormous number of apoptotic cells resulting from the rapid turnover of effector T cells during inflammatory diseases contributes to establishing CIA, based on the following 3 pieces of evidence [2, 5, 9, 10]. First, apoptotic cells derived from activated T cells retain the expression of CD40 ligand (L) and, in contrast to CD40L− apoptotic cells (eg, those derived from epithelial cells), can condition CD40+ dendritic cells (DCs) to acquire high stimulatory capacities [11–13]. Second, apoptotic T cells that have been phagocytosed by DCs can release, within phagosomes, caspase-cleaved fragments, particularly from actin cytoplasmic 1 (ACTB), heterogeneous nuclear ribonucleoprotein, lamin B1, nonmuscle myosin heavy chain 9 (MYH9), vimentin (VIME), proteasome component C2, rho guanosine diphosphate dissociation inhibitor 2, and 60S acidic ribosomal protein P2. Third, these fragments are efficiently translocated into cytosol, processed, and, ultimately, cross-presented as distinct epitopes (apoptotic epitopes) on major histocompatibility complex class I molecules to a wide repertoire of autoreactive apoptotic epitope–specific CD8+ T cells [2, 5]. In chronic HIV or acute hepatitis C virus (HCV) infections, the proportion of apoptotic epitope–specific CD8+ T cells correlates with the proportion of circulating apoptotic CD4+ T cells in vivo and with disease progression, supporting the opinion that apoptotic T cells increase in relation to the level of T-cell activation during the disease evolution and represent a preponderant source of the apoptotic antigens [2, 3]. The emergence and the maintenance of these responses contribute to intensify the CIA [1–3], not only in viral infections but also in autoimmune diseases such as multiple sclerosis [14] or rheumatoid arthritis [15], through
their capacity to produce high levels of inflammatory cytokines in response to apoptotic epitopes. Therefore, CIA induced by CD8+ T-cell autoimmunity to apoptotic epitopes seems to be a general mechanism in both viral and autoimmune diseases.

To examine in depth the multifaceted aspects of the amplification and spreading of CIA, we studied the dynamics of both apoptotic epitope–specific and virus-specific CD8+ T-cell responses in chronic HCV infection. Despite the recent availability of highly effective direct-acting antiviral agents providing complete viral clearance [16], HCV infection remains, to date, a unique example of a natural, extremely widespread infection that can be exploited to understand the intersecting mechanisms of chronic immunopathology. Studies on the natural history of HCV infection indicate that chronic evolution correlates with the acquisition of HCV-specific T-cell dysfunction [17–23], which has been particularly associated with the expression of programmed death 1 (PD-1) receptor and/or T-cell immunoglobulin mucin 3, inducing T-cell exhaustion upon contact with the corresponding ligands [23–28].

Our data show that the frequency of apoptotic epitope-specific (but not HCV-specific) CD8+ T cells correlates with the evolution of chronic HCV infection and the level of various IFN-stimulated genes (ISGs), suggesting that ISGs can regulate apoptotic epitope–specific CD8+ T cells in establishing CIA and that apoptotic epitope–specific CD8+ T cells may ultimately amplify liver immunopathology, particularly by the production of tumor necrosis factor α (TNF-α).

PATIENTS AND METHODS

Please refer to the Supplementary Materials for more-detailed descriptions.

Patients

Twenty HLA-A2+ patients (11 males and 9 females) with chronic HCV infection were enrolled in this study for diagnostic evaluation and follow-up assessment. The clinical characteristics of these patients are shown in Supplementary Table 1.

Samples and Processing

The isolation of peripheral blood mononuclear cells (PBMCs) and liver-infiltrating lymphocytes (LILs) and the detection of T-cell apoptosis are detailed in the Supplementary Materials.

Synthetic Peptides

The descriptions of procedures and lists of synthetic peptides (Supplementary Tables 2 and 3) are reported in the Supplementary Materials.

Ex Vivo and In Vitro Assays

The methods for assays of enzyme-linked immunospot (ELISPOT), PD-1–dependent inhibitory function, RNA purification, and real-time reverse-transcription polymerase chain reaction analysis of ISGs are described in the Supplementary Materials.

Flow Cytometry

The descriptions of procedures for T-cell surface or intracellular staining and for functional assays, as well as a list of antibodies or dextramers used for flow cytometry, are reported in Supplementary Table 4. Monocytes, natural killer cells, B cells, and dead cells were excluded by staining with anti-CD14, -CD16, -CD19, and -CD56 monoclonal antibodies (mAbs) and Fixable Viability Dye eFluor 780 (dump channel).

Statistical Analyses

The collected data underwent statistical analysis with GraphPad Prism 6 software (GraphPad Software). Comparisons between healthy donors and patients and between patients with different responses to therapy were analyzed with the Mann–Whitney U test. Comparisons between patients at different time points were analyzed with the Wilcoxon matched-pairs signed rank test. Correlations between tests and clinical data were analyzed with linear regression analysis or the Spearman rank coefficient test. In all tests, a P value of <.05 was considered statistically significant.

RESULTS

HCV- and Apoptotic Epitope–Specific CD8+ T Cells Parallel in Patients with Chronic HCV Infection

The capacity to form IFN-γ spots (as detected by the ELISPOT assay) in response to both HCV epitope and apoptotic epitope pools by CD8+ T cells was significantly higher and wider in patients with chronic HCV infection than in healthy donors (Supplementary Figure 1A and 1B). However, despite the multispecificity of these IFN-γ–producing CD8+ T cells in patients with chronic HCV infection, they appeared at very low frequencies. No correlation was found between the ELISPOT IFN-γ responses to HCV epitopes or apoptotic epitopes and various clinical markers of hepatitis activity (alanine aminotransferase [ALT] level, HCV load, and histological activity index [HAI]). Thus, we enumerated directly ex vivo the entire HCV- or apoptotic epitope–specific CD8+ T-cell populations, irrespective of their function or differentiation phase, by using both dextramers of HLA-A*0201 molecules complexed to MYH9741-749, VIME78-87, VIME225-233, or ACTB266-274 peptides and dextramers of HLA-A*0201 molecules complexed to HCV−NS31073-1082, −NS31406-1415, −NS4b1807-1817, −NS5b2727-2736, or −Core132-140 peptides (Figure 1A), in combination with anti-CD8 mAb. Circulating frequencies of these cells were quantified using a gating strategy that excluded dead cells, monocytes, natural killer cells, and B cells in a dump channel containing Fixable Viability Dye eFluor780 together with anti-CD14, anti-CD16, anti-CD56, and anti-CD19 mAbs conjugated to APCeFluor780 (Supplementary Figure 2A). Control dextramers complexed to a nonnatural irrelevant peptide (ALIAPVHAV) were unable to stain CD8+ T cells in all samples tested (Figure 1A and Supplementary Figure 2B): these analyses represented the basis on which we set up dextramer+ cell gates of antigen-specific CD8+ T cells.
T cells, allowing us to exclude cells with unspecific staining. Interestingly, 20 HLA-A2–positive patients with chronic HCV infection (including the 14 studied for the ELISPOT assay; Supplementary Table 1) presented parallel frequencies of peripheral apoptotic epitope– or HCV–specific (dextramer+) CD8+ T cells that were significantly higher than in 13 healthy donors studied, calculated both as a single dextramer percentage (Figure 1B) and as the sum of dextramer percentages by a single patient or healthy donor (Figure 1C). It is notable that the relatively high frequency of HCV–specific CD8+ T cells in some healthy donors (Figure 1B), likely due to the cross-reactivity between NS3_1073-82 and influenza virus neuraminidase231-239 epitopes known to increase both the response and frequency of these CD8+ T cells [29, 30]. In addition, apoptotic epitope–specific (but not HCV–specific [data not shown]) CD8+ T cells positively correlated with HAI staging (ie, the HAI of liver fibrosis; Figure 1D) but not with ALT level or viral load (data not shown). A considerable proportion of both HCV–specific and apoptotic epitope–specific CD8+ T cells tested expressed CD95 and, to a lesser degree, the late activation marker HLA-DR, instead of the early activation marker CD69, indicating that they are long-term experienced memory T cells, as expected in a chronic infection setting (Figure 2A). Interestingly, dextramer+ T cells from patients with chronic HCV infection expressed an activation phenotype similar to the dextramer+ T cells (Figure 2A). Activated (HLA-DR+) HCV–specific (but not apoptotic epitope–specific [data not shown]) CD8+ T cells positively correlated with ALT level (representing a reliable marker of hepatocyte lysis; Figure 2B), which suggests that they are involved in the lysis of hepatocytes expressing the viral epitopes. In addition, a notable proportion of both apoptotic epitope–specific and HCV–specific CD8+ T cells expressed the proliferation marker Ki67 (in contrast to the dextramer+ cells), suggesting that they likely proliferated in response to the relevant

Figure 1. Comparison of characteristics of apoptotic epitope (AE)–specific and hepatitis C virus (HCV)–specific dextramer+ CD8+ T cells in individuals with chronic HCV infection and healthy donors (HDs). A, Flow cytometry of dextramer+ CD8+ T cells specific to the indicated AEs (left) or HCV epitopes (right) in a patient and a HD. The gates of dextramer+ antigen–specific CD8+ T cells were set up on the basis of the control dextramers complexed to a nonnatural irrelevant peptide (dextramer–) that were unable to stain CD8+ T cells. B, Percentage of AE–specific (left) or HCV–specific (right) dextramer+ CD8+ T-cell populations in 13 healthy donors and 20 patients (each symbol represents a single dextramer+ CD8+ T-cell percentage, and the gray symbols represent dextramer+ CD8+ T cells specific to NS3_1073-82). C, Sum of the percentages of AE–specific (left) or HCV–specific (right) dextramer+ CD8+ T-cell populations in 13 healthy donors and 20 patients (each symbol represents a single patient). ****P < .00001. D, Correlation between the sum of percentages of the various AE–specific CD8+ T-cell populations and histological activity index staging. Data are mean values ±SD, unless otherwise indicated. *P < .01 and ****P < .0001, by the Mann–Whitney U test (B and C) and Spearman correlation (D).
antigenic epitopes in vivo (Figure 2C). In support of this possibility, both the HCV- and apoptotic epitope–specific CD8⁺ T cells tested were capable of degranulation (as detected by CD107a up-regulation) in response to the relevant peptides ex vivo (Figure 2D), thereby clearly validating their antigen specificity.

**Divergent Functional Capacity of Peripheral and Intrahepatic Apoptotic Epitope–Specific or HCV-Specific CD8⁺ T Cells**

Both apoptotic epitope–specific and HCV-specific CD8⁺ T-cell populations were mostly represented by effector memory (TEM) T cells (CCR7⁻CD45RA⁺) and by terminal effector memory RA (TEMRA) T cells (CCR7⁻CD45RA⁺) containing significantly higher percentages of cells coexpressing both the nuclear transcription factor eomesodermin and the transcription factor T-box (Eomes⁺/T-bet⁺) than cells expressing eomesodermin but not T-box (Eomes⁺/T-bet⁻; Figure 3A–C). As a control, CCR7, CD45RA, EOMES, and T-bet gates in the total CD8⁺ population from patients with chronic HCV infection are reported (Supplementary Figure 3). As expected, Eomes and T-bet were barely represented in the naive T (TN) or central memory T (TCM) cell populations within both apoptotic epitope–specific and HCV-specific CD8⁺ T cells (Figure 3B). Although the percentage of PD-1⁺ cells was nearly similar within the total apoptotic epitope–specific and HCV-specific CD8⁺ T cells (Supplementary Figure 4A), the Eomes⁺/T-bet⁻ subset within both apoptotic epitope–specific and HCV-specific CD8⁺ T-cell populations expressed significantly more PD-1 than did the Eomes⁺/T-bet⁻ subset, providing evidence that Eomes⁺/T-bet⁻ cells are mostly PD-1⁻ (Figure 4A and 4B) [31, 32]. The different HCV genotypes of our patients did not seem to influence PD-1 expression, based on the following observations: (1) no significant difference in PD-1 expression by HCV-specific CD8⁺ T cells was observed between HCV genotype 1–infected individuals and HCV genotype 2– or 3–infected individuals (Supplementary Figure 4B); and (2) PD-1 was significantly (or tended to be) less expressed by Eomes⁺/T-bet⁻ cells than by Eomes⁺/T-bet⁻ cells in both patient populations (Supplementary Figure 4C). However, although the majority of both apoptotic epitope–specific and HCV-specific CD8⁺ TEM or TEMRA cell populations expressed T-bet, a major transcription factor mediating IFN-γ production [33], they produced very low levels of IFN-γ (as we had also determined with the ELISPOT assay [Supplementary Figure 1A and 1B]) in the periphery (Figure 5A–C). To investigate whether IFN-γ–producing cells migrated into the inflamed tissue to perform their effector functions, we studied
the phenotype and function of LILs isolated from the liver biopsy specimens from patients with chronic HCV infection. Interestingly, both CD8\(^+\) LIL subsets produced significantly higher levels of TNF-\(\alpha\) in response to the related peptides ex vivo than the counterparts in the periphery (Figure 5A–C). Notably, apoptotic epitope–specific CD8\(^+\) LILs were significantly more efficient than HCV-specific CD8\(^+\) LILs in performing this function (Supplementary Figure 5) and produced significantly higher levels of interleukin 2 (IL-2) than the peripheral counterparts (Figure 5A–C). Interestingly, the percentage of apoptotic epitope–specific CD8\(^+\) LILs producing TNF-\(\alpha\) parallels, although in a nonsignificant fashion, the total HAI (grading plus staging) score (Figure 5D). In addition, greater percentages of both apoptotic epitope–specific (Figure 6A) and HCV-specific (data not shown) CD8\(^+\) TEM cells expressing HLA-DR, CD69, or PD-1 accumulated within the LILs than in the periphery in all patients tested (Figure 6A–C). Similarly, a greater percentage of dextramer CD8\(^+\) T cells that had a TEM phenotype and expressed the various activation markers analyzed accumulated in the liver than in the periphery (Figure 6B and 6C). Antigen-specific CD8\(^+\) LILs increased their capacity to produce TNF-\(\alpha\) and IL-2 upon 1 round of stimulation with autologous irradiated PBMCs in the presence of the relevant peptide for 10 days in IL-2–conditioned medium in vitro, confirming that they are mainly represented within LIL-derived memory T cells (Supplementary Figure 6). Nevertheless, PD-1 provided inhibitory signals, as evidenced by the improved capacity to produce IFN-\(\gamma\) by both peripheral apoptotic
epitope– and HCV-specific CD8+ T cells (expressing PD-1) in the presence of a blocking antibody specific to PD-L1 in vitro (Supplementary Figure 7).

Apoptotic Epitope–Specific CD8+ T Cells, CIA, and ISGs

To provide evidence of the interaction between CIA and IFN-I signaling [7, 8] in chronic HCV infection, we correlated the frequency of apoptotic epitope– or HCV-specific CD8+ T cells with the expression of the following ISG transcripts in T cells from patients with chronic HCV infection and healthy donors: protein kinase R (PKR) – an IFN-inducible double-stranded RNA–activated protein kinase [34]; myxovirus resistance A (MxA), an IFN-I–induced protein expressed in various cell types and known for its antiviral activity against orthomyxoviruses [35]; and ISG15, an IFN-I–inducible ubiquitin-like protein conjugated with many intracellular substrates via

Figure 4. Prevalent expression of programmed death 1 (PD-1) in the Eomes+/T-bet− subset of apoptotic epitope (AE)–specific or hepatitis C virus (HCV)–specific CD8+ T cells. A, Representative flow cytometry of PD-1+ cells within the Eomes+/T-bet− or Eomes+/T-bet+ cells present in AE- or HCV-specific CD8+ T cells from a patient. B, Mean of percentages of PD-1+ cells within the Eomes+/T-bet− or Eomes+/T-bet+ cells present in AE-specific (empty bars) or HCV-specific (filled bars) dextramer+ CD8+ T cells from 10 patients. *P<.01 and **P<.001, by the Wilcoxon matched-pairs test.

Figure 5. Differential functions of peripheral and liver-infiltrating apoptotic epitope (AE)–specific or hepatitis C virus (HCV)–specific CD8+ T cells. Flow cytometry of the indicated cytokines produced by AE-specific (A) or HCV-specific (B) CD8+ T cells, stained with the pool of the related dextramers, in peripheral blood mononuclear cells (PBMCs) or liver-infiltrating lymphocytes (LILs) for a patient, with or without stimulation with the pool of the relevant peptides. Dot plot analyses are gated on dextramer+ CD8+ T cells and show percentages of cytokine-producing cells. C, Percentage of cells producing the indicated cytokines in AE-specific (left) or HCV-specific (right) CD8+ T cells in response to the related peptides from PBMCs or LILs from 13 and 5 patients, respectively. Data are represented with background (no stimulation) subtracted. D, Relationship between histological activity index (HAI) and percentage of AE-specific CD8+ T cells producing tumor necrosis factor α (TNF-α). **P<.001, ***P<.0001, and ****P<.00001, by the Wilcoxon matched-pairs test. Abbreviations: IFN-γ, interferon γ; IL-2, interleukin 2; IL-17, interleukin 17.
ISGylation [36]. We evaluated the baseline and the day 30 time point (after 1 month of weekly administration of pegylated IFN alfa plus ribavirin), representing a more chronic IFN-I exposure (and thus chronic IFN-I signaling) and corresponding to 2 days after the fourth (weekly) IFN administration. The ISG transcript levels significantly increased following pegylated IFN alfa plus ribavirin therapy, indicating that T cells are susceptible to IFN-I signaling in vivo (Figure 7A). Both PKR transcript and MxA transcript levels directly correlated with the percentage of apoptotic epitope–specific (but not HCV-specific [data not shown]) CD8+ T cells in patients with chronic HCV infection at baseline (Figure 7) and after 1 month of therapy (data not shown), supporting previous evidence showing an association between sustained ISG expression (including MxA and PKR) and CIA in persistent infections [7, 8, 37]. By contrast, the ISG15 level was inversely correlated with the apoptotic epitope–specific CD8+ T-cell frequency (Figure 7), consistent with the recent evidence showing a strict association of ISG15 deficiency with increased immunopathology [38].

DISCUSSION

Our findings suggest that apoptotic epitope–specific CD8+ T cells play an important role in establishing CIA and disease progression in chronic HCV infection. The finding that both
antigen-specific (apoptotic epitope– or HCV-specific) dextramer+ CD8+ T cells and dextramer– CD8+ T cells expressed an activation phenotype (particularly when they migrated into inflamed liver) suggests that a global (polyclonal) T-cell activation including both activated apoptotic epitope–specific and HCV-specific CD8+ T cells contributes to CIA development during chronic HCV infection. Virus-specific and apoptotic epitope–specific CD8+ T cells may favor the recruitment of independent CD8+ T-cell populations and, likely, inflammatory innate immune cells, which contribute to magnify CIA via the production of a storm of cytokines (including IFNs and TNFs) [39, 40]. The evidence showing that activated (HLA-DR+) apoptotic epitope–specific (but not HCV-specific) CD8+ T cells correlated with the liver fibrosis score suggests that they may be involved in amplifying liver inflammation, ultimately driving the progression to fibrosis, likely via the production of multiple cytokines, including TNF-α [2, 5]. These data are consistent with results showing that the frequency of apoptotic epitope–specific CD8+ LILs producing TNF-α (which was produced by a significantly greater percentage of apoptotic epitope–specific CD8+ T cells, compared with HCV-specific CD8+ T cells in the liver) parallels the HAI (including grading [ie, inflammatory index] and staging [ie, fibrosis index]), thus supporting the possibility that they can help mediate immunopathology ultimately leading to fibrosis, likely via the activation of hepatic stellate cells [41].

The possibility that apoptotic epitope–specific CD8+ T cells may contribute to CIA development is emphasized by the correlation between their frequency and some ISG transcript levels. The direct correlation between apoptotic epitope–specific CD8+ T-cell frequency and MxA or PKR is consistent with previous experimental data showing that sustained expression of ISGs (including MxA and PKR) is associated with CIA development and disease progression in persistent infections and that blockade of IFN-I signaling diminishes CIA and facilitates clearance of the persistent infection [7, 8, 37, 42]. On the contrary, our observation of an inverse correlation between the ISG15 level and the apoptotic epitope–specific CD8+ T-cell frequency suggests that ISG15 counterbalances MxA and PKR functions, ultimately helping to limit chronic inflammation [38]. In support of this hypothesis, recent data indicate that inherited ISG15 deficiency in humans is, paradoxically, associated with increased IFN-I immunity via the destabilization of ubiquitin–specific protease 18 (USP18; itself an ISG), which is a negative regulator of the IFN-I responses [38]. Collectively, our findings support the idea that the balance between proinflammatory (eg, MxA and PKR) and antiinflammatory (eg, ISG15/USP8 axis) ISGs may contribute to establishing a status of low-grade/long-lasting CIA in chronic HCV infection. Studies are required to screen additional ISGs [43] for possible correlations with the generation of CIA in a larger cohort of patients with chronic HCV infection.

Recently, the division of virus-specific CD8+ T cells into Eomes+/T-bet– and Eomes+/T-bet+ cells has been validated to discriminate dysfunctional and functional cells, respectively, in various chronic viral infections [22, 44], based on previous experimental data showing that T-bet expression in memory T cells is associated with the control of chronic infection through downregulation of PD-1 [31, 32] and that Eomes is associated with memory and with the control of chronic

![Figure 7](https://academic.oup.com/jid/article-abstract/213/4/674/2459401/674)
infection when it is coexpressed with T-bet [32]. We found that the majority of both apoptotic epitope–specific and HCV-specific CD8+ T cells (in both the periphery and the inflamed livers) were confined within TEM and TEMRA cell subsets displaying a dysfunctional T-helper type 1–like program because they produced very low or undetectable amounts of IFN-γ, despite the notable expression of the related transcription factor T-bet. However, both HCV-specific and, to a significantly higher extent, apoptotic epitope–specific CD8+ T cells increased their capacity to produce TNF-α and IL-2 in response to the relevant antigenic peptides when they migrated into the inflamed liver, despite a concomitant increase in PD-1 expression on these cells. The dichotomy between the poor IFN-γ or IL-17 production and the notable TNF-α production by intrahepatic CD8+ T cells may be determined by the possibility that these cells have lost the polyfunctional capacity that was previously observed in patients resolving acute HCV infection [3], likely due to the progressive establishment of the T-cell exhaustion process in chronic infections [28]. In the present study, apoptotic epitope–specific CD8+ LILs were more efficient than virus-specific LILs in producing TNF-α. This finding is likely because apoptotic epitope–specific CD8+ T cells interact with tissue-resident antigen-presenting cells that cross-present apoptotic T cells and deliver stimulatory (in addition to inhibitory) signals, whereas HCV-specific CD8+ T cells target infected hepatocytes that simultaneously express viral antigenic stimuli and various inhibitory (but not stimulatory) receptor ligands.

Our data differ substantially from those of recent studies showing that the majority of virus-specific CD8+ T cells are dysfunctional and associated with low T-bet expression in chronic hepatitis B virus (HBV) and HCV infections [22], as well as chronic HIV infection [44]. These contradictory findings may reflect the heterogeneity of these patients, thus underscoring the need to verify whether a genetic background predisposing to viral persistence harbors variants that may foster these differences. These discrepancies may also be attributed to the different functional assays (eg, in terms of cytokines produced by T cells) of peripheral and tissue-recruited lymphocytes that were performed in the various studies. The finding that TNF-α– or IL-2–producing cells were mainly recruited to the liver compartment may explain why they were visualized to a lesser extent in the periphery. In addition, some of the discrepancies could be the consequence of a mismatch of the HCV-epitope sequence in the autologous virus as a result of mutational escape of the epitope, which may profoundly change the virus-specific CD8+ T-cell phenotype, including the PD-1 expression. Further studies are required to ascertain the potential impact of such epitope mismatches on these differences.

Here we propose that both intrahepatic HCV-specific and apoptotic epitope–specific CD8+ T cells be defined as semifunctional cells because they can perform certain functions but not others: they have the ability to produce IL-2 and, in particular, TNF-α (a potent inflammatory cytokine that even contributes to hepatocellular carcinoma promotion [45, 46]), but they do not have polyfunctional capacities, including production of high levels of IFN-γ or IL-17 in response to the relevant epitopes. Our results are consistent with reports proposing that intrahepatic virus-specific CD8+ T cells are not completely exhausted in chronic HCV infection [47] and that PD-1+ T cells in chronic infections or tumors conserve a crucial level of responses that are instrumental in controlling pathogen or tumor spread and in causing minimal tissue damage [48]. Under these conditions, the persistence of functionally competent apoptotic epitope–specific CD8+ T cells would strongly promote CIA and immunopathology, particularly via their notable production of TNF-α. In this context, it should be taken into account that the application of immunotherapy inhibiting PD-1/PD-L1 interaction, despite potentially being capable of improving antiviral immune responses [49, 50], may also increase CIA when PD-1– apoptotic epitope–specific CD8+ T cells are disengaged from the PD-1–dependent control. Nevertheless, this therapy is limited in chronic HCV infection by the recent development of highly effective direct-acting antiviral agents providing complete HCV eradication [16]. Future studies will be planned to ascertain the possibility that direct-acting antiviral agents that elicit complete HCV eradication may, as a consequence, reduce/eliminate the immune cell activation that is represented by the substrate-inducing apoptotic epitope–specific T cells [2, 5].

**Supplementary Data**

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

**Notes**

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