

Bachelor Thesis

IMPAIRED HCV ANTIBODY RESPONSE
IN HIV/HCV CO-INFECTED PATIENTS

A POSSIBLE ROLE FOR TGF-B

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INTRODUCTION

Human immunodeficiency virus (HIV) is known to be a major health problem throughout the world, especially in non-developed countries. Approximately 34 million people worldwide are infected with HIV [Hernandez MD, 2011; Rider PJ, 2012]. HIV causes a disease known as AIDS (acquired immunodeficiency syndrome), which is characterized by a profound suppression of the host's immune system and is associated with opportunistic infections causing multiple severe diseases [Abbas AK, 2007]. No cures have been found yet for AIDS or HIV, but due to effective combination anti-retroviral therapy (cART), which was introduced in 1996, an enormous reduced mortality from HIV infection is seen in developed countries. This also resulted in a decline in development of AIDS and AIDS related deaths in HIV infected patients [Joshi D, 2011]. Now, more than 50% of all deaths in HIV infected individuals have causes other than AIDS.

The most frequent causes of non-AIDS related death in HIV infected individuals, are shown to be liver-related [The D:A:D Study Group, 2006], of which 66% are the consequence of the hepatitis C virus [Joshi D, 2011]. Prior to the cART in HIV infected patients, these patients did not survive long enough to even suffer disease caused by chronic infection with HCV. However, since cART, patients infected with HIV do live long enough to be affected by chronic HCV co-infection [Rider PJ, 2012]. This chronic infection can cause liver fibrosis, cirrhosis, liver cancer and by doing so even death.

HIV and HCV can be transmitted via similar routes of infection, for example via the blood borne route and via sexual transmission. Because of these shared routes of transmission, co-infection with HCV is a common phenomenon in patients infected with HIV [Joshi D, 2011]. It is estimated that about 30% of people infected with HIV are co-infected with HCV [Hernandez MD, 2011]. Patients who are co-infected with HIV and HCV are known to progress faster to end-stage liver disease than patients infected with HCV alone. Liver fibrosis, which is scarring of the liver, is accelerated in co-infected individuals [Joshi D, 2011]. After acute infection with HCV, 90% of patients infected with HIV will develop chronic HCV disease, whereas 70% of individuals with HCV mono-infection will develop chronic HCV disease. This shows that the risk of chronic disease caused by HCV is much higher in patients co-infected with HIV [Hernandez MD, 2011].

If someone is infected with a virus is usually tested based on the presence of antibodies against that virus in that person's blood. Antibody testing is also one of the main screening methods for HCV infection in HIV infected individuals [WHO]. However, it is known that there can be an impaired antibody response against HCV antigens in HIV-infected patients [George SL, 2002; Chamot E, 1990; Cribier B, 1995]. Because of this impaired antibody formation, antibodies against HCV are not detectable and false-negative results occur in patients who are co-infected. These false negative results may cause a delay in detection of HCV in HIV-infected patients, which may reduce the chance of successful treatment [Thomson EC]. Because of this, it is important to create more knowledge about how HIV-associated impaired immune responses affects HCV infection and, more importantly, the formation of antibodies against HCV antigens. I try to do so in this bachelor thesis by researching the available literature and some common sense.

HEPATITIS C VIRUS (HCV)

The hepatitis C virus (HCV) causes viral hepatitis. Hepatitis C can range in severity from a few weeks lasting mild malady to a serious chronic condition, which can lead to liver cirrhosis and, liver cancer and, by doing so, death. An estimated 130-170 million people have a chronic HCV infection. Hepatitis C-related liver diseases cause the death of more than 350,000 people each year, even though it is curable using effective antivirals. A lot of research is performed regarding HCV, but no vaccine has been developed yet. Transmission of HCV occurs most commonly through exposure to infectious blood, for example through blood transfusions, organ transplants and injections given in health care settings or given for drug use. Also, HCV infected mothers can transmit the virus to their children. Less commonly, HCV is transmitted through sex with an infected person. HCV is not transmitted through casual contact, food or water [WHO].

Acute HCV infection is generally asymptomatic in patients who are immune-competent, and because of this, the true incidence of HCV infection is underestimated [Zylberberg H, 1996; Joshi D, 2011]. HCV is asymptomatic in about 80% of the people who get infected with HCV. People who do exhibit symptoms after acute infection often show fever, fatigue, nausea, vomiting and jaundice. Because of the high percentage of asymptomatic patients, HCV infection is often not diagnosed. HCV is generally diagnosed with the presence of antibodies against HCV, anti-HCV antibodies, in someone's blood. However, presence of antibodies in the blood does not mean that a patient has an active infection, since the presence of antibodies can also indicate past infection [WHO].

When anti-HCV antibodies are present for more than six months, the diagnosis of chronic infection is made. When a chronically infected person exhibits symptoms, it might indicate liver disease in advanced stage. About 65% of people who are diagnosed with chronic HCV develop chronic liver disease of which 5%-20% can develop in cirrhosis, which can ultimately lead to liver cancer and death [WHO]. Also, in the Western World, chronic HCV is one of the major causes of liver transplantation [Gerlach JT, 2002].

HCV is an enveloped positive stranded RNA virus, which belongs to the *Flaviviridae* family [Rivière Y, 2012; Logvinoff C, 2004] and the *Hepacivirus* genus. HCV has been classified into 6 genotypes with more than 100 subtypes [Attallah S, 2001]. Replication of HCV occurs mainly in the liver, and about 30% of HCV infected individuals spontaneously clear the virus. When not cleared spontaneously, more progressive liver disease may be the result, because the infection progresses to a chronic stage. This occurs in 70% of HCV infected individuals [Rivière Y, 2012; Logvinoff C, 2004]. Occurrence of chronic infection in such high frequency suggests that initiation of an effective antiviral immune response to HCV fails to be initiated or maintained, possibly because of virus-mediated immune escape strategies [Logvinoff C, 2004].

The immune response against viruses can consist of two different kinds of immune responses, which have interactions with each other. The first of the two immune responses is the cellular immune response, which is mediated by T-cells and includes CD4+ T-cells and CD8+ T-cells. The other immune response is the humoral immune response. This response is characterized by antibodies produced by B-cells. In case of

HCV, the interaction between these two kinds of responses is important. This is because HCV antigens are protein antigens, and these kinds of antigens need to be recognized by helper T-cells, which are commonly CD4+CD8-, in order to generate a sufficient humoral immune response. After recognition of the antigen, helper T-cells activate B-cells with cytokines to differentiate into antibody secreting cells against that antigen (figure 1) [Abbas AK, 2007]. A major role in the protection from virus infection, after natural infection or vaccination, is played by neutralizing antibodies (nAbs). It was found that in HCV infection, strain-specific and cross-reactive nAbs responses are elicited, which develop late in chronic phase of infection [Logvinoff C, 2004]. But also, lack of detectable antibodies in patients who were HCV RNA positive has been reported by several groups. These false-negative results were particularly found among patients with chronic liver disease [George SL, 2002]. How these false-negative results occur, is not clear yet.

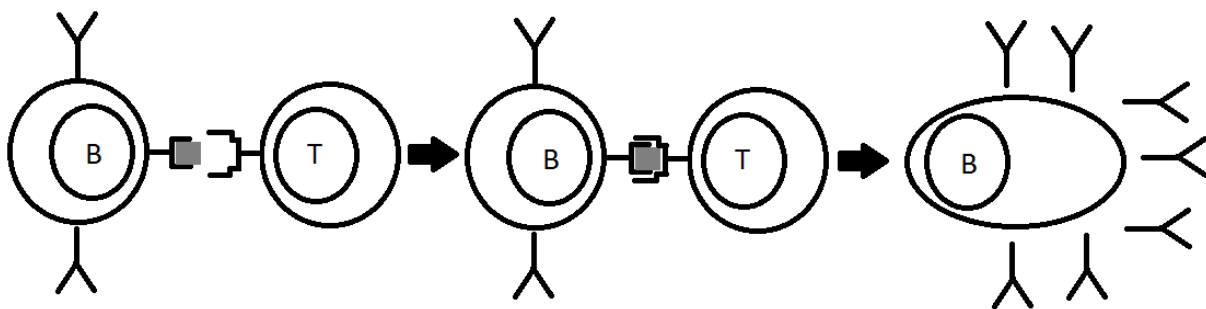


FIGURE 1: NORMAL HELPER T-CELL DEPENDENT B-CELL ACTIVATION. After recognition of the HCV antigen, an activated B-cell and an activated T-cell interact with each other. The T-cell then activates the B-cell, resulting in B-cell proliferation and differentiation into antibody secreting cells against the HCV antigen [Adapted from Abbas AK, 2007].

Chen *et al.* (1999) studied the specific humoral immune response to structural and non-structural proteins of HCV in acute and chronic phase of the disease. It has been shown previously that in chronic and viral diseases, the most commonly detected antibodies are anti-viral IgG1 and IgG3 antibodies [Chen M, 1999]. Chen *et al.* found that antibody response to all HCV proteins, except the HCV core protein, were highly restricted to the IgG1 antibody isotype. The same observation was made by Netski *et al.* (2005). This might not be expected to mediate efficient neutralization and clearance of the virus. Chen *et al.* also found that low titers of antibodies against the HCV antigens are produced compared to patients with chronic hepatitis B virus. Also, they showed a delay in appearance of antibody production. No significant levels of anti-HCV antibodies were found during the acute phase, the first 6 months, of HCV infection. They suggest that the level of antigen stimulation may be too low to reach threshold to induce and maintain a humoral immune response which is sufficient enough [Chen M, 1999].

Also, it was shown that CD4+ T cell play an important role in the clearance of HCV. The loss of HCV specific CD4+ T cell response in HCV infected individuals was promptly followed by HCV recurrence. The results indicate that a virus specific CD4+/Th1+ T cell response that eliminates the virus during acute phase of disease has to be maintained permanently to achieve long-term control of the virus [Gerlach JT, 2002].

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV is nowadays a global health problem with major impact. The virus was discovered in 1981 and since then, millions of people have died from the disease HIV causes: acquired immunodeficiency syndrome (AIDS) [SOA AIDS NL]. Approximately 33.3 million people are infected and living with HIV, and an estimated 1.8 million people die each year from AIDS-related deaths [UNAIDS Global Report 2010].

HIV can be transmitted through body fluids, such as blood, semen and vaginal secretions. Also, it can be transmitted through breast milk. Symptoms of HIV vary according to the stage of the infection. In the first few weeks after infection, symptoms may include a flu-like illness expression fever, headache, rash or sore throat. However, the first few weeks of infection can also occur asymptotically. Symptoms change as the infection progressively weakens the immune system of the infected person. Symptoms include swollen lymph nodes, weight loss, fever and diarrhea. If HIV infected persons are not treated, severe diseases can develop, such as cancer and opportunistic infections, among which tuberculosis, meningitis [WHO].

HIV belongs to the Lentivirus family. This family belongs to animal retroviruses, which are RNA tumor viruses. Lentiviruses produce fatal diseases, which slowly progress and which can be latent. This includes central nervous system degeneration and wasting syndromes, which includes progressive loss of weight and muscle tissue. HIV-1 and HIV-2 are two closely related types of HIV which have been identified. AIDS is commonly caused by the HIV-1 type, but HIV-2, which differs from HIV-1 in genomic structure and antigenicity, causes a clinical syndrome which is very similar to AIDS [Abbas AK, 2007].

HIV is diagnosed by detecting the presence of antibodies to HIV antigens in blood. In the first 3 to 12 weeks, antibodies against HIV antigens are still being produced and may not be detectable yet. Because of this, retesting should be done after three months to confirm infection [WHO]. For earlier diagnosis, it is possible to use PCR testing.

HIV infection causes diverse complications in the immune system of the host [Netski DM, 2007]. Not long after HIV was discovered, it became clear that *in vitro*, HIV could replicate within human CD4+ cells [McCune JM, 2001]. These include T-cells, monocytes and macrophages, which all play important roles in the immune system [Abbas AK, 2007]. Also, the protein of the viral envelope is able to bind to CD4 and as HIV progresses, circulating CD4+ T-cells decrease in number. It also seems that CD4+ T helper cells have a crucial role in the coordination of cellular and humoral immune responses against exogenous antigens [McCune JM, 2001]. So, depletion of these cells causes severe immune deficiencies. Cures for HIV and AIDS are not available yet, despite all ongoing research, but with treatment with antiretroviral drugs, it is possible for patients to control the virus and enjoy a healthy life [WHO].

HIV/HCV CO-INFECTION

Due to effective combination anti-retroviral therapy (cART), which was introduced in 1996, an enormous reduced mortality from HIV infection is seen in developed countries. This resulted in a decline in AIDS and AIDS related deaths in HIV infected patients [Joshi D, 2011]. Now, more than 50% of all HIV-related deaths have causes other than AIDS. The most frequent cause of non-AIDS related death in HIV infected individuals, are shown to be liver-related [The D:A:D Study Group, 2006]. 66% of these liver-related deaths are the consequence of the hepatitis C virus [Joshi D, 2011].

HCV infection occurs in approximately 20%-30% of the 33.3 million people who live with HIV. This is called co-infection. A characteristic that HIV and HCV share is that they result in high-grade chronic viraemia within the host that can last indefinitely, unless treated. Also, the disease course in both cases is initially asymptomatic, but after years to decades, they result in destruction of the immune system (in case of HIV) or severe liver disease (in case of HCV) [Kim AY, 2009].

Co-infection of HIV and HCV appears to be more common among certain populations. These include hemophiliacs, men having sex with men (MSM) and intravenous drug users (IDUs). Among IDUs, the prevalence of HIV/HCV co-infection is as high as 90%. This is because the main source of transmission of both HIV and HCV is the blood borne route [Hernandez MD, 2011]. However, the risk of transmission of HCV is approximately 10-fold higher than the risk of HIV transmission in IDUs [Hernandez MD, 2011; Kim AY, 2009]. This might be related to the greater concentrations of HCV than HIV in the blood. Because of this, patients with HIV/HCV co-infection were usually infected first with HCV, instead of the other way around [Kim AY, 2009; George SL, 2002]. The prevalence of HCV is substantially lower among patients who become infected with HIV via sexual transmission than via the blood borne route. This is because transmission of HIV is far more efficient via sexual exposure than HCV transmission is, because HIV is adapted to cross the mucosa found at rectal and genital surfaces [Kim AY, 2009].

90% of patients who are already infected with HIV develop chronic disease after acute infection with HCV. This is much higher than is the case in HCV mono-infected patients, where approximately 70% of acutely infected patients develop chronic disease [Hernandez MD, 2011]. Prior to the cART, patients infected with HIV did not survive long enough to suffer disease caused by chronic infection with HCV [Rider PJ, 2012], since the time between infection with HCV until the development of severe liver disease is usually many years to decades [Nelson K]. However, since effective anti-retroviral therapy, patients infected with HIV live long enough to actually be affected by chronic HCV co-infection [Rider PJ, 2012]. Because of this prolonged survival, the impact of HCV on the morbidity and mortality of HIV is increasing. Individuals co-infected with HCV and HIV have a more frequent and more accelerated progression to severe liver damage than HCV mono-infected individuals have [Page EE, 2011; Graham CS, 2001]. Also, it has been shown that liver abnormalities can appear 8 years earlier in patients co-infected with HIV and HCV than in HCV mono-infected patients [Zhang Y-H, 2004].

HCV seronegativity in co-infected patients

Whether someone is infected with HIV and/or HCV can be determined by the presence of antibodies against the virus antigens in someone's blood. This can be done by performing serological tests. Given the clinical importance of HCV co-infection in HIV infected patients, it is recommended to perform serological tests in HIV-infected patients in order to screen for antibodies against HCV [Chamie G, 2007]. Available serological tests to detect HCV antibodies are enzyme linked immunosorbent assay (ELISA) and recombinant immunoblot assay (RIBA) [Tashkandy MA, 2007].

Whereas in acute HCV mono-infected patients seronegative results can occur during the period from exposure to the virus until the development of detectable antibodies against HCV (seroconversion), also delayed antibody response and even false-negative results are shown in chronic HCV mono-infected patients [Chen M, 1999; Chamie G, 2007; Netski DM, 2005; Schroeter M, 2005]. This is also shown in experimental chimpanzee-models of HCV infection [Bassett SE, 1998]. These impaired antibody responses against HCV have also been described in people who are already infected with HIV [Cribier B, 1995; Chamot E, 1990; Bonacini M, 2001; Valdez H, 2000; George SL, 2002; Thompson EC, 2009]. It has been shown that about 9% of HIV-positive, HCV RNA positive patients were HCV antibody negative. These negative antibody responses could last up to 6 years [George SL, 2002]. In addition to delayed antibody responses against HCV in co-infected individuals, reversal of a positive serology (seroreversions) of anti-HCV antibodies have been observed in IDUs and hemophiliacs, after which they were seronegative [Chamot E, 1990; Ragni MV, 1993]. Although one study showed that HCV viremia in the absence of anti-HCV was rare, HIV patients may fail to mount or sustain an antibody response to HCV proteins and therefore may harbor the HCV virus in the absence of a positive antibody [Bonacini M, 2001].

To establish which groups of HIV infected subjects were at risk for being anti-HCV negative while being HCV RNA positive, several studies searched for possible factors associated with seronegative HCV infection in HIV patients. Chamie *et al.* (2007) performed a large combined cohort study regarding these possible factors. They examined predictors of HCV RNA positivity among HIV infected individuals whom were anti-HCV negative. They showed that anti-HCV negative subjects, who were HIV positive, were more likely to be HCV RNA positive when they had 1) a history of injection drug use, 2) a CD4+ cell count below 200 cells/uL or 3) elevated alanine aminotransferase (ALT) levels, which is a clinical marker for liver health. These findings were confirmed by several other studies. Bharti *et al.* (2011) also reported history of IDU and an elevated ALT level as a clinical variable for predicting HCV seronegativity in HCV/HIV co-infected individuals, and George *et al.* (2002) showed that lower CD4+ cell counts were associated in anti-HCV negative HCV RNA positive patients, but in contrast showed lower ALT levels in anti-HCV negative HCV RNA positive patients.

Because of the false-negative results found with serological tests such as ELISA and RIBA, many studies suggest that in individuals at risk for HCV infection, HCV RNA testing should be performed in order to prevent seronegative results in these at risk patients, and to diagnose HCV infection early [Thompson EC, 2009; Chamie G, 2007; Schroeter M, 2005; Tashkandy M, 2007].

Influence of HIV infection on HCV seronegativity in co-infected patients

As discussed before, in order to generate a sufficient humoral immune response against HCV, HCV antigens need to be recognized by helper T-cells. These helper T-cells, which are commonly CD4+, can activate B-cells to proliferate and differentiate into antibody secreting cells against the virus [Abbas AK, 2007]. The production of these HCV specific antibodies may be inhibited by HIV through immune suppression, leading to false-negative results in serological tests [Bonacini M, 2001]. How this specific antibody response is inhibited by HIV, is not quite clear.

HIV can infect different cell types, such as macrophages and dendritic cells, and it causes diverse immune system abnormalities. However, infection, destruction and functional impairment of the CD4+ T-cell subset is the major defect in the immune system that HIV causes [Abbas AK, 2007; Netski DM, 2007]. In this way, HIV infection and destruction of CD4+ T-cells might cause the impaired antibody response against HCV in co-infected individuals [Netski DM, 2007]. An important loss of CD4+ T-cells in HIV infected people is the direct cytopathic effect of infection in these cells by HIV. This production of virus in infected cells is a major cause of the decline in CD4+ T-cells, especially in the acute phase of HIV infection [Abbas AK, 2007]. However, this is not the only explanation for a decline in numbers of CD4 + T-cells. Accelerated destruction and impaired production of CD4+ T-cells can also contribute to depletion of these cells. Also, the fraction of circulating cells may decrease if viral infection results in the redistribution of the cells out of the intravascular space, into the confines of lymphoid organs. This might give the appearance of loss [McCune JM, 2001].

Netski et al (2007) showed a CD4+ T cell-dependent decrease of antibodies to HCV antigens caused by HIV. Their study continues on results showed in multiple studies regarding low CD4+ levels in seronegative HIV/HCV co-infected patients. They compared anti-HCV titers to HCV Core, NS3, NS4 and NS5 antigens before and after HIV infection at which the subject's CD4+ T cell count was at its lowest point (nadir). They compared 3 groups: CD4+ T cell counts A) >500 cells/mm³, B) 200-499 cells/mm³ and C) <200 cells/mm³. Among the first group, no significant difference in anti-HCV titers was found before and after HIV infection. In the second group, a significant difference was found for all antigens after seroconversion. The last group showed a highly significant difference in Core, NS3 and NS4 and an approaching significant difference in NS5 (figure 2). With these results, they show that HIV causes a CD4+ T cell dependent decrease in levels of antibodies to HCV antigens in HCV infected individuals co-infected with HIV. They show that the effect of HIV on anti-HCV titers is not restricted to specific antigens, and that the effect on the drop in anti-HCV titers increases as CD4+ T lymphocyte cell counts decrease. However, they did not show such a decrease in anti-HCV titers that they were undetectable. They explain this with the fact that their study consists of only IDUs, whom may be repeatedly exposed to HCV by active drug use. In this way, their immune system gets a boost with every repeated exposure. This is supported by their finding that higher antibody titers were associated with current drug use [Netski DM, 2007]. If this research will be performed in patients who have no or minimal risk of repeatedly exposure to HCV, a decrease in anti-HCV antibodies to a level where they are undetectable may be found.

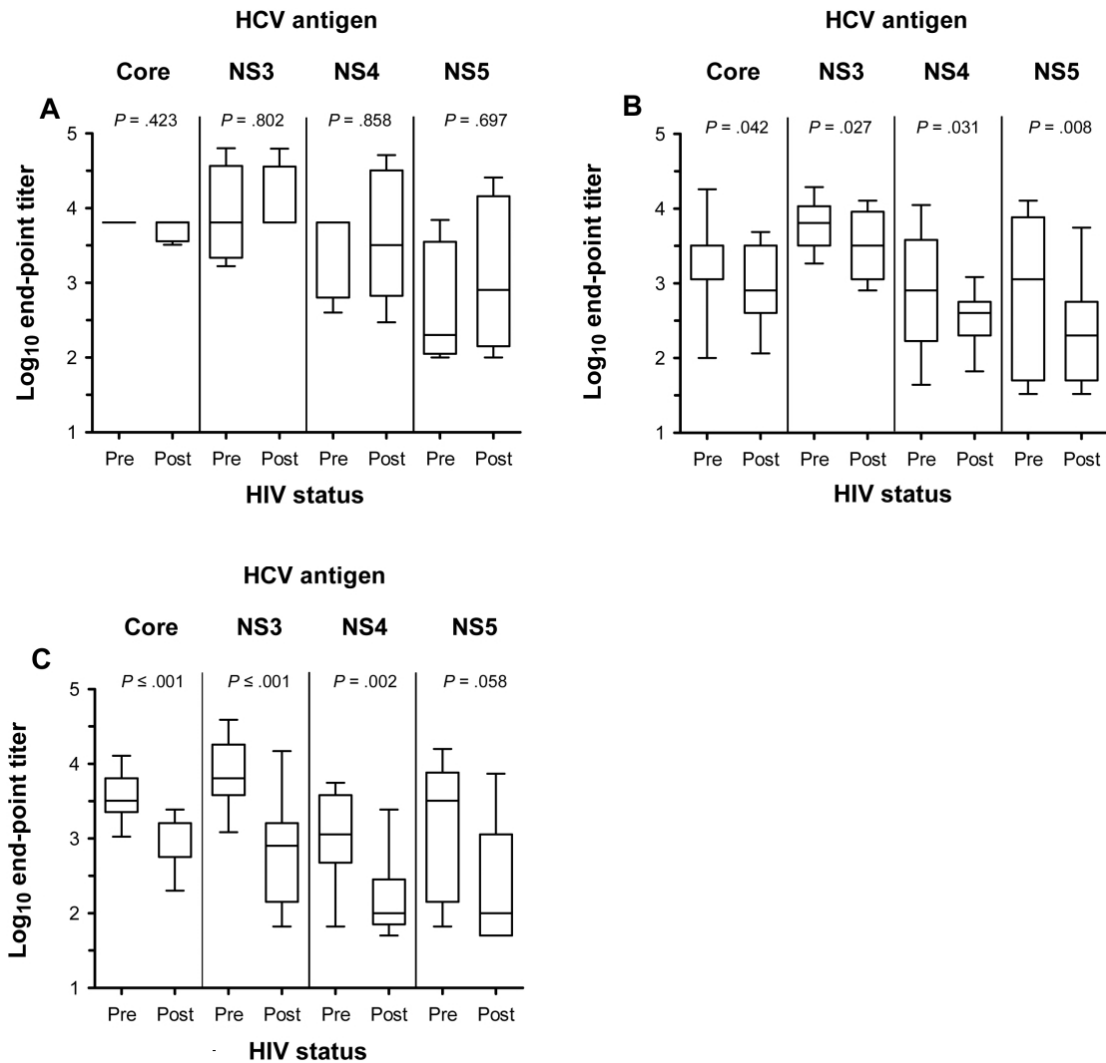


FIGURE 2: ANTIBODY RESPONSE AGAINST HCV ANTIGENS. Anti-HCV end point titers against different hcv antigens are shown before HIV-infection (pre-HIV) and after HIV infection (post-HIV). Subjects were divided in groups based on their nadir CD4+ T-cell count: A) >500, B) 200-499 and C) <200 cells/mm³. Pre and post-HIV HCV end-point antibody titers were compared using a paired T-test, and if P<0.05, it was considered as significant [Netski et al. 2007].

The role of TGF- β in HCV seronegativity in co-infected patients

HCV specific T cell responses could be markedly and specifically augmented by the neutralization of regulatory cytokines, primarily TGF- β . It has been shown that blockade of TGF- β and IL-10, another cytokine, could enhance peripheral HCV specific T-cell responses, even in the presence of HIV (figure 3) [Alatrakchi N, 2007]. It is also documented that HIV infection is associated with elevated levels of TGF- β in HCV-positive individuals, but not in HCV mono-infected individuals [Blackard JT, 2007]. Besides this, it is shown that the lack of TGF- β production by HCV specific T-cells is associated with HCV clearance in HIV/HCV co-infected individuals [Harfouch S, 2012]. Although recent studies report that TGF- β blockade might not be a potent way to promote clearance of persistent virus infections [Garidou L, 2012; Boettler T, 2012], these findings indicate there might be an indirect role for TGF- β in the delayed production of antibodies against HCV in HIV/HCV co-infected individuals.

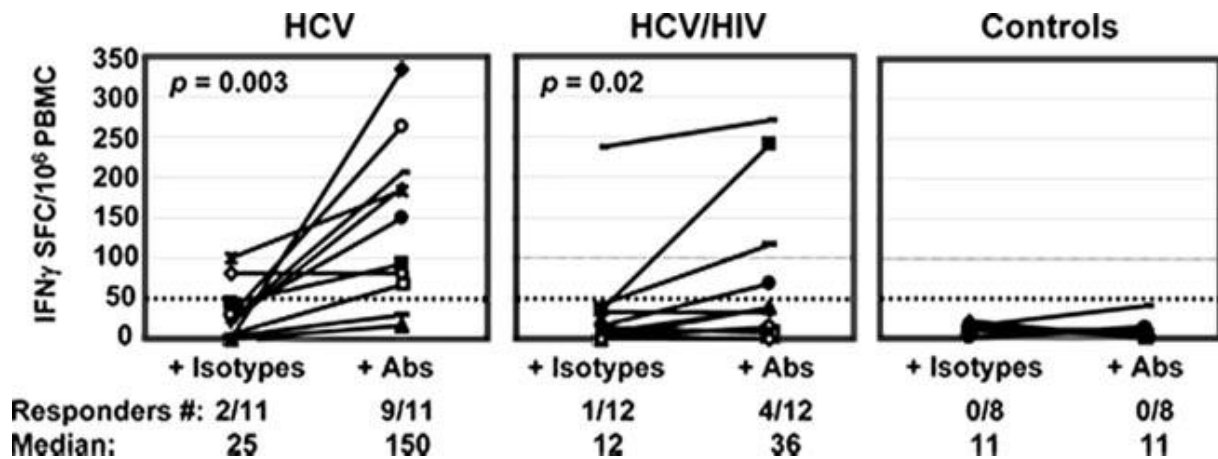


FIGURE 3: THE EFFECT OF BLOCKING TGF- β ON T-CELL RESPONSE. IFN- γ was used as an indication for T-cell response. IFN- γ enzyme-linked immunospot assays against HCV core peptides in the presence of isotype controls and the blocking antibodies anti-TGF- β and anti-IL-10 was performed. Each line represents a subject. Results were considered positive if a minimum of 50 antigen-specific spot forming cells (SFC) were observed over background. After the addition of blocking antibodies, a significant increase of the median HCV-specific T-cell responses was seen in both the mono-infected (HCV, n=11) and co-infected (HCV/HIV, n=12) group. P values below 0.05 were considered to be significant. PBMC: peripheral blood mononuclear cells [Alatrakchi et al. 2007].

Many investigators have demonstrated the importance of the immunosuppressive cytokine TGF- β in the homeostasis of T cell regulation [Tiemessen MM, 2003]. TGF- β has a lot of immunosuppressive effects, more than other cytokines. Reported inhibitory effects are on B-cells, CD4+ T-cells, cytotoxic T lymphocytes (CTLs) and NK cells [Prud'homme GJ, 2000]. In this case, the effects of TGF- β on the B-cells and CD4+ T-cells are most interesting. The effect on T-cells includes inhibition of proliferation and effector functions. The effect on B-cells includes also inhibition of proliferation [Abbas AK, 2007].

It is reported that NK cell derived TGF- β induces activated CD8+ T-cells to reduce antibody production by blocking the initiation of this response [Horwitz DA, 1999]. Also, TGF- β inhibits secretion of IgG and IgM antibodies by B lymphocytes [Prud'homme GJ, 2000]. Co-culture of T-cells with NK cells is shown to induce a CD8+ dependent down regulation of IgM and IgG synthesis. Besides this, the helper activity of CD4+ T-cells activated in the presence of TGF- β is significantly decreased [Horwitz DA, 1999]. As mentioned previously, anti-HCV antibodies have been reported to be restricted to the IgG subtype [Chen M, 1999; Netski DM, 2005]. Since TGF- β is upregulated in co-infected patients and it downregulates the synthesis of IgG antibodies, this might be an important cause of the impaired antibody response against HCV.

It is shown that in HCV mono-infected and HIV/HCV co-infected patients CD8+ regulatory T-cells (Tregs) are elevated [Hartling HJ, 2012]. It has also been shown that HCV-specific CD8+ T-cells produce TGF- β [Alatrakchi N, 2007]. And, as mentioned above, the activity of CD4+ helper T-cells is significantly decreased when activated in the presence of TGF- β [Horwitz DA, 1999]. These facts, combined with the fact that HIV does not infect CD8+ T-cells but does infect CD4+ T-cells, might indicate an accumulating effect which causes the impaired antibody response in HIV/HCV co-infected individuals against HCV antigens.

CONCLUSION

It seems that the impaired antibody response in patients co-infected with HIV and HCV depends on multiple factors. The depletion of CD4⁺ T-cells as a cause of HIV infection has a major impact on the cellular immune response against HCV, and contributes to the impaired antibody response against HCV antigens, probably because of a decrease in B cell activation. Also, the impaired antibody response against HCV antigens seems to be influenced by TGF- β , which is elevated in HIV/HCV co-infected patients. This cytokine seems to, among other things, down regulate the synthesis of IgG antibodies. HCV antibodies are, along with the IgM subtype, restricted to the IgG subtype. So down regulation of the synthesis of the IgG subclass might contribute to the impaired antibody response. TGF- β also seems to decrease the activity of CD4⁺ T-cells. This decreased activity might accumulate on the already decreased amount of CD4⁺ T-cells and cause an impaired antibody response against HCV antigens.

All results mentioned above may indicate that there is an important role for TGF- β in the impairment of antibody response against HCV in HIV/HCV co-infected patients. A lot of research needs to be performed regarding the exact mechanisms by which HIV influences the humoral response against HCV, in order to be able to restore the immune response against HCV. TGF- β might be a good start because it is elevated in HIV/HCV co-infected individuals and because of its many immunosuppressive features. In addition, in HIV infected individuals who are at risk for being co-infected with HCV, PCR screening needs to be performed in addition to the serological tests for a more certain diagnosis of HCV infection.

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