

Chronic immune activation and decreased CD4 cell counts associated with hepatitis C infection in HIV-1 natural viral suppressors

Mohammad M. Sajadi, Roopa Pulijala, Robert R. Redfield and Rohit Talwani

We have established a cohort of natural viral suppressors (NVS) who can suppress HIV-1 replication to less than 400 copies/ml in the absence of therapy (similar to Elite Controllers/Elite Suppressors). Of the 59 patients currently in the NVS cohort, 45.8% have chronic hepatitis C virus (HCV) infection, thereby presenting a unique opportunity to study immune activation and the interaction between HCV and HIV. NVS with chronic HCV infection had elevated levels of immune activation (CD38-positive HLA-DR-positive CD8 cells) compared to NVS without chronic HCV ($P=0.004$). The increased levels of immune activation were not associated with sex, HLA B57 status, or injection drug use. NVS patients with chronic HCV had lower mean CD4 cell counts, CD4 percentage, and CD4/CD8 ratios than NVS without chronic HCV infection ($P=0.038$, $P=0.008$, and $P=0.048$, respectively). The difference in CD4 cell count appeared to occur early in HIV infection with no difference observed in CD4 slopes between groups. Among all NVS, there was a direct correlation between mean CD4 cell count, mean CD4 percentage, and mean CD4/CD8 ratio with percentage of CD38⁺ HLA-DR⁺ CD8 cells ($P=0.0018$; $P=0.0069$; and $P=0.0014$, respectively). This study suggests a relationship between HCV infection, immune activation, and CD4 cell counts in the NVS, with chronic HCV infection associated with lower CD4 cell counts and higher levels of immune activation. Further studies are needed to determine if successful HCV treatment lowers immune activation levels and/or increases CD4 cell counts in these patients. © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

AIDS 2012, **26**:1879–1884

Keywords: elite controller, hepatitis C virus, HIV, HIV-1 nonprogressors, immune activation

Introduction

Natural viral suppressors (NVS) are HIV-infected patients who have the ability to naturally suppress HIV-1 to undetectable levels in the absence of therapy [1–3]. Multiple factors appear to contribute to this natural suppression [4]; however, the precise mechanisms remain

poorly understood. Recently, some specific immune defects have been described in these patients [5], however, these individuals still maintain normal CD4 cell counts for many years [1,4,6].

We previously reported that within this NVS cohort, patients with active hepatitis C virus (HCV) infection had

Institute of Human Virology at the University of Maryland School of Medicine, Baltimore, Maryland, USA.

Correspondence to Mohammad M. Sajadi, MD, Institute of Human Virology at the University of Maryland School of Medicine, 725 West Lombard St., N548, Baltimore, MD 21201, USA.

Tel: +1 410 706 1779; fax: +1 410 706 1992; e-mail: msajadi@ihv.umaryland.edu

Received: 30 April 2012; revised: 3 July 2012; accepted: 10 July 2012.

DOI:10.1097/QAD.0b013e328357f5d1

lower CD4 cell counts and CD4 cell count percentages than NVS without HCV infection [3]. Given the substantial increase in the number of patients in this cohort, we sought to verify this earlier work, determine if markers of immune activation were increased in the coinfecting patients, and if this correlated with CD4 cell count decline.

Methods

Study patients

The NVS cohort has been described in detail elsewhere [1–3]. Briefly, after informed consent was obtained, NVS patients had to be confirmed HIV-1 positive by western blot and proviral DNA and have demonstrated viral loads less than 400 copies/ml for a 2-year period without the use of antiretroviral therapy (one viral load >400 copies/ml in a 2-year period was allowed provided the subsequent values were <400 copies/ml). All patients in the NVS cohort were required to have a hepatitis C antibody or PCR test performed. For those with a positive hepatitis C antibody, a follow-up PCR (quantitative or qualitative) was required.

Several cohorts were created for comparison with the NVS cohort. These included HIV/HCV-negative individuals, HCV monoinfected patients, and non-NVS HIV and HIV/HCV-coinfecting patients. These cohorts were matched closely for race but not other parameters. HIV/HCV-negative individuals were exclusively noninjection drug use (IDU), whereas the majority (88%) of the HCV monoinfected had a history of IDU.

Demographic and laboratory data collected

The demographic data collected included the following: age, sex, race, date of diagnosis of HIV, and risk factor for HIV. For the NVS earliest and latest CD4 cell count, and mean CD4 cell count, CD4 percentage, and CD4/CD8 ratios were recorded. alanine aminotransferase (ALT) and aspartate aminotransferase platelet ratio index (APRI) scores were calculated based on available data closest to date of sample tested for immune activation. HIV-1 proviral copy number was performed as described previously [2]. Flow cytometry was performed by using

FACSCalibur flow (BD Biosciences, San Jose, California, USA) with gating for CD3, CD8, HLA-DR, and CD38. PBMCs were stained by using CD3 PerCP, CD8 APC, CD38 PE, and HLA-DR FITC (BD Biosciences). All of the dyes were used according to manufacturer's specifications and optimized according to the experiment. Data was analyzed by using FlowJo software (Ashland, Oregon, USA).

Statistical analysis and definitions

'Chronic HCV infection' was defined as demonstrating a positive plasma PCR for HCV. Those 'without chronic HCV infection' were defined as those having a negative HCV antibody, or a positive HCV antibody with a negative HCV PCR. CD3⁺ CD8⁺ cells with dual expression of HLA-DR⁺ and CD38⁺ were defined as activated.

For data with normal distribution Student's *t*-test was performed; otherwise, the Mann-Whitney test was used. Fischer's exact test was used for contingency table testing. All *P* values were two-tailed and considered significant if less than 0.05. All data were analyzed with GraphPad Prism software (San Diego, California, USA).

Results

Patient characteristics

We have established a cohort of 59 NVS patients, of which 45.8% have chronic HCV infection. We further characterized the NVS cohort into those with chronic HCV and those without chronic HCV infection. The only statistically significant difference between the two groups was IDU as a risk factor for HIV, with 85.7% in the HCV positive versus 28.1% in the HCV negative (*P*=0.0001). The demographics of these two groups within the NVS cohort are shown in Table 1. Of note, 10 patients who had positive HCV ELISAs were PCR negative suggesting that they had cleared infection (one of these patients had cleared with HCV therapy). Excluding the one patient who received HCV therapy, the spontaneous HCV clearance rate in the NVS is 25%, slightly higher than our earlier findings [3,7].

Table 1. Demographic information on the natural viral suppressors cohort based on chronic hepatitis C virus status.

| Characteristic | Chronic HCV (n = 27) | Without chronic HCV (n = 32) | <i>P</i> |
|---------------------------|----------------------|------------------------------|----------|
| Age | 54 | 52 | 0.07 |
| Race | 96.9% AA | 100% AA | 1.0 |
| Sex | 37% female | 56.3% female | 0.19 |
| Median HIV diagnosis date | 1994 | 1995 | 0.86 |
| HIV risk factor | 85.2% IDU | 28.1% IDU | 0.0001 |
| Incidence of cancer | 22.2% | 9.4% | 0.28 |

AA, African-American; HCV, hepatitis C virus; IDU, injection drug use. NVS = HIV-1 infected individuals with HIV-1 viral loads less than 400 copies/ml without therapy. NVS patients were divided into those with chronic HCV (HCV PCR positive) and those without chronic HCV (HCV antibody negative, or HCV antibody positive with a negative HCV PCR).

Immune activation in the natural viral suppressors and controls

Immune activation was measured in the NVS ($n=53$), HIV/HCV-negative controls ($n=12$), HCV-monoinfected ($n=10$), HIV-monoinfected ($n=9$), and dual HIV/HCV-infected patients ($n=4$). Immune activation was lowest in the HIV/HCV-negative and HCV-monoinfected controls, followed by sequentially higher levels in the NVS without chronic HCV, NVS with chronic HCV, and then HIV and HIV/HCV control groups. Within the NVS cohort, level of immune activation correlated with chronic HCV status, with those NVS with HCV infection having a statistically significant elevation in percentage of CD38⁺ HLA-DR⁺ CD8 cells ($P=0.004$). NVS without chronic HCV had a statistically significantly elevated percentage of CD38⁺ HLA-DR⁺ CD8 cells compared to HIV/HCV-negative controls ($P=0.003$), and NVS with chronic HCV had a statistically significant elevated percentage of CD38⁺ HLA-DR⁺ cells compared to those with HCV mono-infection ($P=0.006$). Overall, HCV-negative NVS, HCV monoinfected, and HIV/HCV negative patients had lower levels of percentage of CD38⁺ HLA-DR⁺ CD8 cells compared to NVS with HCV, or dual HIV/HCV-infected individuals. A summary of the above is shown in Fig. 1a.

Within the NVS, levels of immune activation did not correlate with sex, IDU history, HLA-B57 status, or viral blips more than 400 copies/ml HIV-1 RNA ($P=0.30$, $P=0.21$, $P=0.16$, and $P=0.09$, respectively) (Fig. 1b). Among NVS with chronic HCV, there was no correlation between percentage of CD38⁺ HLA-DR⁺ CD8 cells and HCV viral load, ALT, or APRI score (data not shown).

HIV-related parameters in the natural viral suppressors based on hepatitis C virus status

Within the NVS cohort, individuals without chronic HCV had a statistically significant elevation in mean CD4 cell count (928 cells/ μ l) compared to those NVS with chronic HCV (766 cells/ μ l) ($P=0.038$). This correlation of CD4 cell count with HCV status was statistically significant for earliest recorded CD4 cell count ($P=0.01$) and there was a trend toward significance with the most recent CD4 cell count as well ($P=0.058$). There was a similarly statistically significant elevation in the mean CD4 percentage and mean CD4/CD8 ratio in those without chronic HCV compared to the chronic HCV group ($P=0.008$ and $P=0.048$, respectively). We found no correlation between chronic HCV status and HIV-1 viral blips to more than 400 ($P=1.0$). In patients with at least six available data points, there was no correlation between chronic HCV status and CD4 slope, CD4 percentage slope, or CD4/CD8 ratio slope ($P=0.37$, $P=0.83$, and $P=0.37$, respectively).

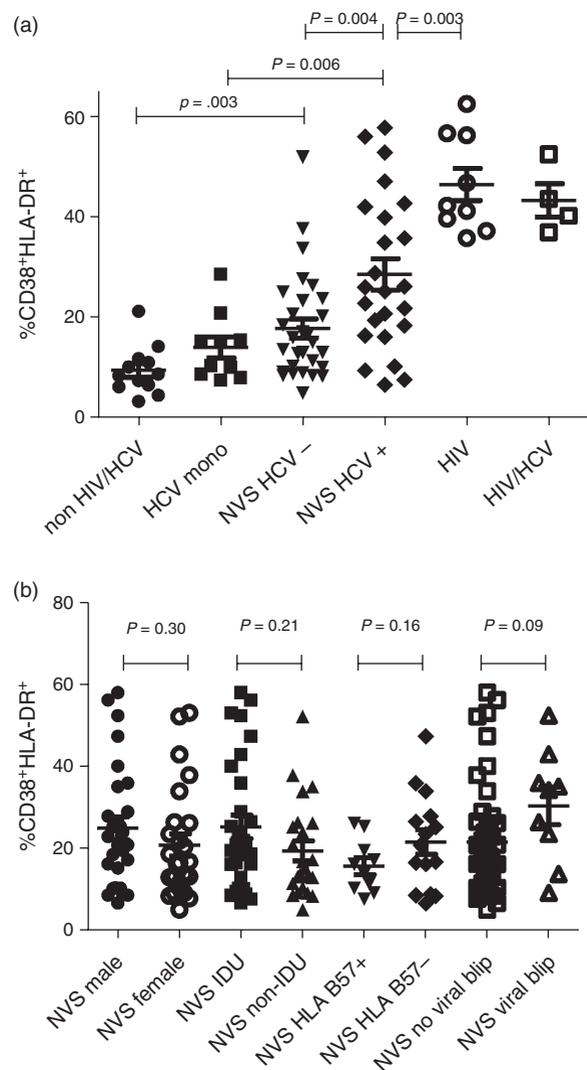


Fig. 1. Effect of HIV and hepatitis C virus on percentage of activated CD8 cells. Percentages are given as proportion of CD38⁺ and HLA-DR⁺ cells among all CD3⁺ CD8⁺ cells. (a) Percentage of CD38⁺HLADR⁺ cells among normal controls, hepatitis C virus (HCV) monoinfected, natural viral suppressors (NVS) without chronic HCV, NVS with chronic HCV, HIV monoinfected, and HIV/HCV dual infected patients. The association of HCV infection with immune activation within the NVS is seen when comparing those with or without chronic HCV infection ($P=0.004$). The association of HIV infection with immune activation in the NVS are demonstrated when comparing NVS without chronic HCV to HIV/HCV-negative controls, or NVS with chronic HCV to HCV monoinfected controls ($P=0.003$ and 0.006 , respectively). (b) Effect of sex, injection drug use (IDU) status, HLA-B57 status, or HIV-1 viral blip more than 400 copies/ml on percentage of activated CD8 cells. There was no statistically significant effect of sex, IDU status, HLA-B57 status, or HIV-1 viral blip on the percentage of activated CD8 cells. NVS are HIV-1 infected individuals with HIV-1 viral loads less than 400 copies/ml without therapy.

Correlation between CD4 cell count and CD8 immune activation

Among all NVS, there was a direct correlation between mean CD4 cell count, mean CD4 percentage, and mean CD4/CD8 ratio with percentage of CD38⁺ HLA-DR⁺ CD8 cells [$P=0.0018$ ($r^2=0.17$); $P=0.0069$ ($r^2=0.13$); and $P=0.0014$ ($r^2=0.21$), respectively] (Fig. 2). On subanalysis, this correlation between immune activation and CD4 cell counts held more often true with the NVS with chronic HCV, with correlations with latest CD4 cell count ($P=0.003$), mean CD4 cell count ($P=0.003$), and mean CD4/CD8 ratio ($P=0.015$), than those NVS without chronic HCV ($P=0.022$ for latest CD4 cell count only).

Discussion

This study suggests a relationship between immune activation, HCV infection, and CD4 cell count in the NVS, a cohort of HIV-1-infected individuals who can control HIV replication to extremely low levels. Overall, the NVS had levels of immune activation between normal controls and HIV-infected individuals agreeing with previous findings [8]. The effect on HIV infection on immune activation can be seen when comparing the NVS with chronic HCV to HCV-monoinfected patients, or when comparing NVS without chronic HCV with HIV/HCV-negative controls. However, what is noteworthy is that an effect of HCV infection can be seen as well. There was a difference in the percentage of CD38-positive HLA-DR-positive CD8⁺ cells within the NVS group, with the chronic HCV group having a median of 25.6% compared to 15.1% activated CD8⁺ cells in those without chronic HCV negative ($P=0.004$). This difference could not be explained by any differences between the groups, including sex and HLA-status. Although there was a statistically significant higher IDU rate in the chronic HCV NVS compared to the chronic HCV-negative NVS, IDU status by itself did not correlate immune activation. Thus, IDU was a confounder for the true marker, which is chronic HCV infection.

We had previously reported that in this cohort, patients with chronic HCV (defined by a positive blood PCR) had lower CD4 and CD4 percentage, than NVS without HCV. In this larger study, we have found lower mean CD4 cell count, mean CD4 percentage, and mean CD4/CD8 ratio in the NVS with chronic HCV compared to those without chronic HCV infection. The difference in CD4 cell count was also noted when comparing the earliest CD4 cell count on record. CD4 cell count slope was not significantly different between those who had chronic HCV and those without chronic HCV. The estimated date of acquisition of HCV infection in this group predates acquisition of HIV by one or more decades [3,9]; thus, in most of these patients,

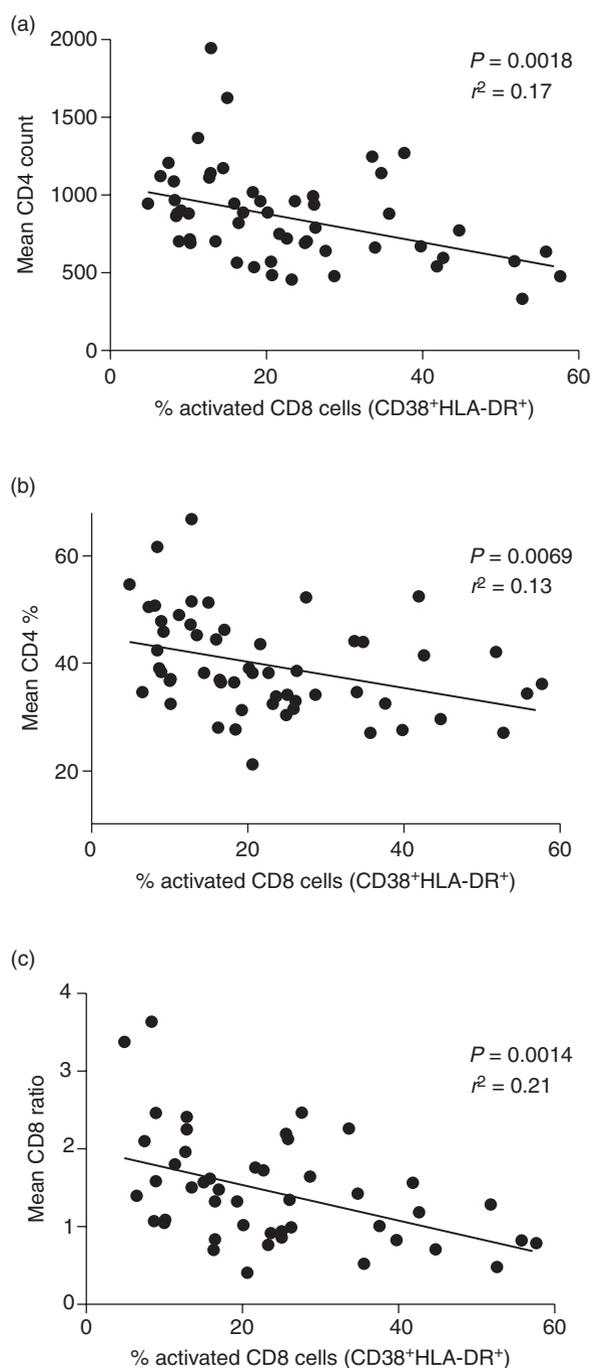


Fig. 2. Correlation between activated CD8 cells and mean CD4 cell count, CD4 percentage, and CD4/CD8 ratio in the NVS. X-axis represents percentages of CD38⁺ and HLA-DR⁺ cells among all CD3⁺ CD8⁺ cells. Y axis represents mean CD4 cells count in cells/ μ l, mean CD4 percentile, or mean CD4/CD8 ratio. (a) Correlation between mean CD4 cell count and percentage activated CD8 cells ($P=0.0018$, $r^2=0.17$). (b) Correlation between mean CD4 percentage and percentage-activated CD8 cells ($P=0.0069$, $r^2=0.13$). (c) Correlation between mean CD4/CD8 ratio and percentage-activated CD8 cells ($P=0.0014$, $r^2=0.21$).

chronic HCV infection was present at the time of HIV infection. Taken together, this suggests that the difference in CD4 cell count between these two groups occur early in infection (and that the presence of chronic HCV may affect the course of acute HIV infection in the NVS), as subsequently the rate of CD4 change is not significantly affected.

In chronic HIV infection, immune activation is felt to play a role in the mechanism of CD4 decline, as patients treated with HAART demonstrate decreased levels of immune activation and a corresponding rise in CD4 cell counts [10–14]. As this effect of HIV on immune activation can be substantial, the contributory effect of other copathogens such as HCV can be masked, unless in situations in which the HIV is controlled, such as in the NVS or patients undergoing HAART therapy. There is evidence that HCV infection can affect the course of HIV infection. Specifically, studies have shown that the presence of HCV can blunt the CD4 recovery. Although there is no agreement on all studies whether HCV coinfection leads to decreased number of CD4 cell recovery [15–23], there does appear to be a delayed CD4 reconstitution associated with HIV/HCV-coinfecting patients undergoing HAART therapy [24–27]. In addition, HCV infection has been cited as a risk factor for developing an AIDS-defining illness [28].

HIV/HCV-coinfecting patients have been shown to have increased levels of CD8 activation compared to HIV-monoinfected patients [29–31]. In one study, HCV treatment in HIV-infected patients on a stable HAART regimen led to decreased levels of immune activation (compared to pre-HCV treatment immune activation levels) [29]. Sandberg *et al.* [32] have suggested that abnormal gut translocation as a result of HIV infection is poorly controlled in HCV-coinfecting patients. The impaired liver function and thus impaired microbial clearance as a result of HCV infection is thought to exacerbate immune activation. The data in this study do not support this, as elevated activation levels were not associated with increased ALT or APRI scores, although this must be corroborated by larger studies.

In this study, the observation that chronic HCV viremia is associated with both decreased CD4 cell counts and elevated levels of immune activation suggests that the mechanism for reduced CD4 cell counts are related to immune activation, similar to findings reported by Gonzalez *et al.* [29]. In addition, as the CD4 loss appears to occur early and stabilize, it is possible that with successful HCV treatment may decrease immune activation, thereby, increasing CD4 cell counts. This may factor into deciding whether these patients should be treated, especially if larger studies show that the chronically high levels of activation could put patients at risk for other complications.

The NVS cohort presents an ideal opportunity to study the effects of HCV on HIV. At baseline, NVS patients have very low HIV-1 viral loads, and levels of immune activation between healthy controls and non-NVS HIV-infected patients. In addition, the NVS lack the widespread immune dysfunction and disease seen in typical HIV-infected patients.

Although this study utilized a large cohort of individuals with HIV suppression and adds to the body of literature of HIV/HCV coinfection, it has certain limitations. This study did not use a HIV/HCV-negative control group that matched IDU risk factor; however, the similar results between this group and the HCV monoinfected (majority IDU) makes it unlikely that IDU in itself could affect immune activation levels. This study also included use of a majority African-American cohort; thus, other studies will have to be done to verify the results and generalizability of these findings.

Conclusion

NVS patients with chronic HCV infection had elevated levels of immune activation (CD38-positive HLA-DR-positive CD8 cells) and lower mean CD4 cell counts, CD4 percentage, and CD4/CD8 ratio than NVS without chronic HCV infection. The difference in CD4 cell count appeared to occur early with no difference in the CD4 slopes between groups. Immune activation directly correlated with mean CD4 cell count, CD4 percentage, and CD4/CD8 ratio. Further studies will be needed to see if HCV treatment leads to lower immune activation levels and rise in CD4 cell counts in these patients.

Acknowledgements

We would like to thank members of the NVS cohort and Becky Boyce, RN, the study coordinator.

M.M.S. was supported by award number 5K23AI084580-02.

Conflicts of interest

There are no conflicts of interest.

References

1. Sajadi MM, Constantine NT, Mann DL, Charurat M, Dadzan E, Kadlecik P, Redfield RR. **Epidemiologic characteristics and natural history of HIV-1 natural viral suppressors.** *J Acquir Immune Defic Syndr* 2009; **50**:403–408.
2. Sajadi MM, Heredia A, Le N, Constantine NT, Redfield RR. **HIV-1 natural viral suppressors: control of viral replication in the absence of therapy.** *AIDS* 2007; **21**:517–519.
3. Sajadi MM, Shakeri N, Talwani R, Redfield RR. **Hepatitis C infection in HIV-1 natural viral suppressors.** *AIDS* 2010; **24**:1689–1695.

4. Pereyra F AM, Kaufmann DE, Liu Y, Miura T, Rathod A, Baker B, *et al.* **Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy.** *J Infect Dis* 2008; **197**:563–571.
5. Bailey JR LK, Yang HC, Quinn TC, Ray SC, Blankson JN, Siliciano RF. **Neutralizing antibodies do not mediate suppression of human immunodeficiency virus type 1 in elite suppressors or selection of plasma virus variants in patients on highly active antiretroviral therapy.** *J Virol* 2006; **80**:4758–4770.
6. Sedaghat AR, Rastegar DA, O'Connell KA, Dinoso JB, Wilke CO, Blankson JN. **T cell dynamics and the response to HAART in a cohort of HIV-1-infected elite suppressors.** *Clin Infect Dis* 2009; **49**:1763–1766.
7. Sajadi MM, Shakeri N, Talwani R, Howell CD, Pakyz R, Redfield RR, Parsa A. **IL28B genotype does not correlate with HIV control in african americans.** *Clin Transl Sci* 2011; **4**:282–284.
8. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, *et al.* **Relationship between T cell activation and CD4⁺ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy.** *J Infect Dis* 2008; **197**:126–133.
9. Villano SA, Vlahov D, Nelson KE, Lyles CM, Cohn S, Thomas DL. **Incidence and risk factors for hepatitis C among injection drug users in Baltimore, Maryland.** *J Clin Microbiol* 1997; **35**:3274–3277.
10. Regidor DL, Detels R, Breen EC, Widney DP, Jacobson LP, Palella F, *et al.* **Effect of highly active antiretroviral therapy on biomarkers of B-lymphocyte activation and inflammation.** *AIDS* 2010; **25**:303–314.
11. Andersson J, Fehniger TE, Patterson BK, Pottage J, Agnoli M, Jones P, *et al.* **Early reduction of immune activation in lymphoid tissue following highly active HIV therapy.** *AIDS* 1998; **12**:F123–129.
12. Lange CG, Lederman MM, Madero JS, Medvik K, Asaad R, Pacheco C, *et al.* **Impact of suppression of viral replication by highly active antiretroviral therapy on immune function and phenotype in chronic HIV-1 infection.** *J Acquir Immune Defic Syndr* 2002; **30**:33–40.
13. Resino S, Galan I, Perez A, Ramos JT, Bellon JM, Fontelos PM, *et al.* **Immunological changes after highly active antiretroviral therapy with lopinavir-ritonavir in heavily pretreated HIV-infected children.** *AIDS Res Hum Retroviruses* 2005; **21**:398–406.
14. Yin L, Rodriguez CA, Hou W, Potter O, Caplan MJ, Goodenow MM, Sleasman JW. **Antiretroviral therapy corrects HIV-1-induced expansion of CD8⁺ CD45RA⁺ CD2⁻ CD11a(bright) activated T cells.** *J Allergy Clin Immunol* 2008; **122**:166–172; 172 e161-162.
15. Al-Harathi L, Voris J, Du W, Wright D, Nowicki M, Frederick T, *et al.* **Evaluating the impact of hepatitis C virus (HCV) on highly active antiretroviral therapy-mediated immune responses in HCV/HIV-coinfected women: role of HCV on expression of primed/memory T cells.** *J Infect Dis* 2006; **193**:1202–1210.
16. Antonucci G, Girardi E, Cozzi-Lepri A, Capobianchi MR, De Luca A, Puoti M, *et al.* **Role of hepatitis C virus (HCV) viremia and HCV genotype in the immune recovery from highly active antiretroviral therapy in a cohort of antiretroviral-naïve HIV-infected individuals.** *Clin Infect Dis* 2005; **40**:e101–e109.
17. Carmo RA, Guimaraes MD, Moura AS, Neiva AM, Versiani JB, Lima LV, *et al.* **The influence of HCV coinfection on clinical, immunological and virological responses to HAART in HIV-patients.** *Braz J Infect Dis* 2008; **12**:173–179.
18. De Luca A, Bugarini R, Lepri AC, Puoti M, Girardi E, Antinori A, *et al.* **Coinfection with hepatitis viruses and outcome of initial antiretroviral regimens in previously naïve HIV-infected subjects.** *Arch Intern Med* 2002; **162**:2125–2132.
19. Greub G, Ledergerber B, Battegay M, Grob P, Perrin L, Furrer H, *et al.* **Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study.** *Lancet* 2000; **356**:1800–1805.
20. Micheloud D, Jensen J, Bellon JM, Gonzalez R, Mellado MJ, Navarro ML, *et al.* **Long-term response to highly active antiretroviral therapy in human immunodeficiency virus and hepatitis C virus coinfecting children: 6 years of follow-up.** *Pediatr Infect Dis J* 2007; **26**:1061–1064.
21. Milazzo L, Foschi A, Mazzali C, Viola A, Ridolfo A, Galli M, Antinori S. **Impact of hepatitis C viral clearance on CD4⁽⁺⁾ T-lymphocyte course in HIV/HCV-coinfecting patients treated with pegylated interferon plus ribavirin.** *AIDS Res Hum Retroviruses* 2012. [Epub ahead of print]
22. Peters L, Mocroft A, Soriano V, Rockstroh JK, Losso M, Valerio L, *et al.* **Hepatitis C virus coinfection does not influence the CD4 cell recovery in HIV-1-infected patients with maximum virologic suppression.** *J Acquir Immune Defic Syndr* 2009; **50**:457–463.
23. Rockstroh JK, Mocroft A, Soriano V, Tural C, Losso MH, Horban A, *et al.* **Influence of hepatitis C virus infection on HIV-1 disease progression and response to highly active antiretroviral therapy.** *J Infect Dis* 2005; **192**:992–1002.
24. Seminari E, Tinelli C, Ravasi G, Ripamonti D, Ladisa N, Marino N, *et al.* **Hepatitis C infection on immune recovery in HIV-positive patients on successful HAART: the role of genotype 3.** *Curr HIV Res* 2010; **8**:186–193.
25. Law WP, Duncombe CJ, Mahanontharit A, Boyd MA, Ruxrungtham K, Lange JM, *et al.* **Impact of viral hepatitis coinfection on response to antiretroviral therapy and HIV disease progression in the HIV-NAT cohort.** *AIDS* 2004; **18**:1169–1177.
26. Macias J, Pineda JA, Lozano F, Corzo JE, Ramos A, Leon E, *et al.* **Impaired recovery of CD4⁺ cell counts following highly active antiretroviral therapy in drug-naïve patients coinfecting with human immunodeficiency virus and hepatitis C virus.** *Eur J Clin Microbiol Infect Dis* 2003; **22**:675–680.
27. Santin M, Mestre M, Shaw E, Barbera MJ, Casanova A, Niubo J, *et al.* **Impact of hepatitis C virus coinfection on immune restoration during successful antiretroviral therapy in chronic human immunodeficiency virus type 1 disease.** *Eur J Clin Microbiol Infect Dis* 2008; **27**:65–73.
28. d'Arminio Monforte A, Cozzi-Lepri A, Castagna A, Antinori A, De Luca A, Mussini C, *et al.* **Risk of developing specific AIDS-defining illnesses in patients coinfecting with HIV and hepatitis C virus with or without liver cirrhosis.** *Clin Infect Dis* 2009; **49**:612–622.
29. Gonzalez VD, Falconer K, Blom KG, Reichard O, Morn B, Laursen AL, *et al.* **High levels of chronic immune activation in the T-cell compartments of patients coinfecting with hepatitis C virus and human immunodeficiency virus type 1 and on highly active antiretroviral therapy are reverted by alpha interferon and ribavirin treatment.** *J Virol* 2009; **83**:11407–11411.
30. Kovacs A, Al-Harathi L, Christensen S, Mack W, Cohen M, Landay A. **CD8⁽⁺⁾ T cell activation in women coinfecting with human immunodeficiency virus type 1 and hepatitis C virus.** *J Infect Dis* 2008; **197**:1402–1407.
31. Kovacs A, Karim R, Mack WJ, Xu J, Chen Z, Operskalski E, *et al.* **Activation of CD8 T cells predicts progression of HIV infection in women coinfecting with hepatitis C virus.** *J Infect Dis* 2010; **201**:823–834.
32. Sandberg JK, Falconer K, Gonzalez VD. **Chronic immune activation in the T cell compartment of HCV/HIV-1 co-infected patients.** *Virulence* 2010; **1**:177–179.