Study on the blood-borne virus co-infection and T lymphocyte subset among intravenous drug users

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AIM: To investigate the features of various blood-borne virus infections and co-infection in intravenous drug users (IDUs), and to examine the correlation of T lymphocyte subsets with virus co-infection.

METHODS: Four hundred and six IDUs without any clinical manifestation of hepatitis and 102 healthy persons were enrolled in this study. HBV-DNA and HCV-RNA were detected by fluorescence quantitative PCR. HBsAg, HBeAg, anti-HBc, anti-HCV, HDV-Ag, anti-HGV, anti-HIV, and HCMV-IgM were assayed by enzyme-linked immunosorbent assay (ELISA) and immunochromatographic tests. The levels of Th1 and Th2 cytokines were measured by ELISA and radioactive immune assay (RIA). The T lymphocyte subpopulation was detected by using fluorescence immunoassay. The similar indices taken from the healthy persons served as controls.

RESULTS: The viral infection rate among IDUs was 36.45% for HBV, 69.7% for HCV, 47.3% for HIV, 2.22% for HDV, 1.97% for HGV, and 3.45% for HCMV. The co-infection rate of blood-borne virus was detected in 255 of 406 (62.81%) IDUs. More than 80% (161/192) of subjects infected with HIV were co-infected with the other viruses, such as HBV, HCV. In contrast, among the controls, the infection rate was 17.65% for HBV and 0% for the other viruses. Our investigation showed that there was a profound decrease in the proportion of CD4/CD8 and the percentage of CD3 and CD4, but not in the percentage of CD8. The levels of PHA-induced cytokines (IFN-γ and IL-4) and serum IL-2 were obviously decreased in IDUs. On the other hand, the level of serum IL-4 was increased. The level of IFN-γ and the percentage of CD4 were continuously decreased when the IDUs were infected with HIV or HIV co-infection. IDUs with HIV and HBV co-infection was 15.1% (29/192). Of those 29 IDU with HIV and HBV co-infection, 51.72% (15/29) and 37.93% (11/29) were HBV-DNA-positive and HBeAg-positive, respectively. But, among IDUs without HIV infection, only 1.68% (2/119) of cases were HBV-DNA-positive.

CONCLUSION: HCV, HBV and HIV infections are common in this population of IDU, leading to a high incidence of impaired Th1 cytokine levels and CD4 lymphocyte. IDUs with HIV and HBV/HCV co-infection have lower expression of Th1 cytokine with enhancement of the Th2 response. HIV may be causing HBV replication by decreasing Th1 function.

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and HGV and examined the association of the virus co-infection with Th lymphocyte response in IDU.

MATERIALS AND METHODS

Study population
In this study, 508 serum samples, including samples from 406 IDUs (heroin addicts, 383 males and 23 females, mean age 32.4 years, range 17-61 years) who had a history of drug use (0.1-0.5 g/d) for 1-18 years and 102 controls (64 males and 38 females; mean age 30.5 years, range 18-58 years) in Southwestern China, were examined. All subjects had no clinical manifestation of hepatitis. One hundred and ninety-nine cases had shared needles with other drug users. Informed consent for inclusion in this study was obtained from each individual. The blood samples were collected from 2002 to 2003 and sera were stored at -40°C or below until use.

Viral marker detection
Samples were detected for markers of virus infection (HBsAg, HBeAg, anti-HBc, anti-HCV, HDV-Ag, anti-HGV and HCMV-IgM) by enzyme-linked immunosorbent assay kit (ELISA, Kehua and Bosai, China). Serum samples were also tested for HCV-RNA and HBV-DNA by fluorescence quantitative polymerase chain reaction (F-Q-PCR Technologies, China). Samples were detected for HIV-1/HIV-2 infection markers by immunochromatographic test kits (Abbott, USA).

Assay for PHA-induced cytokines and serum cytokines
Peripheral blood mononuclear cells (PBMCs) were isolated from 3 mL of fresh heparinized blood samples obtained from each subject by Ficoll centrifugation. The PBMCs were suspended in RPMI 1640 (PHA-M added to 20 μg/mL) and were placed in culture plates at 1 × 10⁶ cells per well for proliferation and cytokine assays. Supernatants were removed after 60-h incubation to determine IFN-γ and IL-4. The levels of IFN-γ and IL-4 were determined by commercial ELISA kit (ELISA, Jingmei, Guangzhou). The serum levels of IL-2 and IL-4 were determined by radioactive immune assay (RIA, 3V Co., China). All commercial kits were used following the manufacturer's instructions.

Assay for T lymphocyte subsets
T lymphocyte subsets were detected by means of fluorescence immunoassay. CD-specific mAb and FTTC-conjugated goat anti-mouse IgG were purchased from Huamei Biotechnical Company of Zhengzhou, China.

Statistical analysis
The results were analyzed by using Student’s t-test and Chi-square test, as appropriate. P < 0.05 was considered statistically significant.

RESULTS

Prevalence and co-infection of blood-borne viruses
Among 406 IDUs, 283 (69.7%) were anti-HCV-positive; among these 283 anti-HCV-positive cases, 55 (19.43%) were positive for HCV-RNA (2.98 × 10⁶ - 2.25 × 10⁸ copies/L). Of 406 IDUs, 148 (36.45%) suffered from HBV infection (anti-HBc-positive); of these 148 cases, 12 (8.11%) and 17 (11.49%) had detectable serum HBeAg and HBV-DNA (4.59 × 10⁶ - 5 × 10⁸ copies/L), respectively. Out of 406 IDUs, 192 (47.3%) suffered from HIV infection (anti-HIV-1/2-positive). The rate of HDV, HGV and HCMV seroprevalence was lower than that of HCV/ HIV/HBV. The rate of virus co-infection was very high (62.81%, 255/406). In IDUs without infection markers, serum HCV-RNA or HBV-DNA could not be detected. Anti-HCV was not associated with HCV-RNA. Blood-borne virus seropositivity among IDUs was associated with needle sharing. Among 102 controls, HBV infection rate was 17.65%, but no other blood-borne viruses were detected (Table 1).

Cytokine levels and T lymphocyte subsets
The percentage of CD3/CD4 and the proportion of CD4/CD8 were obviously lower in IDUs (HIV-negative) than in healthy controls (P < 0.01). The levels of PHA-induced IFN-γ and IL-4 were lower in IDUs than in healthy controls. The level of serum IL-2 was lower, but IL-4 was higher (Table 2).

Co-infection of HIV and HBV
IDUs with HIV and HBV co-infection were 15.1% (29/192). Twenty-four (5.91%) patients were HBsAg-positive and 12 of these (50%) had detectable HBeAg as a marker for HBV replication. Serum HBV-DNA was detected in 17 of the 24 HBsAg-positive patients (70.83%), with a mean concentration of 4.59 × 10⁶ - 5 × 10⁸ copies/L. Serological markers of HBV infection in all IDUs according to the relation to HIV-infection are shown in Table 3. Among 29 IDUs with HIV-infection, 15 (51.72%) were HBV-DNA-positive and 11 (37.93%) were HBeAg-positive. But, among IDUs without HIV infection, only 1.68% (2/119) cases were HBV-DNA-positive.

Co-infection and T lymphocyte subsets
Among 192 subjects with HIV infections, 29 (15.1%) and 154 (80.21%) were co-infected with HBV and HCV, respectively. In IDUs, HIV infection and co-existence of HBV/HCV infection were related to the decreased level of PHA-induced IFN-γ and CD3/CD4 percentage. But, higher virus load was not correlated with lower IFN-γ level. Serum cytokines showed no significant difference between HIV-infected IDUs and non-HIV-infected IDUs. The IFN-γ level and CD4 percentage showed no significant difference between virus gene-positive IDUs and virus gene-negative IDUs (Tables 4 and 5).

DISCUSSION

Drug abuse has spread quickly since reemerging as a national problem in the world in the late 1970s. Addiction spread like a plague, especially heroin abuse. One major drug-related problem is the spread of HIV and HCV, causing a major social and economic damage in many
Drug abuse has led to many problems, in particular HIV/AIDS, HCV and sexual transmission diseases in China. Heroin addicts have an increased susceptibility to a variety of infectious diseases, and alterations in a wide variety of immune parameters also have been reported among them[10].

Our data showed that the viral infection rates among IDUs were considerably higher than in the non-IDU population. Repeated co-infection with different viruses probably is common in IDU[10]. HBV, HCV, HIV and HGV share transmission routes. The viruses enter the host by drug use through injection and blood transfusion. Drug abuse has led to many problems, in particular HIV/AIDS, HCV and sexual transmission diseases in China. Heroin addicts have an increased susceptibility to a variety of infectious diseases, and alterations in a wide variety of immune parameters also have been reported among them[10].

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was observed. Only 45 (11.08%) of the subjects had no blood-borne virus infection, whereas 361 (88.92%) were infected with one or more than one viruses. These results are in agreement with the findings reported recently\[^{[3]}\].

Specific antiviral immunity includes two effectors mechanisms: cellular immune response and humoral immune responses. In general, effectors CD4 and CD8 T cell responses subside as the virus infection is resolved, but antibody production can last for several months to years. Viruses are small, obligate intracellular parasites which cause infection by invading cells of the body and replication within them. Cell-mediated immune response plays a very important role in the resistance and prophylaxis in viral infection. In IDU, a profound decrease in the proportion of CD4/CD8 and the percentage of CD3 and CD4, but not in the percentage of CD8 has been observed. The present study also confirmed the observation of other scholars that drug abuse suppresses immune function and disturbs T lymphocyte regulation. Other studies also suggested that morphine attenuated the mitogen-induced lymphocyte proliferation in a dose-dependent manner. In addition, morphine inhibited proliferation of PHA-IL-2 activation of na\’ive mice thymocytes in a dose-dependent manner \textit{in vitro}\[^{[4]}\]. Morphine-mediated effects on the immune system operate through central processes\[^{[5]}\]. Opiates behave like cytokines, modulating the immune response by interaction with their receptors in the central nervous system and in the periphery. Potential mechanisms by which central opioids modulate peripheral immune functions may involve both the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system. The HPA axis is necessarily involved in all opioid-mediated immunosuppressive effects. There are opioid receptors on cells of the immune system. These results provide evidence for the local and systemic activity of opioids on immunocompetence and immune homeostasis\[^{[6]}\].

CD4+ Th lymphocyte plays an important role in the immune response against intracellular viral, bacterial, and parasitic pathogens. It is known that the subsets of Th cell could be distinguished by the pattern of cytokine co-expression. Th1 cells produce IFN-γ, TNF-β and IL-2, and Th2 cells produce IL-4, IL-5 and IL-10. IFN-γ and IL-2 are cytokines that play important roles in the development of CD4+CD8+ lymphocyte activity and cellular immune response, such as enhancing activities of cytotoxic T lymphocytes (CTL), NK and Th1. IL-4 inhibited the production of Th1 cells, modulated humoral immunity, and resulted in immune pathologic diseases\[^{[7,8]}\]. The advances in the knowledge on Th1 and Th2 cells revealed that Th1 cells produce cytokines that stimulate the proliferation of CTL. Th2 cells produce cytokines that are responsible for the activation of the humoral immune response in healthy people. The Th1 and Th2 subtypes are stable and represent significant function differences between two sets of Th cells. Clerici and Shearer\[^{[9]}\] presented a hypothesis, “the Th1 → Th2 switch hypothesis”, whereby Th1 cell activity declines and Th2 activity increases in HIV-infected people.

Our investigation showed that the levels of PHA-induced IFN-γ, IL-4 and serum IL-2 were obviously decreased in IDUs, but the levels of serum IL-4 were increased (\(P < 0.01\)). IDUs have the imbalance of Th1 and Th2. Virus entry into blood might also be facilitated due to the dysfunction of the T lymphocyte subpopulation in IDU. Conflicting results, both decreased induced-IL-4 and increased serum IL-4, have been detected concerning the altered immune function in IDUs. There are other pathogens (parasites, fungi, viruses) infections, and modulation of surface markers on T cells\[^{[10,11]}\]. Divaricating results, in part, may be due to the specific mechanisms responsible for opiate-induced changes in the immune system being undefined.

The prevalence of HBV in patients infected with HCV is common in chronic hepatitis, hepatocirrhosis and hepatocellular carcinoma\[^{[12]}\]. According to different routes of transmission, 10%-15% of the HBV-infected patients presented with detectable antibodies against hepatitis C. Among 406 IDUs, HBV/HCV co-infection rate was 27.83% (113/406). There are reports on the changes of viral behavior and accelerated progression to more advanced liver disease after the co-infection of several viruses in IDU, but there is good understanding of the critical roles of these viruses, of whether the phenomenon of interference among viruses exists, and of how to evaluate the pathogenesis of the viruses. Gilson et al\[^{[13]}\] believed that the co-infection of HCV and HBV could increase the expression of anti-HBs and DNA polymerase. They proposed that co-infection of both HCV and HBV in IDU might augment the level of HBV replication and aggravate the damage to the liver. But our reports concerning co-infection of HCV and HBV yielded different results and no significant correlation was observed between HBV-DNA and HCV infection. The discrepancy might be due to the small number of our samples (17 HBV-DNA-positive cases). We can hypothesize that this may be important in the presence of an additional infection of the body with, for example, parasite or fungi infection\[^{[10]}\]. However, part of the difference was likely due to the immune system dysfunction.

Heroin abusers are a high-risk group for the development of HIV, HCV and HBV infection. According to different routes of transmission, 64%-77% of the HIV-infected patients present with detectable antibodies against hepatitis C and 10%-21% are positive for anti-HBe antibodies\[^{[14]}\]. Co-infection with HIV and hepatotrophic viruses causes complex interactions. The results from our study indicate that more than 80% (161/192) of subjects infected with HIV were co-infected with HBV/HCV (15.1% with HBV, and 80.21% with HCV). Chung et al\[^{[15]}\] reported the prevalence of chronic HBV infection varies between the risk groups for transmission of HIV (21% for homosexuals, 14% for heterosexuals, 7% for drug addicts, and 14% for hemophiliacs). HIV-induced impairment of the cell-mediated immunity causes higher replication of hepatotropic viruses. Additionally, HBV leads to enhanced transcription of HIV through an NF-kB element in the long terminal repeat of HIV\[^{[16]}\]. Our data showed that among 29 IDUs with HIV-infection, 51.72% (15/29) were HBV-DNA-positive and 37.93% (11/29) were HBeAg-positive, whereas only 1.68% (2/119) of the IDU
without HIV infection were HBV-DNA-positive. These observations confirm previous reports of increased viral replication of hepatotropic virus in immunocompromised patients.

The prevalence of hepatitis C in HIV-infected patients is strongly related to the parenteral route of infection, and therefore drug addicts present the highest rate of co-infection. The problem of co-infection of HCV and HIV will become more relevant. The permanent state of chronic immune dysfunction related to the persistent hepatitis C virus favors transcription of HIV in infected cells and causes a more rapid destruction of Th lymphocytes. HIV-induced impairment of the cell-mediated immunity causes higher HCV replication. Recent studies have shown significantly higher HCV-RNA levels in HIV-positive patients than in HIV-negative controls. In contrast, no correlation between anti-HIV and HCV-RNA was observed in our study. We can hypothesize that this may be asymptomatic, additional co-infection and immune status of IDU.

Bias in cytokine responses has been proposed as a contributing mechanism to pathogenesis in persistent HIV or HCV infections. Lee et al demonstrated that HCV and HIV co-infection affected cytokine mRNA levels in PBMC, but did not shift the Th1/Th2 balance. Rodrigues et al suggested the balance favorable to Th2 was associated with the presence of HIV and parasite co-infection. Eyster et al showed, in a group of hemophiliacs, a negative correlation between CD4 count and HCV-RNA levels. In the IDUs studied here, reduced CD4 percentage and induced-IFN-γ in the HIV as well as in the HIV co-infection group were associated with a significantly reduced Th1 function compared to the negative group. But a reduced Th1 function in the HIV as well as in the HIV co-infection group was not associated with HBV-DNA/HCV-RNA. Becker suggested that asymptomatic HIV co-infection importantly alters the HCV-specific cytokine response towards a greater production of pro-inflammatory type 1 cytokines. Moreover, the antiviral activity of type 1 cytokines may be modified by an increased production of type 2 cytokines in the CD30 subset. The altered cytokine pattern may contribute to the adverse natural course of hepatitis C in HIV co-infection. Therefore, in IDUs, the balance of Th lymphocyte subsets was upset, and this was especially true for HIV infection and co-infection.

In conclusion, our study demonstrates that the prevalence of HCV, HIV and HBV infection and co-infection is high in IDU. The infection inversely accelerates the disturbance of Th function. IDUs with HIV and HBV/HCV co-infection have lower expression of Th1 cytokine without enhancement of the Th2 response. HIV infection leads to a high incidence of impaired Th1 cytokine levels. Larger trials are needed to study appropriate prevention and treatment strategies. Blood-borne virus prevention schemes include blood bank safety programmes, and immunization of high-risk groups.

REFERENCES

1. Vallejo R, de Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. Am J Ther 2004; 11: 354-365
2. Ahlenstiel G, Iwan A, Nattermann J, Bueren K, Rockstroh JK, Brackmann HH, Kupfer B, Landt O, Peled A, Sauerbruch T, Spengler U, Woitas RP. Distribution and effects of polymorphic RANTES gene alleles in HIV/HCV coinfection -- a prospective cross-sectional study. World J Gastroenterol 2005; 11: 7631-7638
3. Wu N, Li D, Zhu B, Zou W. Preliminary research on the co-infection of human immunodeficiency virus and hepatitis virus in intravenous drug users. Chin Med J (Engl) 2003; 116: 1318-1320
4. Roy S, Chapin RB, Cain KJ, Charboneau RG, Ramakrishnan S, Barke RA. Morphine inhibits transcriptional activation of IL-2 in mouse thymocytes. Cell Immunol 1997; 179: 1-9
5. Theodorou S, Haber PS. The medical complications of heroin use. Curr Opin Psychiatry 2005; 18: 257-263
6. De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ. Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. Eur J Neurosci 1998; 10: 3565-3571
7. Doherty DG, Norris S, Madrigal-Estebas L, McEntee G, Schoffelmeer AN, Binnekade R, Mulder AH, Singh S. Hepatitis B and C virus co-infections in drug users. J Hepatol 2003; 39: 480-487
8. Lee JR, de Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. Am J Ther 2004; 11: 354-365
9. Li JR, de Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. Am J Ther 2004; 11: 354-365
10. Miraglia del Giudice M, Decimo F, Leonard S, Maioello N, Amelio R, Capasso A, Capristo C, Capristo AF. Immune dysregulation in atopic dermatitis. Allergy Asthma Proc 2006; 27: 451-455
11. Clerici M, Shearer GM. A Th1→Th2 switch is a critical step in the etiology of HIV infection. Immunol Today 1993; 14: 107-111
12. Renia L, Potter SM. Co-infection of malaria with HIV: an immunological perspective. Parasite Immunol 2006; 28: 599-595
13. Thorpe LE, Frederick M, Pitt J, Cheng I, Watts DH, Buschur S, Green K, Zorrika C, Landesman SH, Harlow RC. Effect of hard-drug use on CD4 cell percentage, HIV RNA level, and progression to AIDS-defining class C events among HIV-infected women. J Acquir Immune Defic Syndr 2004; 37: 1423-1430
14. Andrade AF, Oliveira-Silva M, Silva SC, Mota JJ, Bonvivino CR. Seroprevalence of hepatitis B and C virus markers among blood donors in Rio de Janeiro, Brazil, 1998-2005. Mem Inst Oswaldo Cruz 2006; 101: 673-676
15. Gilson RJ, Hawkins AE, Beacham MR, Ross E, Waite J, Briggs MC, McNally T, Kelly GE, Tedder RS, Weller IV. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. AIDS 1997; 11: 597-606
16. Gupta S, Singh S. Hepatitis B and C virus co-infections in human immunodeficiency virus positive North Indian patients. World J Gastroenterol 2006; 12: 6879-6883
17. Chung RT, Kim AY, Polsky B. HIV/Hepatitis B and C coinfection: pathogenic interactions, natural history and therapy. Antivir Chem Chemother 2001; 12: Suppl 1: 73-91
18. Miller AO. Management of HIV/HBV coinfection. MedGenMed 2006; 8: 41
19. Dutoit V, Ciufrreda D, Comte D, Gonvers JJ, Pantaleo G. Differences in HCV-specific T cell responses between chronic HCV infection and HIV/HCV co-infection. Eur J Immunol 2005; 35: 3493-3504
20. Anthony DD, Yonkers NL, Post AB, Aakira R, Heinzelf FP, Lederman MM, Lehmann PV, Valdez H, Hunter RC. Effect of hard-drug use on CD4 cell percentage, HIV RNA level, and progression to AIDS-defining class C events among HIV-infected women. J Acquir Immune Defic Syndr 2004; 37: 1423-1430
21. Lee S, Watson MW, Clark B, Flexman JP, Cheng W, French MA, Price P. Hepatitis C virus genotype and HIV coinfection affect cytokine mRNA levels in unstimulated PBMC but do not shift the T1/T2 balance. Immunol Cell Biol 2006; 84: 390-395
22. Rodrigues DB, Correia D, Marra MD, Giraldo LE, Lages-Silva E, Silva-Vergara ML, Barata CH, Rodrigues Junior V. Cytokine serum levels in patients infected by human immunodeficiency
virus with and without Trypanosoma cruzi coinfection. Rev Soc Bras Med Trop 2005; 38: 483-487

21 Eyster ME, Fried MW, Di Bisceglie AM, Goedert JJ. Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. Multicenter Hemophilia Cohort Study. Blood 1994; 84: 1020-1023

22 Becker Y. The changes in the T helper 1 (Th1) and T helper 2 (Th2) cytokine balance during HIV-1 infection are indicative of an allergic response to viral proteins that may be reversed by Th2 cytokine inhibitors and immune response modifiers—a review and hypothesis. Virus Genes 2004; 28: 5-18