# Immunocompetent Expression of CD4+ T Cell and CD8+ T Cell, TNF-α and INF-γ in Patient with Chronic Hepatitis C

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**ABSTRACT**

***Aim:*** *To investigate the expression CD4+ T cell and CD8+ T cell as well as TNF-α and INF-γ level on chronic hepatitis C.*

***Methods:*** *This is a cross-sectional study. Forty patients with chronic hepatitis C based on laboratory examination, who were collected from blood transfusion centers at Dr. M. Djamil Hospital. The control group used forty healthy samples.*

***Results:*** *There were 40 chronic hepatitis C cases satisfying the inclusion criteria. We found that CD4+ T cells count 50.35 ± 3.18%; CD8+ T cells count 59.37 ± 3.52%; TNF-α level 22.03 ± 3.72 pg/ml and INF-γ level 4.47 ± 1.47 pg/ml.*

***Conclusion:*** *The chronic infection hepatitis C virus have given the effects on the immunocompetent cells which increased of CD4+, CD8+, TNF-α level and INF-γ level.*

***Keywords:*** *hepatitis C virus, CD4+ T cell, CD8+ T cell, cytokines TNF-α, INF-γ*

## INTRODUCTION

Hepatitis C virus (HCV) is one of the main causes of chronic hepatitis; possibly followed up to be the liver cirrhosis and hepatocellular carcinoma. This disease has been a serious health problem of all society in the entire world including Indonesia. It is estimated nowadays that there are more than 170 millions of citizens in the world are having a chronic infection HCV[1,2,3,4,5] and around 40,000 new cases of people infected by HCV every year in USA.[3] Globally reported that there are more than 2% of the world's population has been chronically infected by HCV.[6]

Together with the trend of drug misuse parenterally, it is estimated that the number of hepatitis C patients will be increased swiftly. The figures of parenteral drug misuse in Jakarta shows that 74.9% of people have been infected by HCV, which means 150-200 times higher than general population.[7]

Severely-acute hepatitis C patient, around 15-20% of the cases, can be cured by eliminating the HCV through host-immune response, while around 80-85% of the patients will be chronic hepatitis C carrier and afterwards will be the liver cirrhosis and even developed into hepatocellular carcinoma.[8]

A person who has been infected by HCV will produce some antibody for a couple of weeks or months after the acute infection and they still exist for a longer period after the recovery. Concerning this matter we need to differentiate whether someone is in an infected condition or not by doing an RNA-HCV test in his or her blood. Generally in an acute infection RNA-HCV can be detected in the first three weeks of infection period, while antibody to the HCV will emerge in around 8-10 weeks later.[9,10]

Hepatocyte is the target of HCV. HCV lives and replicates itself in the liver cell. The intake and the process of the virus antigen by the antigen presenting cells (macrophage and *Kupffer* cell) in liver are of vital importance for recognition to specific virus cell; the CD4+ T cell at the starting point of infection. The CD4+ T cell is activated by recognizing the virus epitope presented by major histocompatibility complex (MHC) class II on the surface of antigen presenting cells (APC).[11]

The activation of CD4+ T-cell caused the development of the T cell helper (Th cell) into Th1 and Th2 cell. Th1 cell produces IL-2, TNF-α and IFN-γ cytokine,[12] in which later stimulates the growth of natural killer cell (NK) and CD8+ T cell.[13] Peptide complex of MHC class I virus which is peeped out at the surface of the liver cell infected by HCV will be recognized by CD8+ T-cell, later then will break the liver cell itself.[14]

INF-γ cytokine as the immunomodulator will improve the activation to T cell macrophage, NK cell and increase the expression of MHC class I at the hepatocyte cell and bile channel.[15] The purpose of this research is to determine the expression of CD4+ T cell, CD8+ T cell, TNF-α and IFN-γ level in patient with chronic hepatitis C.

## METHODS

A kind of cross-sectional research was done in the Department of Clinical Pathology, Sub-division of Immunology of Dr. M. Djamil Hospital Padang and Laboratory of Airlangga Tropical Disease Centre for RNA-HCV examination in Surabaya against chronic hepatitis C patients (January-June 2005). The patients are divided into different groups; 40 patients of chronic hepatitis C coming from the participants of blood donor at The Indonesian Red Cross-Blood Transfusion Division Padang Branch Office, with positive anti-HCV and RNA-HCV; and healthy control group of 40 patients. The amount of research samples met the requirements of calculation by using the formula of the amount of sample in research. Inclusion criterion includes chronic hepatitis C patients with anti-HCV and positive RNA-HCV after 6 months of anti-HCV examination at first-time checked, male and female, of 17-45 years of age and voluntarily ready to follow the research by signing the approval letter (informed consent form). Exclusion criterion includes hepatitis B, HIV, autoimmune disease history patients and persons of virus infection experiences, bacterium, etc.

ELISA method was used to examine the anti-HCV plasma by utilizing third-generation Human anti-HCV with kit number of 51,250, while PCR was used to examine RNA-HCV. Enumeration CD4+ T cell and CD8+ T cell with isolated lymphocyte cell through Centrifuged Ficoll Gradient and coloration of the cell in immunohistological way by utilizing the kit of Ultra Vision Detection System from USA. The measurement of TNF-α and INF-γ plasma used the ELISA kit from Bender Med System A-1030 Vienna Austria.

The data was manually, quantitatively processed; programmed by SPSS version 12.1 and presented in forms of tables and histograms. The research was continued with t-test and the result of statistic analysis was expressed meaningful if the value had reached $p < 0.05$.

## RESULTS

Starting from January-June 2005, 40 patients of chronic hepatitis C within the criterion of positive anti-HCV and positive RNA-HCV have been examined. There are 37 males (92.5%) and 3 females (7.5%) in the age ranged 26.20 ± 6.23 years old.

**Table 1. Characteristic data of chronic hepatitis C patients and control**

| Variables | Chronic Hepatitis C | Healthy Control |
|---|---|---|
| Sex | | |
| Male | 37 (92.5%) | 23 (57.5 %) |
| Female | 3 (7.5%) | 17 (42.5 %) |
| Age (mean ± SD) | 26.20 ± 6.23 | 28.70 ± 1.9 |
| Anti HCV | 40 positive | 40 negative |
| RNA-HCV | 40 positive | not examined |

In table 2, we can see that the dominant age of the samples was around of 21-25 years old (27.5%) and 26-30 years old (22.5%), while the major gender samples were male as many as 37 patients (92.5%).

**Table 2. Age and sex range of chronic hepatitis C patients**

| Age (years old) | Male (%) | Female (%) |
|---|---|---|
| 17 – 20 | 6 (150) | 1 (2.5) |
| 21 – 25 | 13 ( 23.5) | 2 (5.0) |
| 26 – 30 | 9 (22.5) | 0 (0.0) |
| 31 – 35 | 4 (10.0) | 0 (0.0) |
| 36 – 40 | 5 (12.5) | 0 (0.0) |
| **Total** | **37 (92.5)** | **3 (7.5)** |

The following figure shows CD4+ T-cell and CD8+ T cell with immunohistochemical color system using the Ab-3 CD4+ and Ab-3 CD8+ antibody onto chronic Hepatitis C patient and normal control group. CD4+ T cell and CD8+ T cell were seen yellowish-brown, while other lymphocyte cells were not or just seen as grey.

In the blood of normal control group the amount of CD4+ T-cells were not much (figure 1). On the contrary there were so much CD4+ T cells in chronic Hepatitis C patients (figure 2).

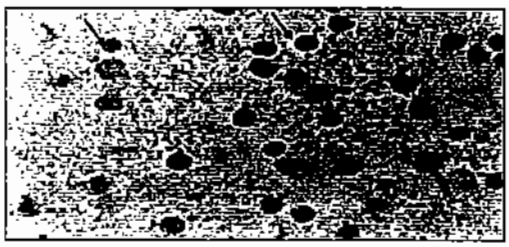

Figure 1. CD4+ T cell inside the blood of normal control group

Figure 2. CD4+ T cell inside the blood of chronic hepatitis C

In figures 3 and 4 there were differences in the amount of CD8+ T cell between the normal control group and the chronic hepatitis C patient.

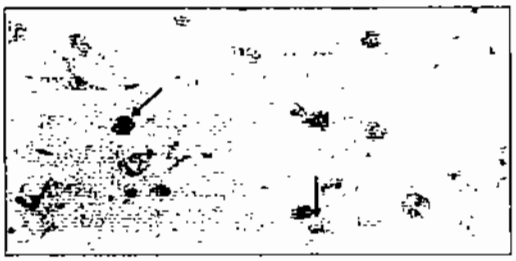

Figure 3. CD8+ T cell inside the blood of normal control group

Figure 4. CD8+ T cell inside the blood of chronic hepatitis C

The research shows that there was a significant increase of CD4+ T-cell from the chronic hepatitis C patients at the average of 50.35% ± 3.18%, while in the normal control group; the amount of the cell was in the normal range (35% - 45%) within the average value of 36.33% ± 1.403%.

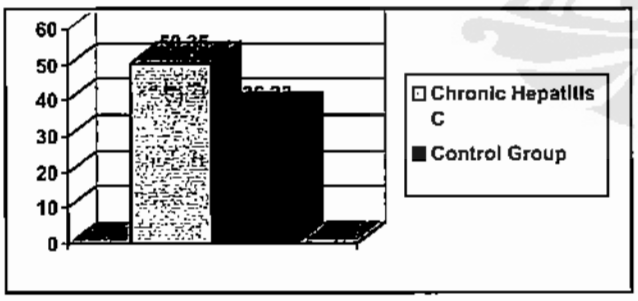


Figure 5. Expression of CD4+ T cell (%) in the chronic hepatitis C and control group

There was an increase of CD4+ T cell with significant difference by the t-test (p = 0.0001) from the CD4+ T cell calculation which was obtained in the blood between chronic hepatitis C patients and the control group.

There was also an increase of CD8+ T cell from the chronic hepatitis C patients compared with those from the healthy control group. There were CD8+ T cells in chronic hepatitis C blood with the average value of 59.37 ± 3.52%, and from the control group there were CD8+ T-cells in normal range (30-40%) within the average value of 35.73 ± 2.60% (figure 6).

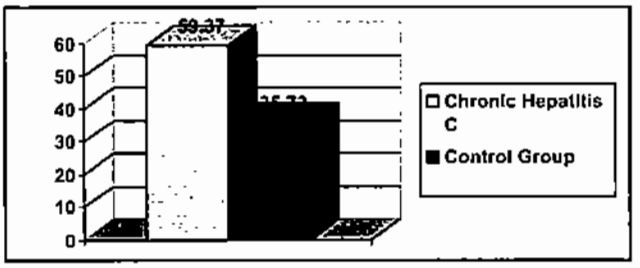


Figure 6. Expression of CD8+ T-cell (%) from chronic hepatitis C and control group

In figure 6, it is clearly seen that there were an increase of CD8+ T cell with a significant difference of meaning (p = 0.0001) with t-test from CD8+ T cell between chronic hepatitis C patients with healthy control group.

It is showed that there was an increase of INF-γ level from chronic hepatitis C compared with those of the control group with the average value of 4.47 ± 1.47 pg/ml, while at the control group the average value of IFN-γ level was around 0.84 ± 0.2 pg/ml (normal < 1.5 pg/ml). We can see it more clearly from figure 7 about it with an increasing level of INF-γ with meaningful difference (p = 0.0001) with t-test between IFN-γ level in chronic hepatitis C blood and the control group.

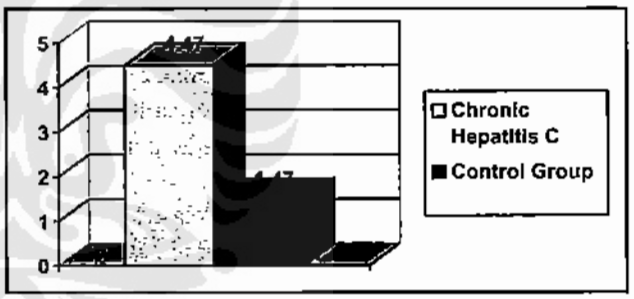


Figure 7. IFN-γ level (pg/ml) from chronic hepatitis C and control group

The INF-α in chronic hepatitis C blood was increased with average value of 22.03 ± 3.72 (pg/ml), while in control group the level could not be detected (table 3).

Table 3. TNF -α level from chronic hepatitis C compared with control group

| Cytokines Level (pg/ml) | Chronic Hepatitis C (Mean ± SD) | Control Group |
|---|---|---|
| TNF-α | 22.03 ± 3.72 | Undetected |

## DISCUSSION

The results of the research indicated that there was an increase in CD4+ T cell in chronic hepatitis C patient. This result tells us that CD4+ Th1 cell has been activated by hepatitis C virus, where in the previous case it has been processed by APC cell (macrophage and *Kupffer* cell in liver) into peptide, and this peptide

formed a complex bind with MHC class II. Afterwards this bind was simultaneously emerged to the surface of the APC cell until it was identified by CD4+ T cell. The activation of CD4+ T cell was also caused by IL-12 produced by APC.[16] The ability of T cell receptor (TCR) is used for one peptide-MHC class II complex bind, the activation of Th cell will be developed intoTh1 and Th2 cells. The Th1 cell produces IL-2, TNF-α and IFN-γ[12] Th2 cell produces IL-4, IL-5 and IL-10, which stimulate the creation of specific antibody to the virus created by B cell.[17] Results of research by Doherty et al[17] also produced the increase of CD4+ T cell caused by APC stimulation, so they were differentiated each other into Th1 cell and Th2 cell. According to Sobue et al (2001), the increase of CD4+ T cell happened in periphery blood and in the liver tissue of chronic hepatitis C patient.

Characteristic response from HCV-specific T cell is from CD4+ T cell and CD8+ T cell from chronic hepatitis C patient. There have been many researches concerning this matter; researchers believe that both T cell have the important role in pathogenesis damage of liver and clearance to virus.[19]

Research shows that the amounts of CD8+ T cell were increasing from the chronic hepatitis C patient. This matter shows that there has been an activation of Th1 cell yielding IFN-γ, which stimulated the NK and CD8+ T cell. IFN-γ also will also result expression improvement of MHC class I molecule in liver cell and bile cell.[14]

Antigen molecule-peptide MHC class I complex on the surface of liver cell will be recognized by specific cell of virus; that is CTLs (CD8+ T cell), then it will lyses the HCV-infected liver cell. This opinion is supported by an invention that the expression improvement of MHC class I is related to the severe liver disease.[20] Research from Bertoletti et al,[21] resulted that the T cell in majority which is insolated from the patient's chronic HCV-infected liver was Th1 cell, and researchers are also consistent with the opinion of the role of Th1 cell as the cause of chronic damaged liver-cell.

Even though the response from the CD8+ T cell from the chronic hepatitis C patient is strong, polyclonal and multi specific, yet it can clean HCV from the patient liver cell. Other researcher also reported that there was a predominant increase from CD4+ T cell at the portal area and CD8+ T cell in the liver lobe, this matter expresses indirectly that both cells cause the damage of the liver cell. In further observation they found the existence of relationship with the increase of alanin aminotransferase (ALT) enzyme rate (ALT) with the severity of the damaged liver cell in a histological way, so that some of the inventions strengthen the opinion that the T cell plays an important role to liver disease pathogenesis.[22]

In the treatment of chronic hepatitis C patient, the improvement of expression from CD4+ and CD8+ can be used as the follow up efficacy to interferon responses, because the purpose of the medication with interferon is to improve the activation to T cell. If there weren't any improvement during the medication with interferon from CD4+ and CD8+, since the first indication there were no responses of what so ever, it would mean that the medication has failed.

In this result, the TNF level was increased in chronic hepatitis C patient's blood, on the other hand, in the control group the TNF-α level was not detected. This shows that the activated immunocompetent cell produced TNF-α cytokines. These cytokines was produced by various types of cell, macrophage, T cell, B cell, astrocyte and *Kupffer* cell. The creation of these cytokines was happened as the responses against the HCV virus and cytokines (IL-1, IL-2, and IFN-γ), immune complex, etc.[23] TNF-α has been proven as the modulator of strong immune responses which chained the adhesive-molecule induction, other cytokine and neutrofil activation.

There were 2 types of TNF receptor which was able to bind TNF-α and TNF-β with strong affinities. TNF Receptor was entangled in immune system and especially in apoptosis control. A culture research of T cell has showed that HCV core protein interaction could be interacted in the lymphotoxin β-receptor (LTR), tumor necrosis factor receptor (TNFR-1)[24,25,26,27] and Fas.[26] The improvement of virus sensitivity was used to influence the apoptosis stimulus so that the apoptosis would not exist, after the lymphotoxin-αβ stimulation complex along with IFN-γ.[24]

Interference from the function will disturb the virus elimination and neutralization. Thereby the core protein of HCV has a role as the key of immunomodulation and that one factor is able to improve the infection persistent from HCV.[27] The results of other researches gave their strong support to the hypothesis that the HCV core protein is a pro-apoptotic activity by influencing the signals of TNFR.[28] Therefore the infected T cell will decrease its apoptosis by core protein through breaking the T-cell activation and the cytotoxic function, by doing so the persistent infection happens.

TNF-α Improvement of rate was gained from most chronic hepatitis C patients but there were no clearance happened to HCV which infected the hepatocyte cell. This research has been done to patients of hepatoma disease, showed the happening of HCV replica, telling us that there is a strong resistance to TNF-α[4]

The results of this research is that there was an increase of IFN-γ level in the patient's blood of chronic hepatitis C. The expression was really meaningful compared with those of control group ($p = 0.0001$). These data was the clear hint that in chronic hepatitis C patients, an activation of Th1 cell has happened as the producer of IFN-γ and IL-2 which has the effect as an anti-virus and stimulate the specific immune responses against the HCV-infected cell.[18,28,29]

## CONCLUSION

This research has resulted that there has been a cell-expression increase of immunocompetent CD4+ and CD8+ and also the improvement of TNF-α and INF-γ cytokines level in patient's blood of chronic hepatitis C. Obviously seen that the inability of patient's immune response to overcome the HCV infection, so the possibility of the infection process will be continued with all of the consequences.

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