

HIV Coinfection Impairs CD28-Mediated Costimulation of Hepatitis C Virus-Specific CD8 Cells

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Background. During human immunodeficiency virus (HIV) infection, reduced proportions of CD8 cells express CD28, the key costimulatory molecule for lymphocyte activation. However, it is unclear whether reduced CD28 expression affects immune responses to non-HIV antigens, potentially contributing to susceptibility to opportunistic infection.

Methods. We measured CD4- and CD8-specific interferon- γ responses to hepatitis C virus (HCV) peptide pools in subjects with chronic HCV mono-infection ($n = 14$), in subjects with chronic HCV/HIV coinfection ($n = 15$), and in healthy control subjects ($n = 10$) by enzyme-linked immunospot assay in the presence and absence of CD28 costimulation.

Results. Anti-CD28 agonist increased the cumulative frequency of HCV-specific CD4 cell responses in the subjects with HCV mono-infection and in those with HCV/HIV coinfection. In contrast, anti-CD28 agonist increased the breadth and cumulative frequency of HCV-specific CD8 cell responses only in the subjects with HCV mono-infection. Additionally, in the presence of anti-CD28 agonist, the proportion of subjects responding, the cumulative frequency, and the breadth of reactive CD8 cells were greater among the subjects with HCV mono-infection than among those with HCV/HIV coinfection. Finally, the HCV/HIV-coinfected subjects had lower proportions of CD8 cells that expressed CD28.

Conclusions. These results indicate that, during HCV/HIV coinfection, memory-effector CD8 cells have reduced responsiveness to CD28 costimulation. This appears to reflect a global effect that HIV has on the activation or differentiation state of CD8 cells that are responsive to other microbial pathogens. This functional defect has implications for the pathogenesis of HCV/HIV coinfection.

Hepatitis C virus (HCV) infection is found in 15%–30% of individuals infected with HIV [1, 2], contributing to increased morbidity and mortality in the HIV-infected host [3, 4]. Moreover, HIV coinfection contributes to faster rates of HCV disease progression

[5–7]. Broad and vigorous T cell immunity directed at HCV during acute infection appears to be critical for viral clearance [8–10], whereas chronic infection is characterized by a paucity of functional HCV-reactive T cells [11–16]. Additionally, HCV-reactive T cells may be even more infrequent during progressive HIV coinfection [14, 17–19], with CD4 cell count being one factor found to affect HCV-specific CD8 effector cell numbers during HCV/HIV coinfection [17]. Although the mechanism by which HIV infection impairs HCV-specific immunity is not clear, HIV infection is characterized by profound decreases in the number of circulating CD4 cells, the functional impairment of both CD4 and CD8 cells [20–24], and the down-regulation of CD3 ζ and CD28 expression [25].

CD28 is expressed on naive and early memory T cells and is key for costimulation during lymphocyte activation [26]. Ligation of CD28 at the time of T cell

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receptor (TCR) engagement leads to increased cytokine and chemokine production, resistance to apoptosis, and the promotion of long-term T cell expansion [27]. In the absence of CD28 ligation, T cells become anergic or undergo apoptosis once exposed to antigen, and mice lacking CD28 develop severe immune deficiency [28]. During HIV infection, the proportion of CD8 cells that express CD28 is reduced [25, 29]. Although this phenotype has been attributed to chronic viral antigen stimulation [25], the precise determinants and consequences of this defect are not understood. Lack of CD28 expression on CD4 and CD8 cells appears to be associated with HIV disease progression [30–32]. Moreover, CD8 cells that express CD28 appear to be important for an effective immune response to HIV infection [33, 34], and the presence of T cells that express CD28 predict a better immune response to neoantigen in HIV-infected patients [35]. In contrast to HIV infection, HCV infection is associated with increased proportions of virus-specific CD8 cells that express CD28 [36]. The setting of HCV/HIV coinfection, therefore, offers a model in which the effect that each chronic viral infection has on CD28 signaling can be explored.

The examination of the function and phenotype of HCV-reactive CD8 cells is difficult, in part because these cells appear infrequently during chronic HCV infection, especially in the presence of HIV coinfection. Previously, we and others demonstrated that stimulation with anti-CD28 agonistic monoclonal antibody (MAb) can enhance T cell responses [37, 38]. Here, we used anti-CD28 agonist to assay CD28 coreceptor function for T cell activation in response to HCV antigens in persons with HCV mono-infection and those with HCV/HIV coinfection. We found that CD28 costimulation enhanced the magnitude and breadth of CD8-specific interferon (IFN)- γ responses to HCV peptides in the HCV-mono-infected subjects but not in the HCV/HIV-coinfected subjects. We also found that CD28-enhanced CD8 cell responses were greater in breadth and cumulative frequency in the HCV-mono-infected subjects than in the HCV/HIV-coinfected subjects. Our results indicate that there is diminished expression or function of CD28 on HCV-reactive CD8 cells during HCV/HIV coinfection.

SUBJECTS, MATERIALS, AND METHODS

Study subjects and institutional review board approval. Participants included adult patients followed at the Cleveland Veterans Affairs Medical Center and University Hospitals of Cleveland. For all participants, HCV infection was defined as detectable serum HCV antibodies and RNA, and HIV infection was defined as a positive result for ELISA or other licensed HIV antibody test, as well as previously detectable plasma HIV RNA; healthy control subjects were negative for both HCV and HIV antibodies. Chronic HCV infection was ascertained on the basis of seropositivity for at least 6 months. Individuals with a

history of HCV treatment were excluded. T cell function experiments were conducted using specimens from healthy control subjects ($n = 10$), subjects with chronic HCV infection ($n = 14$), and subjects with chronic HCV/HIV coinfection ($n = 15$). Flow-cytometric experiments were conducted using specimens from overlapping groups of subjects: 13 healthy control subjects, 20 subjects with chronic HCV mono-infection, 12 subjects with HIV mono-infection (who met the same criteria as described for the HCV/HIV-coinfected subjects, with the exception of the criteria for HCV infection), and 15 HCV/HIV-coinfected subjects. Of these, 8 HCV-mono-infected, 5 HCV/HIV-coinfected, and 5 healthy control subjects were included among those who provided specimens for the T cell function experiments. All study subjects provided written, informed consent, and all experiments were performed with the approval of the institutional review boards for human studies at the Cleveland Veterans Affairs Medical Center and University Hospitals of Cleveland.

Flow-cytometric analysis. Flow-cytometric analysis was performed on a FACScalibur flow cytometer (Becton Dickinson) with CellQuest software (version 3.3; Becton Dickinson), using peripheral-blood mononuclear cells (PBMCs) that had been freshly prepared with ficoll-hypaque (1×10^6 PBMCs were analyzed per specimen). PBMCs were stained with anti-CD8, anti-CD3, and anti-CD28 MAbs (Becton Dickinson) for 20 min at 25°C and then washed in PBS plus 0.01% bovine serum albumin, fixed in 1% paraformaldehyde, and stored at 4°C until analysis. Lymphocytes were identified by forward and side light scatter, and CD8 cell frequency was determined as the proportion of live lymphocyte-gated cells that stained for the CD8 and CD3 cell markers. Analysis of CD4 cell phenotype was not performed.

HCV peptide pools. Peptides ($n = 441$; 18 aa in length, each overlapping by 11 aa) representing the entire HCV-1a H77 sequence were supplied by the National Institutes of Health AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases). Each peptide was dissolved in 100% dimethyl sulfoxide (DMSO; Sigma). The peptides were pooled together into 10 pools (27–61 peptides/pool) according to viral protein region (core, peptides 1–27; E1, peptides 28–55; E2, peptides 56–107; NS2 plus P7, peptides 108–147; NS3-1, peptides 148–193; NS3-2, peptides 194–239; NS4, peptides 240–287; NS5A, peptides 288–348; NS5B-1, peptides 349–394; and NS5B-2, peptides 395–441). Peptide pools were assayed at a final concentration of 2.7 $\mu\text{g}/\text{mL}$ each peptide ($\leq 0.5\%$ DMSO).

IFN- γ ELISPOT assays. PBMCs (3×10^5 cells/well, in duplicate) or CD8 cell-depleted PBMCs (3×10^5 cells/well; >90% depletion by use of the RossetteSep CD8 Depletion Cocktail [StemCell Technologies]) were plated onto 96-well IFN- γ ELISPOT plates with each of the 10 peptide pools in the presence

Table 1. Clinical characteristics of the study subjects.

Characteristic	HCV monoinfection (n = 14)	HCV/HIV coinfection (n = 15) ^a
HCV genotype, no. of subjects		
1	12	12
2/3 ^b	1	1
4	1	0
Unknown	0	2
Plasma RNA level		
HCV, IU/mL	1,579,249 ± 1,500,035	2,040,105 ± 2,724,529
HIV, IU/mL	NP	57,314 ± 192,965
CD4 cell count, 10 ⁶ cells/L	NP	397.8 ± 215.9
ALT level, IU/L	89 ± 53	78 ± 76
Albumin level, g/dL	4.1 ± 0.4	3.4 ± 0.5
Total bilirubin level, mg/dL	0.6 ± 0.5	0.5 ± 0.2
Platelet count, 10 ³ cells/mm ³	157 ± 51	206 ± 70

NOTE. Data are means ± SDs, unless otherwise specified. ALT, alanine aminotransferase; HCV, hepatitis C virus; NP, not performed.

^a Of the 15 HCV/HIV-coinfecting subjects, 10 were receiving antiretroviral therapy.

^b Both subjects were infected with genotype 3.

or absence of anti-CD28 agonist (final concentration, 1 µg/mL; BD Pharmingen), incubated for 20 h at 37°C, and then developed and analyzed as described elsewhere [38–42]. In the present study, a response was considered to have occurred when both the frequency of IFN-γ production was 3-fold greater than the mean background frequency (in the presence or absence of anti-CD28 agonist, as appropriate) and a level of ≥15 spot-forming units (sfu)/1 × 10⁶ PBMCs or CD8 cell–depleted PBMCs was observed. Using similar criteria, we have previously observed no responses in healthy control or disease control subjects [15, 38, 40, 42]. Background wells (with medium) incubated with anti-CD28 agonist did not contain significantly greater numbers of spots than those incubated without anti-CD28 agonist for the healthy control, HCV-monoinfected, and HCV/HIV-coinfecting subjects (*P* > .05). On the basis of the 90% depletion of CD8 cells produced by our method and an assumption of well-to-well variability of 15% in the ELISPOT assay, we considered a T cell response to be CD4 cell dominant or CD8 cell dominant as follows: a response was considered to be CD4 cell dominant when IFN-γ production was reduced by ≤30% or emerged after CD8 cell depletion; a response was considered to be CD8 cell dominant when IFN-γ production was reduced by ≥70% after CD8 cell depletion; and a response was considered to be a combination CD4/CD8 cell response when IFN-γ production was reduced 31%–69% after CD8 cell depletion. Only 2 responses were identified as combination CD4/CD8 cell responses (data not shown); these responses were considered to be PBMC responses and were not included in either the CD4 cell– or the CD8 cell–dominant response category for analysis. Notably, because we measured responses to 10 separate peptide pools, it was possible to identify both CD4

cell– and CD8 cell–dominant responses for the same subject. Additionally, because some CD4 cell–dominant responses were observed only after CD8 cell depletion, it was possible for some subjects to have a CD4 cell–dominant response in the absence of a PBMC response. Cumulative frequencies of HCV-specific IFN-γ responses were determined for each subject by summing the numbers of IFN-γ spot-forming units above background for each pool targeted. These values were calculated for each subject in each cell compartment separately. Of the 10 healthy control subjects analyzed for HCV-specific IFN-γ–producing PBMC responses, only 1 had a low-frequency response (39 sfu/1 × 10⁶ PBMCs).

Statistical analysis. We compared continuous variables by the Mann-Whitney *U* test and categorical data by Pearson’s χ^2 or Fisher’s exact test, as needed. To explore possible associations between continuous variables, we used Spearman’s rank correlation and, when necessary, nonparametric partial correlations, to adjust for the effect of an intervening variable. To test for significant differences between values obtained before the addition of the CD28 costimulatory signal and those obtained after, we used Wilcoxon’s signed rank test for continuous variables and Liddell’s exact version of McNemar’s test for dichotomous variables. All tests were 2-sided, and *P* ≤ .05 was considered to be statistically significant.

RESULTS

Characteristics of the study subjects. The clinical characteristics of the subjects evaluated for HCV-specific T cell function are listed in table 1. HCV genotype frequencies, serum alanine aminotransferase (ALT) levels, albumin levels, total bilirubin

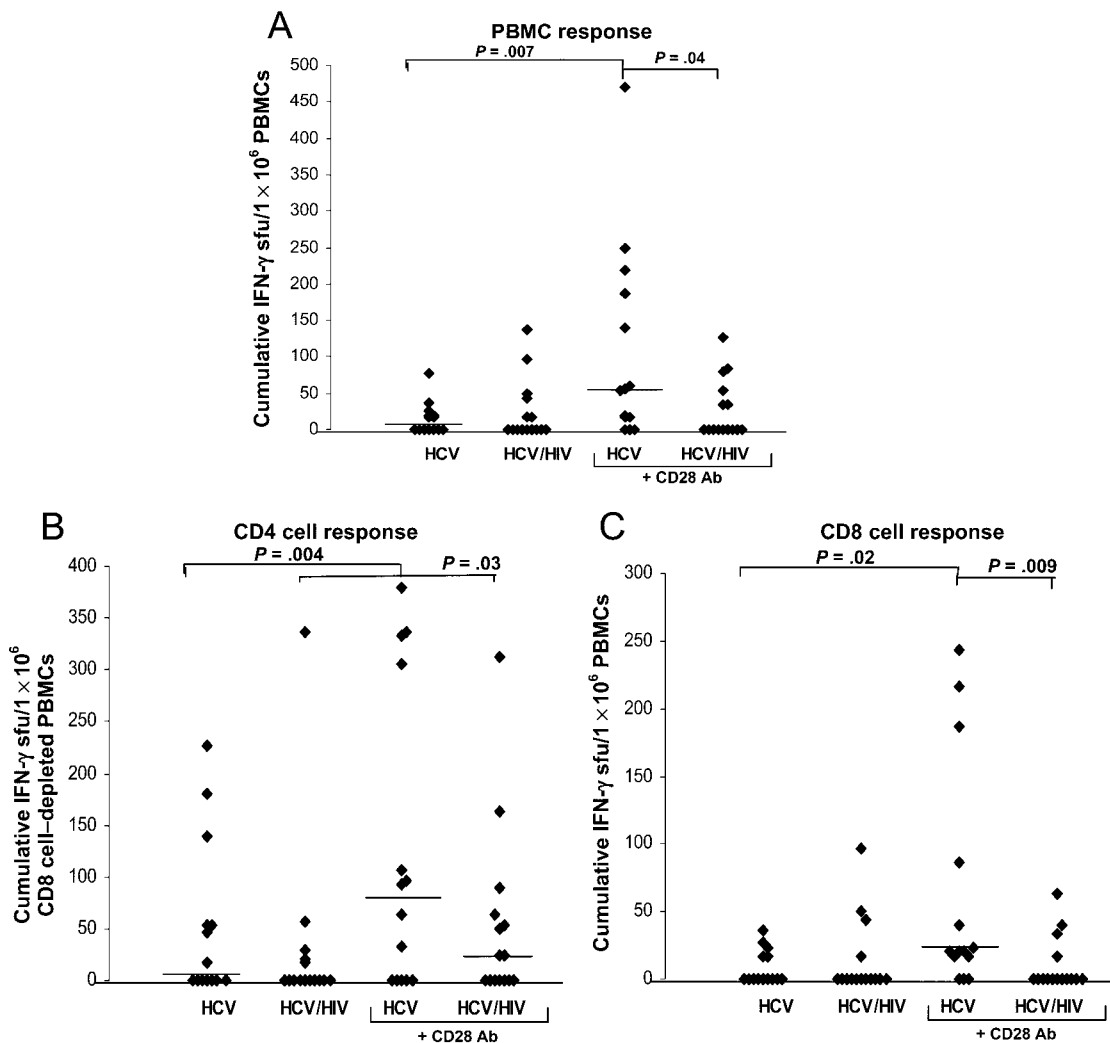


Figure 1. Increase in the cumulative frequencies of hepatitis C virus (HCV)-specific interferon (IFN)- γ -producing peripheral-blood mononuclear cells (PBMCs) after costimulation with anti-CD28 agonistic antibody (CD28 Ab) in HCV-monoinfected subjects but not HCV/HIV-coinfected subjects. Cumulative frequencies (the sum of all responses to HCV peptide pools) are shown for each subject; bars represent median values. *A*, PBMC response. *B*, CD4 cell response. *C*, CD8 cell response. *P* values were calculated by the Wilcoxon signed rank test. sfu, spot-forming units.

levels, plasma HCV RNA levels, and platelet counts were similar in the 2 groups. Among the 15 HCV/HIV-coinfected subjects, CD4 cell counts ranged from 26 to 935 cells/ μ L, and plasma HIV RNA levels ranged from <50 to 750,000 IU/mL. Of the 15 coinfecting subjects, 5 had plasma HIV RNA levels below the limit of detection (50 IU/mL), and 10 were receiving antiretroviral therapy. Age was similar in the 2 groups (mean [range] of 53 [48–61] years for the HCV-monoinfected subjects and of 51 [38–63] years for the HCV/HIV-coinfected subjects).

Increase in the magnitude of HCV-specific CD8 cell responses in HCV monoinfection, but not HCV/HIV coinfection, after CD28 costimulation. To determine whether HIV coinfection impairs the function of HCV-reactive T cells, we examined IFN- γ production in response to the HCV 18mer peptide pools representing the entire HCV-1a H77 sequence. To

explore the role played by CD28 cell function in this setting, assays were performed in the presence and absence of anti-CD28 agonist (figure 1). Because the magnitude of T cell responses may influence the outcome of viral infection, we first compared the cumulative frequencies of HCV-reactive T cells, as determined by the sum of the numbers of HCV-specific T cells that expressed IFN- γ in response to each of the 10 HCV peptide pools (figure 1A). The addition of anti-CD28 agonist significantly increased HCV-specific cumulative T cell frequencies in the HCV-monoinfected group (median, 8 vs. 55 sfu/1 $\times 10^6$ PBMCs; *P* = .007) but not in the HCV/HIV-coinfected group (median, 0 vs. 0 sfu/1 $\times 10^6$ PBMCs). In particular, the cumulative frequencies of HCV-specific T cell responses were increased by the addition of anti-CD28 agonist in 4 of the 14 HCV-monoinfected subjects by 2.6–15-fold and in 2 of the 15

HCV/HIV-coinfected subjects by 1.6–5-fold. Also, the addition of anti-CD28 agonist allowed for newly identified T cell responses in 5 of the 14 HCV-monoinfected and 2 of the 15 HCV/HIV-coinfected subjects. Moreover, in the presence of anti-CD28 agonist, there were greater cumulative frequencies of HCV-reactive T cells in the HCV-monoinfected subjects, compared with those in the HCV/HIV-coinfected subjects (median, 55 vs. 0 sfu/ 1×10^6 PBMCs; $P = .04$).

In the CD4 cell compartment, an increase in cumulative HCV-specific CD4 cell frequencies was observed with the addition of anti-CD28 agonist in the HCV-monoinfected subjects (median, 8 vs. 78 sfu/ 1×10^6 CD8 cell-depleted PBMCs; $P = .004$) (figure 1B). This was a result of new responses detected in 2 of the 14 HCV-monoinfected subjects and of increased cumulative frequencies observed in 7 of the 14 subjects (1.4–7.1-fold enhancement). Similarly, for the HCV/HIV-coinfected group, 4 of the 15 subjects had new responses detected, and 3 of the 15 subjects had increased cumulative frequencies of responding cells (1.5–5.4-fold enhancement); this resulted in a significant increase for the group in the cumulative frequency of CD4 cell responses (median, 0 vs. 23 sfu/ 1×10^6 CD8 cell-depleted PBMCs; $P = .03$).

In the CD8 cell compartment, after the addition of anti-CD28 agonist, an increase in response frequencies was observed in the HCV-monoinfected subjects (median, 0 vs. 20 sfu/ 1×10^6 PBMCs; $P = .02$) but not in the HCV/HIV-coinfected subjects (median, 0 vs. 0 sfu/ 1×10^6 PBMCs; $P = .7$) (figure 1C), and the cumulative frequencies of CD28-enhanced HCV-reactive CD8 cells were significantly greater in the HCV-monoinfected subjects than in the HCV/HIV-coinfected subjects (median, 20 vs. 0 sfu/ 1×10^6 PBMCs; $P = .009$). This difference appeared to be mainly attributable to newly detected responses in the HCV-monoinfected, but not in the HCV/HIV-coinfected, subjects. After CD28 costimulation, new CD8 cell responses were more commonly identified in the HCV-monoinfected subjects than in the HCV/HIV-coinfected subjects (7/14 for HCV monoinfection vs. 1/15 for HCV/HIV coinfection; $P = .02$), and the proportions of new responses to all possible peptide pools were greater in the HCV-monoinfected subjects than in the HCV/HIV-coinfected subjects ($P < .001$).

When we focused on the proportions of subjects within each group with HCV-specific T cell responses, the frequencies of responses to HCV in the absence of anti-CD28 agonist in the HCV-monoinfected and HCV/HIV-coinfected groups were similar (50% vs. 40%; $P = .6$) (figure 2). In contrast, after the addition of anti-CD28 agonist, the frequency of responses was greater in the HCV-monoinfected group than in the HCV/HIV-coinfected group (79% vs. 40%; $P = .04$). This apparent enhancement was largely attributable to a differential effect on CD8 T cells. In both groups, the frequencies of CD4 T cell responses were modestly but nonsignificantly increased in the

presence of anti-CD28 agonist, whereas the agonistic antibody enhanced the CD8 T cell response only in the HCV-monoinfected subjects (36% vs. 79%; $P = .07$), to a level that exceeded that in the HCV/HIV-coinfected subjects (79% vs. 26%; $P = .005$).

Increase in the breadth of HCV-specific CD8 responses in HCV monoinfection, but not HCV/HIV coinfection, after CD28 costimulation. Diverse CD4 and CD8 cell responses are also thought to influence disease outcome in HCV infection [12]. Therefore, we asked whether CD28 costimulation enhanced the breadth of peptide recognition in HCV monoinfection or in HCV/HIV coinfection. In the absence of CD28 costimulation, the breadth of peptide recognition in the HCV-monoinfected and HCV/HIV-coinfected groups was similar ($P = .8$) (figure 3A). Subjects from both groups demonstrated a narrow pattern of recognition for the HCV genome, with subjects recognizing only 0–2 peptide pools each. In the presence of CD28 costimulation, the HCV-monoinfected subjects had a significant increase in diversity (0–9 peptide pools targeted; $P = .009$), whereas the HCV/HIV-coinfected subjects showed little enhancement (0–3 peptide pools targeted; $P = .3$). The increased breadth of response in the HCV-monoinfected group was observed in the CD8 cell compartment ($P = .02$) (figure 3C), and a trend toward the same was observed in the CD4 cell compartment ($P = .06$) (figure 3B); however,

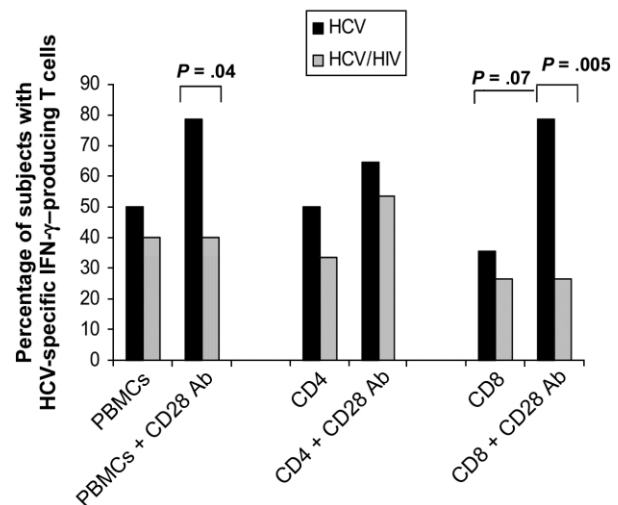


Figure 2. Greater proportion of hepatitis C virus (HCV)-specific CD8 cell responses in the presence of anti-CD28 agonistic antibody (CD28 Ab) in HCV-monoinfected subjects than in HCV/HIV-coinfected subjects. Peripheral-blood mononuclear cells (PBMCs) or CD8 cell-depleted PBMCs from HCV-monoinfected ($n = 14$) and HCV/HIV-coinfected ($n = 15$) subjects were analyzed for HCV-specific interferon (IFN)- γ -producing responses. The proportion (%) of subjects within each group where HCV-specific PBMC, CD4 cell, and CD8 cell responses were observed is represented in both the presence and absence of anti-CD28 agonist. For between-group analysis, the χ^2 test was used; for within-group analysis, Liddell's exact version of McNemar's test was used.

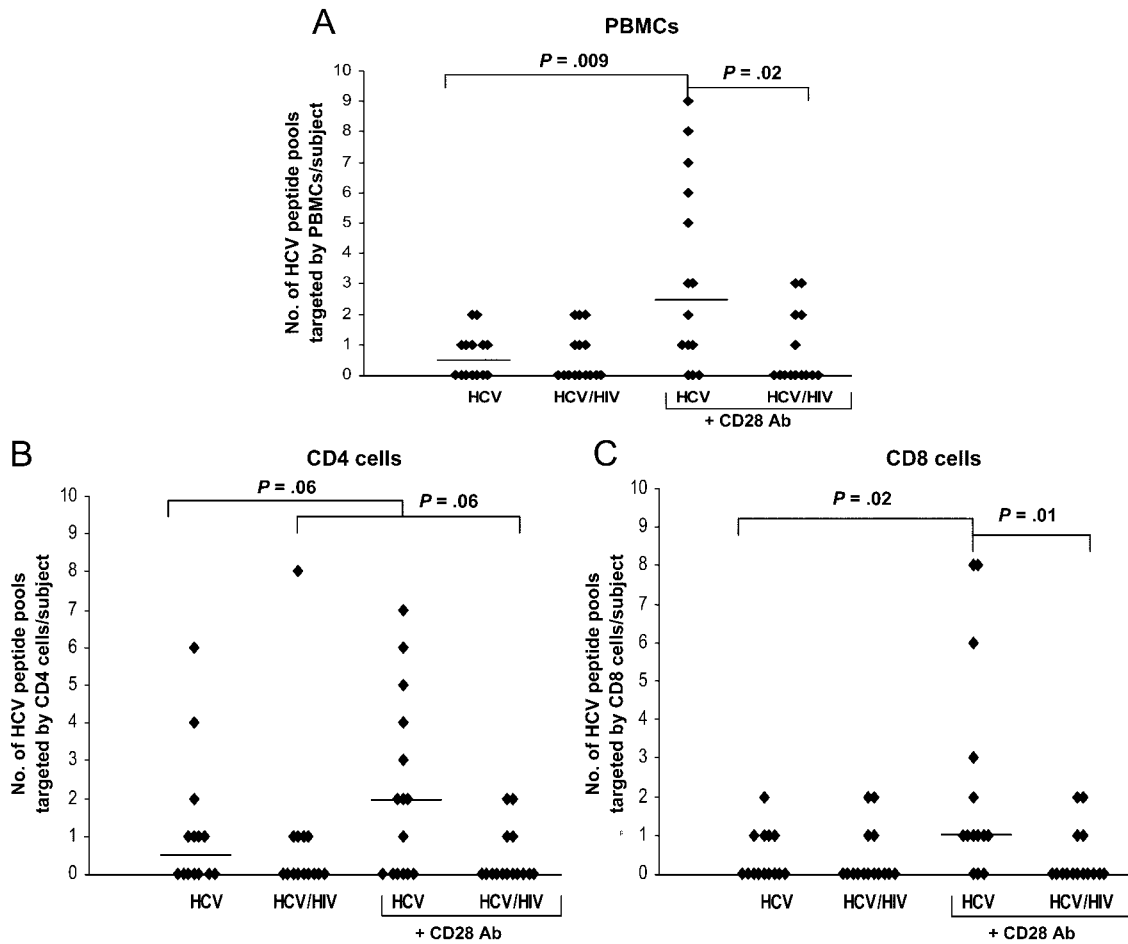


Figure 3. Increase in the breadth of hepatitis C virus (HCV)–specific CD8 cell responses after costimulation with anti-CD28 agonistic antibody (CD28 Ab) in HCV-monoinfected subjects but not in HCV/HIV-coinfected subjects. The no. of peptide pools recognized is shown for each subject; bars represent median values. *A*, Peripheral-blood mononuclear cell (PBMC) response. *B*, CD4 cell response. *C*, CD8 cell response. *P* values were calculated by the Wilcoxon signed rank test.

in the HCV/HIV-coinfected subjects, a marginally significant increase was observed only in the CD4 cell compartment ($P = .06$) and not at all in the CD8 cell compartment ($P = 1$). In the presence of anti-CD28 agonist, the HCV-monoinfected subjects had a significantly more diverse CD8 cell response than did the HCV/HIV-coinfected subjects ($P = .01$).

Responses to immunodominant antigens may play an important role in the control of viremia. Dividing the 441 HCV peptides into 10 pools corresponding to the various protein regions allowed us to identify the regional specificity of responses (figure 4). There did not appear to be any single immunodominant region recognized by the CD4 or the CD8 cell compartment in either group.

No correlations were observed between CD4 cell count, plasma HCV RNA level, plasma HIV RNA level, or serum ALT level and HCV-specific CD4, CD8, or T cell responses in this population ($P > .05$, for each comparison). We tested for responses to HCV genotype 1–based peptide sequences in some

subjects who did not have genotype 1 infection. Altogether, there were 5 subjects infected with non-genotype 1 HCV or with HCV of an unknown genotype; of these subjects, only 1 responded to the test peptides. When analysis of our data set was restricted to subjects with known genotype 1 infection only, all statistically significant observations remained, with statistical significance modestly increased in some comparisons.

Reduction in the proportion of CD8 cells that express CD28 in HIV infection. CD8 cell frequency is increased in HIV infection, whereas the proportion of CD8 cells that express CD28 is reduced [14, 25, 29]. We asked whether this is the case in persons with HCV/HIV coinfection as well. In overlapping cohorts of subjects, we investigated the frequencies of CD8 cells and the proportions of CD8 cells that express CD28. Our results are in agreement with those of previous reports, showing a greater frequency of CD8 cells in HCV/HIV-coinfected subjects than in HCV-monoinfected subjects (median, 32.6% vs. 17.3%; $P < .001$) (figure 5A). Additionally, the pro-

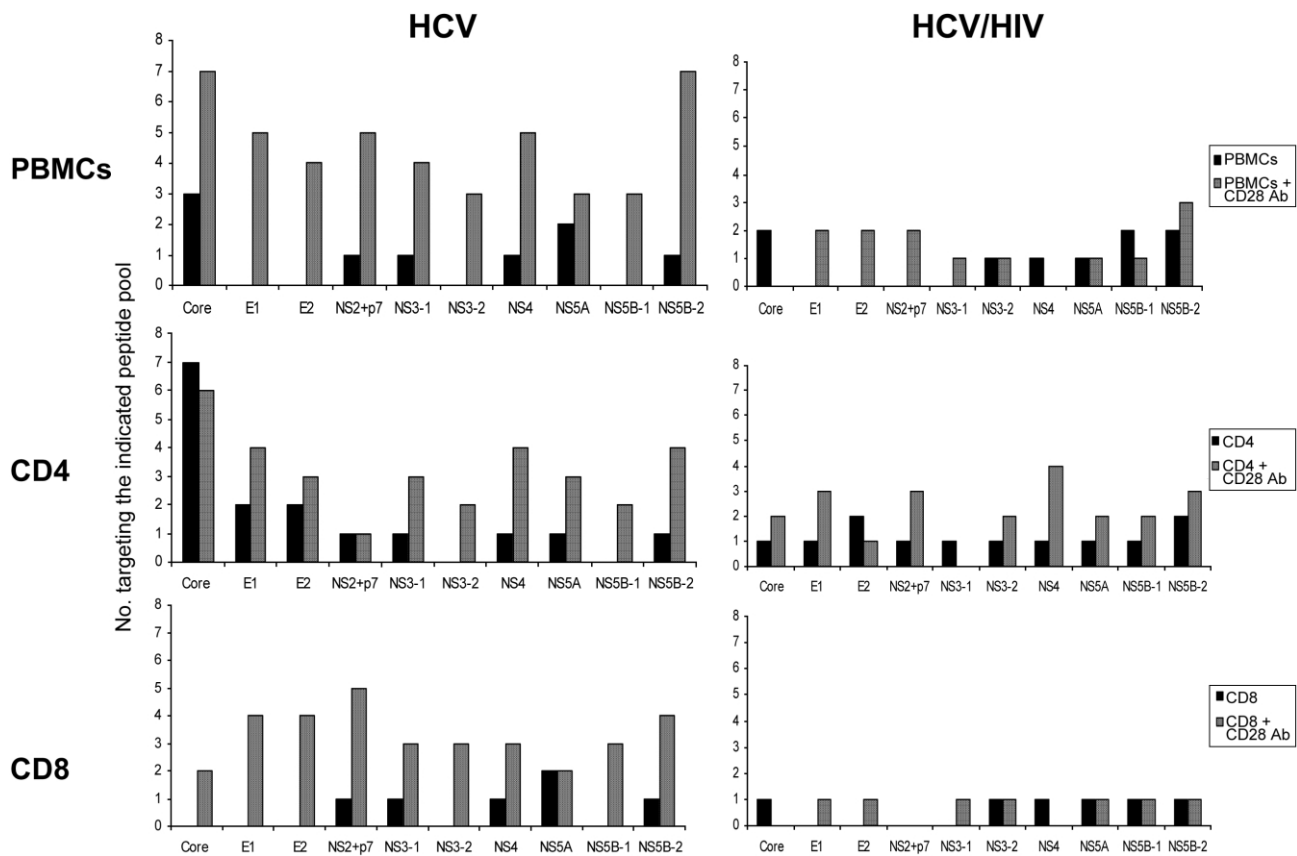


Figure 4. Lack of targeting of any specific protein region by hepatitis C virus (HCV)-specific T cell responses. The no. of individuals targeting each protein region in the peripheral-blood mononuclear cell (PBMC), CD4 cell, and CD8 cell fractions in the presence and absence of anti-CD28 agonistic antibody (CD28 Ab) are shown for the HCV-monoinfected and HCV/HIV-coinfected groups.

portions of CD8 cells that expressed CD28 were lower in the HCV/HIV-coinfected subjects than in the HCV-monoinfected subjects (median, 48.6% vs. 67.3%; $P = .003$) (figure 5B), with the proportions in the HCV/HIV-coinfected subjects being similar to those in the HIV-monoinfected subjects (median, 37.6%) and the proportions in the HCV-monoinfected subjects being similar to those in the healthy control subjects (median, 62.9%). Therefore, the previously described global effect that HIV infection has on the proportion of CD8 cells that express CD28 is extended to HCV/HIV coinfection.

DISCUSSION

CD28 ligation is known to affect the transcription and stability of interleukin-2 and IFN- γ mRNA [43, 44]. Therefore, reduced CD28 expression may have downstream effects on CD8 cell function at the time of TCR engagement. Here, when we evaluated the effect that CD28 costimulation has on HCV-specific T cell responses, we found that CD4 and CD8 cells were responsive in HCV-monoinfected subjects, whereas only CD4 cells were responsive in HCV/HIV-coinfected subjects. These results have direct implications for the ability of the HCV/HIV-

coinfected host to form an optimal immune response to HCV infection.

One possible explanation for this observation is reduced CD28 expression on CD8 cells, including HCV-specific CD8 cells, in HCV/HIV-coinfected individuals. In fact, we found a global reduction in the expression of CD28 on CD8 cells in our HCV/HIV-coinfected subjects, indicating that this abnormal phenotype, previously described in HIV infection [25], is extended to HCV/HIV coinfection. Although the proportions of CD4 cells that express CD28 are also modestly reduced in HIV infection, the magnitude of the difference is substantially less than that observed in the CD8 cell compartment [45, 46], perhaps accounting for our observation that there was no impairment in CD28-enhanced CD4 cell function. The lack of CD28-enhanced CD8 cell function in HCV/HIV coinfection may, alternatively, result from a defect in anti-CD28 agonist engagement of other CD28-bearing cells, such as CD4 cells, which, in turn, help facilitate CD8 cell responses. However, we found that anti-CD28 agonist directly enhanced HCV-specific CD4 cell responses in both subjects with HIV coinfection and those without, making this possibility unlikely. Because we did

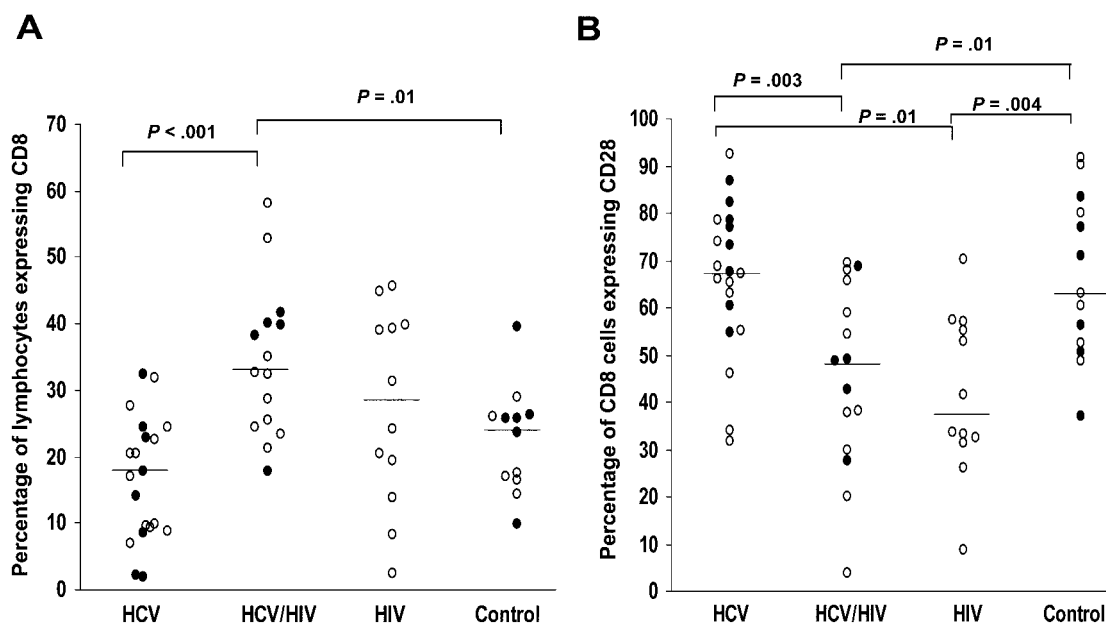


Figure 5. Greater frequencies of CD8 cells (A) and reduced proportions of CD8 cells expressing CD28 (B) in hepatitis C virus (HCV)/HIV-coinfected subjects. Peripheral-blood mononuclear cells (PBMCs) were stained with conjugated anti-CD8 and anti-CD28 monoclonal antibodies to measure, by flow-cytometric analysis, the frequencies of CD8 cells and CD28-expressing CD8 cells among lymphocyte-gated PBMCs in HCV-monoinfected ($n = 20$), HCV/HIV-coinfected ($n = 15$), HIV-monoinfected ($n = 12$), and healthy control ($n = 13$) subjects. Black circles represent subjects who were also studied in functional assays, and white circles represent additional subjects studied for this phenotypic analysis; bars represent median values. P values were calculated by the Mann-Whitney U test.

not measure CD28 expression directly on HCV-reactive CD8 cells, we can not exclude the possibility that CD28 signaling, and not CD28 expression, is impaired in HCV-reactive CD8 cells in the setting of HIV coinfection. Regardless of which of these possibilities accounts for our observation, it is clear that the impairment in CD28-dependent costimulation of HIV-reactive CD8 cells is also found in HCV-reactive CD8 cells in persons with HCV/HIV coinfection.

A pervasive effect of one virus infection on the phenotype of CD8 cells specific for other viruses in the same host has been described previously [47]. Although this phenomenon is not thought to be common during acute viral infection [47, 48], bystander effects have been observed in HIV and HCV infection [49–51]. In fact, during primary HIV infection, Epstein-Barr virus (EBV)-specific CD8 cells tend to express less CD28 than do EBV-specific CD8 cells found in healthy control subjects [49]. Here, we observed that the effect that HIV infection had on the function of HCV-specific CD8 cells during chronic HCV/HIV coinfection was dominant; such dominance implies that the mechanisms of bystander effects differ between HCV and HIV infection. Decreased CD28 expression on CD8 cells in HIV-infected individuals has been attributed to chronic antigen stimulation [25], implying terminal differentiation and senescence of antigen-specific CD8 cells [52]. Sustained viremia also occurs during chronic HCV infection, yet reduced proportions of CD8 cells that express CD28 are not found [36,

51]. Moreover, increased CD28 expression has been reported among HCV-specific CD8 cells. With regard to the possibility of senescence, we analyzed antigen-specific CD4 and CD8 cell proliferation (by the carboxyfluorescein succinimidyl ester dye-dilution method) from a subset of our subjects in whom IFN- γ responses were observed. We detected proliferative responses (only in the presence of anti-CD28 agonist) in HCV-monoinfected subjects (2/4) but not in HCV/HIV-coinfected subjects (0/4) (data not shown), indicating that agonist can augment HCV-specific T cell proliferative responses in at least some HCV-monoinfected subjects.

An alternative explanation for the dramatic difference in CD28 expression and function among CD8 cells in HCV and HIV infection may relate to differences in the functions of antigen-presenting cells in these 2 infections. Dysfunction of antigen-presenting cells has been well described in both HIV [53–55] and HCV [19, 56–60] infection. One or more of the distinct differences in dendritic-cell abnormalities between HCV and HIV infection may result in a difference in T cell priming, activation, or differentiation. Another alternative explanation may relate to the different cytokine environments in HCV and HIV infection [61, 62], which may exert different bystander effects on T cell phenotype and function, given that cytokines are known to affect the activation or differentiation state of T cells [63]. Additionally, viral proteins may directly affect CD28 expression on T cells—in fact, the down-regulation

of T cell CD28 expression by HIV Nef protein has been described [64]. Another possible explanation includes HIV-related immune deficiency resulting in impaired host control of HCV infection, which, in turn, results in an altered activation or differentiation state of HCV-reactive CD8 cells. Another possibility is that cross-reactivity between HCV- and HIV-specific CD8 cells results in an altered activation state of HCV-reactive CD8 cells. Finally, a combination of these potential mechanisms may be at work.

In conclusion, we found reduced proportions of CD8 cells that express CD28 in HCV/HIV-coinfected subjects. These proportions were similar to those previously described in HIV mono-infection. Furthermore, this phenotype was associated with reduced IFN- γ production by HCV-specific CD8 cells in response to CD28 costimulation. These results indicate that HIV infection affects CD28 signaling and the function of non-HIV-specific CD8 cells. Because CD28 signaling may play a key role in an adaptive response to HCV infection, HIV coinfection may directly interfere with the ability of the HCV-infected host to mount an optimal response to HCV and, thus, contribute to the increased morbidity observed for HCV/HIV coinfection.

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