The Role of Specific Cellular Immune System in Chronic Hepatitis C

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ABSTRACT

Hepatitis C virus is a RNA virus with very high speed replication. The clinical course of chronic hepatitis C is frequently asymptomatic like other hepatitis viruses. Infection of hepatitis virus will activate the immune system specifically as well as non-specifically. Mechanism of the immune system regulation is controlled by tissues consisting of antibodies cells and cytokines. In the process, all of the immune systems integrate and coordinate with the main agent-lymphocytes. Lymphocytes recognize antigens through the specific-surface antigen receptors. Following exposure to viral chronic hepatitis virus, viremia takes place within 1-2 weeks. In immuno-competent hosts, viremia will be preceded with the increase in transaminase enzyme and delayed seroconversion of antibodies will occur.

Unlike other immunologic processes, these established antibodies are not protective in nature but serve only as the sign that someone has been infected by hepatitis C. In most cases of hepatitis C virus infection, this virus cannot be eradicated in the acute phase. Approximately 80-90% of acute infection progresses to be chronic infection and in 50% of the cases, there is an increase in transaminase enzyme that reveals that there is still liver cell damage. The degree of liver tissue damage in hepatitis depends on the number of virus infecting and the activity of cytotoxic T cells.

***Keywords**: hepatitis C virus, humoral immune response,cellular immune response*

INTRODUCTION

Hepatitis C virus (HCV) is the main cause for chronic liver disease, liver cirrhosis and hepatocellular carcinoma. This disease has infected 170 million people worldwide and around 2.7 million Americans. Approximately 40% of the chronic liver disease in the United States is caused by hepatitis C virus. In South-East Asia, the prevalence of hepatitis C is 2.5-4.9% of people.

According to WHO, the prevalence of hepatitis C in Indonesia is around 1-2.4% whereas in Jakarta, according to Nurul Akbar (1994), it was 3.9% with even distribution of gender and the most prevalent age was > 50 years (11.4%). The survey conducted at Dr. Cipto Mangunkusumo General National Hospital and one private hospital in Jakarta in 1999 by Rino et al revealed that there were increasing numbers of HCV cases by 74.9% due to drug abuse (positive anti HCV with the mean age 21.2 $\pm$ 4 years). It revealed that there was shifting of onset age so that the risk for liver cirrhosis and hepatoma increased.[1,2,3]

ETIOLOGY

Clinical spectrum of hepatitis C

The clinical course of hepatitis C infection can be seen in the above picture. The incubation period is around 7 weeks, namely around 2-30 weeks. Acute hepatitis C is usually asymptomatic and 80-90% will progress to chronicity. Around 20-30% of chronic HCV patients will develop cirrhosis within 10-20 years and 5-10% of them will suffer from late-stage liver disease with the risk for hepatoma around 1-4% annually from those with cirrhosis.

The general symptoms are not typical such as lethargy, anorexia, nausea, upper quadrant abdominal pain, brownish urine and itch can appear and will resolve or disappear while the progression of the disease goes on to be chronic. Generally, acute HCV patients will have increasing levels of aminotransferase enzyme and only 25-30% of them have normal levels. This increase will fluctuate 1.5 up to 10 times the normal level and intermittently can decline approaching the normal value.

Several trials have proven that around 1/3 of chronic HCV patients have normal ALT enzyme levels. Previously, these patients were regarded as healthy virus carriers, but since the biopsy results have proven that there is change in the histopathological findings, this opinion has been out of date. Now, these patients are regarded as subclinical HCV patients.

Characteristics of hepatitis C virus

Hepatitis C virus is a RNA virus with positive genome that is derived from the family of flaviviridae. The diameter is around 30-60 nm with the length 9.4 kb or 9,413 nucleotides and possesses an open reading frame (ORF). This virus managed to be identified in 1987 and is postulated to account for 80-90% of the cause of viral hepatitis infection-non A, non B. Replication of this virus occurs through a RNA-dependant RNA polymerase. The speed of the virus replication is very high, ranging from 10^{10} - 10^{12} copies of virus daily. Heterogenicity occurs due to mutation during replication. Consequently, HCV infected patients can harbor hepatitis C virus with different nucleotide matrix. This enables the virus to avoid the host's immunologic response in the efforts to survive. Heterogenicity also has a role in influencing the course of the disease, response to anti-viral agents and difficulty in inventing a vaccine against HCV.

Right now, 6 genotypes of HCV have been identified with 11 subtypes. Genotype 1 is abundant in North America (60%) followed by genotypes 2 and 3. Genotypes 4 are frequently found in Egypt, whereas genotype 5 is found in North Africa and 6 in South-East Asia. These genotypes are correlated with the therapy response but not correlated with the severity of the disease and levels of alanine aminotransferase enzyme.

The clinical course of chronic hepatitis C is frequently asymptomatic like other hepatitis viruses. The most often abnormality found is the increase in ALT levels in some patients, but the level is still normal in some other patients.

Since May 1990, there has been an antibody test against HCV with the method Enzyme-Linked Immunoassay (ELISA). The sensitivity of this test is very high; almost up to 95% in high-risk population and 25% in low-risk population. False positive is often found in blood donors so that recombinant immunoblast assay (RIBA) has been developed lately. It can be used to confirm the positive result of ELISA test in low-risk population. However, with the development of RNA detection method, either qualitatively or quantitatively, this test is seldom used now.

Qualitative RNA test is the most sensitive test and can defect the number of virus eventhough it is in very low quantities. (< 100 copies of HCV RNA/IU). This examination is very useful in determining the presence of HCV virus in immunocompromised patients and the presence of persistent infection in patients with positive antibodies.

Immun response to hepatitis virus

Infection of hepatitis virus, like other viral infections, will activate the immune system specifically as well as non-specifically. The non-specific immune system is an immune system that works directly without any exposure to the previous antigen and is frequently referred to as natural immune system. This immune system is the first defense that consists of physical/mechanical defense, namely skin, mucosa, cilia, cough/sneeze, physiological defense and biochemistry (gastric pH, lactopherine, lisozim) cellular non-specific defense occurs through the production of interferon (IFN) and activation of natural killer cells (NK) whereas the specific defense consists of humoral and cellular immune responses.[1-4]

Mechanism of the immune system regulation is controlled by tissues consisting of antibodies cells and cytokines. In the process, all of the immune systems integrate and coordinate with the main agent-lymphocytes. Lymphocytes recognize antigens through the specific-surface antigen receptors.

Virus serves as an antigen that causes local accumulation of mononuclear cells that exit from the blood vessels. This occurs through adhesion based on the signal of the endothelial cells and target cells. The cellular factors and cytokines involved are:

- CD4+ CD8+
- ICAM-1 (Intercellular adhesion molecule 1)
- Cytokines : interleukin, TNF, interferon

Humoral immune response

Following exposure to HCV virus, viremia takes place within 1-2 weeks. In immunocompetent hosts, viremia will be proceeded with the increase in transaminase enzyme and delayed seroconversion of antibodies will occur. Unlike other immunologic processes, these established antibodies are not protective in nature but serve only as the sign that someone has been infected by hepatitis C.

In most hepatitis C virus-infected patients, the host's immune system fails to eliminate the virus in the acute phase. This condition causes almost 80% of HCV infection to progress to chronicity.

If viral infection occurs, NK cells will recognize the infectious cells non-specifically and destroy them. These infected cells will secrete interferon (alpha and beta) that will cause repression of the replication process of virus. If this process fails, cytotoxic T cell lymphocytes (CTL), together with neutralizing antibodies will take over the role in destroying these infected cells.

Neutralizing antibodies will bind to particles of specific virus that exist in the body's fluid and eliminate them. However, some trials have proven that the neutralizing antibody response is very strain-specific in nature. In addition to that, the quasispecies nature of this virus can cause the emergence of other strains when the predominant strain is repressed by the immune system. This nature will create an obstacle for inventing a vaccine against hepatitis C infection.

Several trials have mentioned that antibody response to the hypervariable protein E2 from the HCV viral structure was correlated with the incidence of resolution from HCV infection.[1]

Cellular and cytokine immune response

Cytotoxic T cells (Cytotoxic T Cell Lymphocyte/CTL) and neutralizing antibodies are directly involved in the eradication process of virus from the body's fluid and kill virus that infects cells. Antibody production and activation as well as proliferation of cytotoxic T cells are controlled by T-helper cells. T-cells are activated on identification of viral antigens that are presented by APC cells. Identification of cells infected with this virus by T cells, CD4+ or CD8+ requires presentation of peptide virus through major histocompatibility complex molecules (MHC)I and II.[3]

CTL will recognize viral antigens expressed onto the surface of the infected cells by antigen presenting cell (APC) and then attack those cells in the effort to eliminate the virus.

Cytokines are peptide molecules secreted by cells and function as a communication tool with other cells. Cytokines produced by lymphocyte cells are referred to as lymphokine. Cytokines that function as the regulators for cell movement toward a lesion (chemotaksis) are called chemonin. Interleukin is a cytokine that serves to send messages among leucocytes. Surface molecules are the sign of cell identity that is named after the function. T lymphocyte cells have 2 kinds of surface molecules that are named CD4+ and CD8+. CD4+ cells generally bear the nature as T helpers and CD8+ as cytotoxic cells.

Response of CD8+ lymphocyte T cells

Based on the cumulative data, it can be seen that response of specific antigen CD8++, cytotoxic T lymphocytes (CTL) in hepatitis C will persist permanently in patients with chronic hepatitis C that has undergone mutation. Some trials have shown that CTI specific HCV can generate in liver cells or in the peripheral blood. Quantitative and qualitative analysis of the cellular immune response in hepatitis C has revealed that T-specific CD8+ cells exist in the peripheral blood cells of patients with the number relatively lower than that in the intrahepatic.

Some studies that investigated the response of CTL, specific HCV correlated with the number of virus revealed that there was correlation between the numbers of virus with the response activity of CTL specific. Nelson et al obtained that in patients with CTL specific activity, the high HCV had the concentrations of RNA HCV below 0.5-1 log. This revealed that CTL specific activity was essential in the elimination process of virus.

Response of MHC II lymphocyte T-cell

CD4+ constituens an induction center to respond to antivirus because it can increase the antibody response by producing B cells and activities the cell activity of CD8+. The latest development in the meaning about the cellular immune response regulation toward infection agents is by identifying 2 subpopulations of lymphocyte

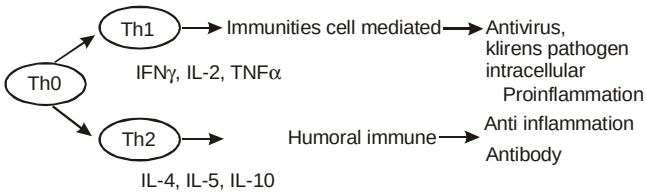

Figure 1. Identifyng CD4+ based on cytokine produced[1]

T helper T cells (CD4+) based on the cytokine produced namely T-helper 1 (Th1) and T-helper (Th2) as well as Th0 that's not active.[1]

The cells will activitate the cellular immune response and cytotoxic T cell response. If activated, Th1 cells will produce IL-2 and IFNγ to precipitate the activation and proliferation of cycotoxic T cells, TNFα and NK cells.

Th2 cells have more roles in the humoral immunity. These cells will produce IL-4, IL-5, IL-6 and IL-10 that will stimulate differentiation of b cells to be antibody plasma cells (APC) as well as to proliferate. APC also produces IL-12 when activated by T cells. These cytokines serve as Th1 cells, T cells and NK cells that work to eliminate and inhibit the replication of virus.[4]

In hepatitis virus A and B, the mechanism of the cellular immune response is like the forementioned mechanism. However, in most cases of hepatitis C virus and some cases of hepatitis B virus, the virus cannot be eradicated in the acute phase. Consequently, a variety of cell damage will keep on depending as the ratio between the numbers of virus as antigens that infect cells with the specific activation of cytotoxic T cells.

Mechanism of hepatocellular injury in hepatitis virus

Cytotoxic T cells are the main agents in the process of liver cell damage following the infection of hepatitis virus. These cells will be activated if they recognize the cells infected by virus and will release a protein called perforin. This perforin will perforate the membrane of the target cell by injecting a proteolitic enzyme produced that is called granzim. Through this mechanism, the cells are killed.

Activated cytotoxic T-cells will increase the expression of Fas ligand (FAS) and TNFα, if these cells are sensitive to Fas ligand or TNFα, activated cytotoxic T cells will damage the cells, either those that are infected or those that are not infected. This process occurs through the release of apoptosis signals mediated by Fas ligand antigen and TNFα.

Cytotoxicity of Fas antigen and Fas ligand system or TNFa is lower than that of perforin. It occurs because this system attacks most uninfected cells, but those cells have obtained sensitivity (immune) because they have undergone damage. The damage occurs through a mechanism that is correlated with the inflammatory process, whereas perforin only damages the virus-infected cells. The molecular mechanism that causes damage in these virus-infected cells has been proven through trials by using cytotoxic T cell clones that are specific for Hepatitis C4 virus.

Mechanism of liver cell damage in infection of hepatitis virus

Lymphocyte cytotoxic T cells that have recognized the liver cells infected by virus will be activated and proliferate under the stimulation of cytokines, such as IL-2 and IFNg that are produced and activated by T helper cells. As explained above, perforin is regarded as an important role in the hepatocellular injury process. Production of Fas ligans and TNFa increases when the cytotoxic T cells are activated. When the increase in the expression of Fas receptors and TNF occurs in the process of hepatitis, liver cells become sensitive to Fas and TNFa receptors even through in fact, it has undergone slight sensitivity in the normal condition. Based on this, these two systems are considered having a correlation with the damage of cells in hepatitis virus infection.

In a trial a patients with chronic hepatitis C virus, expression of Fas antigen would increase at the site of the severe inflammation process. In that trial, the involvement of perforin. Fas and TNFα was also reported in the severe hepatocellular damage in hepatitis experimental models. INFγ secreted by cytotoxic T cells and activated by T helpers can activate macrophages. This macrophage activation will secrete TNFα so that mobilization of inflammatory cells, like neutrophils, occurs through the activation of endothelial cells and fibroblasts. Activation of these inflammatory cells will cause virus hepatitis infection to be more aggressive.

The severity of hepatitis varies depending on the potential replication of virus, antigenicity of virus and host's immune response cells. Potential replication and anti-genicity of hepatitis virus A and B are very high that can cause hepatitis to be fulminant. In hepatitis C, virus does not replicate fast and the antigenicity is not too high so that it is seldom fulminant in the HCV infection.

In HBV and HCV infection, the viral infection that will persist will regress the host's immune response.

Depletion, tolerance and participation of T suppressor cells are postulated in the suppression mechanism of immune cells even though the exact mechanism has not been clear yet. If persistent viral infection occurs concomitantly with the cellular immune response that is not adequate to eradicate the virus, chronic liver damage will occur even through it is not as severe as the damage in acute hepatitis.

Despite the fact, that several mechanisms that have the role in the hepatocellular injury and liver cell damage, have been explained, but how hepatitis can occur in healthy carriers and the suppression mechanism of the cellular immune system in chronic hepatitis has still not been elucidated.[1,4]

Immunopathogenesis of hepatitis C

a. Host's immune/response in the infection of Hepatitis C virus

In 1994 Gonzales stated that liver cell damage in the HCV infection was postulated not to be caused by the cytopathic nature of the virus, but by the interaction of the virus with the host's immune response. The mechanism of hepatocellular damage in hepatitis C has not been clearly understood, there is interaction of factors that influence the immune response and it is very individual those factors include the virus factor per se such as the cytopathic effect, the ability to replicate and heterogenicity. The host factor includes the specific and non-specific immune systems as well as production of local and systemic cytokines. The environment factor like immunosuppression and alcoholism also has the role in determining the progressivity of disease.

The host's immune response in the infection of hepatitis C virus will involve the non-specific and specific immune systems. The specific immune has cellular components (T lymphocyte and humoral B lymphocyte or immunoglobulin). The cellular component consists of leucocytes, mast cells, macrophagues, T cells (Th0, h1, h2) and B cells. The cellular immune response is activated by activation of CD4+ and CD8+ cells.

According to Alvarado (1999) based on his trial, it was proven that the immune response had a role in the pathogenesis of liver disease in (HCV), but the immune response of B cells and TCD4+ cells and CD8+ that are not effective cannot prevent the course of the disease so that it can cause chronic infection.

Up to now, the mechanism of immunity has not been able to be explained. The role of lymphocyte T cells, in this context, CD4+ and CD8 are still being investigated. Some trials have mentioned that CD4+ lymphocyte T cells had more roles in the elimination process of hepatitis C virus infection compared with CD8+ in acute infection. In addition to that, heterogenicity of HCV also causes antibodies to neutralize the immune system that is not capable of protecting the body against immunological response. Chang et al in his trial on chimpanzees obtained that quantitatively the level of CD8+ did not adequately cause infection to be persistent, but the role of CD8+ T cell response in human beings has not been well understood. Like in other virus infections, CD4+ T cell and CD8+ cell response are correlated. CD4 is required to activate CD8+ even though the correlation between the cellular immune response and the clinical condition or virology in hepatitis has not been clear yet.

Immune response in the infection of hepatitis virus is initiated when infection of liver cells occurs that stimulates the production of interferon a and b. Both of them will inhibit the replication of virus. If the infection cannot be control, the role to eliminate this virus will be taken over by neutralizing antibodies and cytotoxic T cells. These antibodies will neutralize virus in the body fluid and cytotoxic T cells will recognize, express and destroy the virus.[3]

In most cases of hepatitis C virus infection, this virus cannot be eradicated in the acute phase. Approximately 80-90% of acute infection progresses to be chronic infection and in 50% of the cases, there is an increase in transaminase enzyme that reveals that there is still liver cell damage. The degree of liver tissue damage in hepatitis depends on the number of virus infecting and the activity of cytotoxic T cells.[1,2,4]

In acute HCV infection, the humoral response has a role with the formation of antibodies against virus. However the role of antibody toward infection has still been not known.

b. The role of cellular response and cytokines in HCV infection

The cellular immune response is produced by CD4+ and CD8+ against the HCV antigens. Cytokines released by CD4+ and CD8+ directly inhibit replication of virus. Compared with hepatitis B, hepatitis C is resistant to the repression effect of cytokines so that the role of other cytokines that cause liver cell damage becomes clear.[1,2]

As explained before, Th1 cells will produce interleukin-2 cytokine (IL-2) and interferon gamma (IFNg). These cytokines are required to increase the host's cellular immune response in killing the virus. Th2 cells produce IL-4, IL-5 and IL-10 that function to augment the production of antibodies for humoral immunity, but also inhibit the Th1 response, especially interferon. The imbalance between cytokine production of Th1 and Th2 has the effect on the progression of disease or irreversible infection. In hepatitis C, the cellular immune response is dominant but the activation patterns are toward activation of Th2. This will cause the elimination process of virus to be ineffective.[1,4]

The occurrence of chronic HCV is caused by other mechanisms such as 'escaped mutant' due to the high speed of mutation besides the imbalance of cytokines. Patients that recover from acute HCV infection and do not become chronic have strong Th1 response and weak Th2 response. Conversely, patients whose infection becomes chronic have a lack of Th1 response and strong Th2 response.[1,2,4-11]

Tsai et al also obtained a correlation between the Th1 response with recovery of HCV infection and Th2 response revealed a going process toward chronic hepatitis.

CD4+ T cell response to viral protein is important to protect the host's cells. CD4+ T cells can control and protect liver cells from hepatocellular damage. Generally, trials are aimed at observing the proli-feration of CD4+ + cells from the peripheral blood compared with the intrahepatic.

Diepolder et al obtained a correlation between clearances of HCV NS3 virus with the specific T helper cellular immune response in trials consisting of 14 patients with acute Hepatitis C Virus.

According to Minutello et al, in patients with active chronic liver, the proliferation response to CD4 was higher than in carriers so that it was postulated that there was correlation between the activities of the disease with CD4+

The role of immune system in the treatment

Principally, the objective of treatment for viral infection hepatitis is to eliminate virus, prevent progressivity as well as to reduce the risk for cirrhosis and hepatoma. The standard treatment with antiviral agents and hepatoprotectors has been believed to be able to prolong the life expectancy of HCV patients.

The role of interferon in viral infection is through, the following process:

- To inhibit replication of virus by inhibiting RNA transcription and replication of DNA
- To increase the expression of MHC 1 molecules (CD8+) to recognize viral antigens
- To stimulate the development of Th1
- To increase the activity of NK cells and macrophagues
- To inhibit the penetration of virus into the cells

A trial conducted in Taiwan proved that ribavirin could increase the cellular immune response in HCV infection and was correlated with the increase in the secretion of IL-12. The sophisticated therapy Th which has been developed is the usage of pegylated interferon. This therapy is regarded as a beneficial one based on the kinetic HCV RNA response that reincreases after 24 hours following the treatment by standard interferon. Pegylated has longer bioavailability in the body because it is gradually absorbed and gradually excreted and is postulated to be able to reduce the immunity reaction of HCV virus.

REFERENCES

1. Nelson DR. The Immunopathogenesis of hepatitis C virus infection. Clinic liver disease 2001; 5(4):(cited 2/26/2002) available from http://home mdconsult.com/das/article/body/1/jorg.
2. Chisari FV. Imunobiology and pathogenesis of viral hepatitis in viral hepatitis and liver disease procedings of IX. Triannial international symposium on viral hepatitis and liver disease. Roma Italy 21 - 25 April 1996. Editione Minerva Medica. Turin 1997.p. 405-15.
3. Wedemeyer H, Rehermann B. Cellular immune responses in hepatitis C virus infection. Viral hepatitis and liver diseases proceeding s of the 10 th International symposium on viral hepatitis and liver diseases. International Medical Press 2002.p.294-3300.
4. Imawari M. Pathogenesis of viral hepatitis. Asian Med J 1999; 42 (4):178-83.
5. Hoofnagle JH. Therapy of chronic hepatitis C. Viral hepatitis and liver diseases proceedings of the 10 th International symposium on viral hepatitis and liver diseases. International Medical Press 2002.p.316-225.
6. Akbar N. Patogenesis dari perjalanan penyakit hepatitis C. Current treatment in internal medicine 2000. Pusat Informasi dan Penerbitan Bagian Ilmu Penyakit Dalam FKUI.
7. Andus T, Bauer J, Gerok W. Effect of cytokines on the liver hepatology. 1991; 13:2.364-73
8. Akbar N. Pedoman penatalaksanaan hepatitis virus akut dan kronik. Current treatment in internal medicine 2000.p.153-5.
9. Ferrari C, Urbaini S, Penna A, Cavalli A, et al. Immunopathogenesis of hepatitis C virus infection. J Hepatol. 1999; 31 (Suppl 1): 31-8.
10. Seef LB, Lindsay KL, Bacon BR, Kresna TF, Hoofnogle JH. Complementary and alternative medicine in chronic liver disease. Journal of hepatology. 2001; 34(1):595-63.
11. Zulkarnain Z. Tinjauan multi aspek hepatitis virus C pada anak. Naskah lengkap pendidikan kedokteran berkelanjutan ilmu kesehatan anak XLII. Jakarta 31 Mei 2000.p.57-72.