

RelationsBetween Interferon α and p24 Antigen Level On Peripheral Blood mononuclear Cells (PBMC) Infected HIV-1

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Abstract : Background : The role of innate immunity in early HIV-1 infections is important to determine the next direction of the disease. At the beginning of the infection, inflammatory mediators such as interferon alpha, which is a type 1 interferon is a cytokine that is produced at the beginning of infection by infected cells and have the ability to inhibit the synthesis of viral proteins.

Objective: The purpose of this study was to observe the activity of interferon alpha in HIV viral replication in peripheral blood mononuclear cells (PBMC) were infected with HIV-1.

Methods: Samples are Peripheral Blood Mononuclear Cells (PBMC) of healthy people which cultured in RPMI 1640 medium equipped with 15% fetal bovine serum (FBS) and added with phytohemagglutinin (PHA) and incubated at 37°C and 5% CO₂. After a sufficient number of cells was 1x10⁶ cells / mL, the culture infected with the HIV-1 MT4 virus. After 7 days, we examined levels of IFN alpha and p24 HIV viral protein antigen.

Results: The correlation test between two variables using the Pearson correlation, the correlation coefficient interferon alpha is 0.354, this is a weak correlation level. Value significance is 0.178, where the value is >0,05, there is no significant relationship between the concentration of HIV-1 p24 protein in cell cultures with levels of IFN alpha.

Conclusion: in this study, the value of $p > 0.05$. Indicates that there is no significant correlation between the amount of HIV p24 antigen proteins with interferon alpha levels produced by the cells of PBMC infected with HIV-1 MT4.

Keywords : HIV virus, infection, interferon alpha, p24, PBMC.

Introduction

HIV attacks the body's immune system, so the role of innate immunity in early infection is important to determine the next course of the disease. At the beginning of the infection, inflammatory mediators and cytokines is an important part in inhibiting viral replication. Inflammatory mediators such as interferon- α , which are groups of type 1 interferon is a cytokine produced early in infection by infected cells and have the ability to inhibit the synthesis of viral proteins.

Type 1 interferon has anti-viral effects against HIV directly, but it has several effects including the regulation of immune system activation and role in cellular apoptosis. Antiviral mechanism of interferon through inhibition HIV viral replication at several stages in the viral life cycle. As a cytokine which is secreted in the natural immune system, interferon has the ability as an immunostimulatory, increasing activation and maturation some cells such as dendritic cells, macrophages, Natural Killer (NK) cells and T lymphocytes¹.

The purpose of this research is to see the expression of interferon- α in cultured of Peripheral Blood Mononuclear Cells (PBMC) were infected with the HIV-1 MT4 virus were able to inhibit the replication of the virus.

Interferon (IFN) are a group of proteins that play a role in inhibiting viral infection and stimulate the immune system against these pathogens. Interferon belongs to a class of glycoproteins known as cytokines, which unlike antibodies, interferon is not a specific virus, but a specific host, so that the virus infection in human cells can only be inhibited by interferon produced by human cell².

Type 1 IFN produced by infected cells as an autocrine or paracrine effect signal for the presence of viral infection. The signal will be delivered through the IFN receptor then activates gene expression of IFN inducible genes simultaneously so as to produce a protein that inhibits viral replication proteins within the cell^{3,4}.

Interferon α has the effect of antiviral against HIV that inhibit viral replication at several stages of replication, the research in invitro mentioned that IFN α can decrease the amount of HIV proviral DNA in cells of monocyte by inhibition of replication through the barriers uncoating of the virus, inhibit the process of budding or through cell activation of immune cells through regulation of APOBEC3G molecules, TRIM5 α and tetherin. IFN α activate latent HIV virus thus decreasing the number of cells infected with the virus through increasing cytotoxic effect of the immune system. Type 1 IFN induction by the HIV virus by binding to the viral RNA Toll Like Receptor (TLR) 3, 7, 8 or 9 involving the adapter molecule myeloid differentiation primary response protein 88 (MyD88), Interferon Regulatory Factor (IRF-3) and also the transcription factor NF κ B⁵.

Antiviral role of IFN α and β in cells infected HIV showed a link between high levels of type 1 IFN produced by the number of low HIV viral load and increase in CD4 T lymphocyte counts and reduced opportunistic infections⁶.

In invitro studies using cells culture that infected with HIV virus, type 1 IFN can inhibit the replication of the virus in cell culture when IFN added to the cell cultures before infected by the HIV virus, the effects of such inhibition on the integrase and reverse transcriptase of the virus. However, if IFN added to the cell culture after infected by HIV, antiviral effects of IFN otherwise lacking. The role of IFN also on the inhibition of assembling viral protein⁷.

IFN 1 plays a role in defense against acute viral infections, but exposure to type 1 IFN in chronic persistent viral infections such as HIV will disrupt a protective immune response against the virus, including the depletion of lymphocyte populations⁸.

Experimental

This study is observational analytic study with a randomized factorial study design. Samples are Peripheral Blood Mononuclear Cells (PBMC) of healthy people which are infected with the HIV-1 MT4 virus.

Preparation of PBMCs

PBMCs isolated from whole blood using Hypaque Ficoll density gradient centrifugation according to the manufacturer's protocol. Blood carefully placed on Ficoll solution hypaque then centrifuge at 1500 rpm for 30 min at 4 ° C. Interphase mononuclear cells were taken and washed three times in phosphate buffered saline solution (PBS)⁹.

The principle of separation of PBMCs from the blood is density gradient centrifugation, which uses the power of a large centrifuge 1500 rpm for 30 minutes at a temperature of 4°C, thus separating the blood components konponen be plated layers according to density. The greater the density of mass (weight) of a component, will be placed at the bottom of the tube.

Cells PBMCs were washed with a solution of PBS (Phosphate Buffer Saline) three times rinsing to remove residual Ficoll solution, by adding 12-13 ml of PBS, shaken to form a solution, then centrifuged 1300 rpm for 5 minutes at a temperature of 4°C. The procedure is performed three times.

PBMCs cultures

After the last flushing, sediment taken in the bottom and the supernatant was discarded. PBMC pellet was then dissolved in 1 ml medium RPMI 1640 10% FBS + 1% Na-bicarbonate, then resuspended pellet and dissolve completely. PBMC culture is transferred to a well plate containing RPMI medium and added to a volume of 2ml and add 2 μ l PHA (of working solution) to each well. Incubated at 5% CO₂ incubator at a temperature of 37°C for 2-3 days. After 3 days, PBMC was resuspended and transferred to a 15 ml tube. Centrifuged at 1500 rpm for 5 minutes, discard the supernatant and add 1 ml RPMI containing IL-2 in the ratio 1: 2000. Calculated PBMC cells, if the concentration was sufficient (10⁶ cells / ml), culture is ready to inoculated with the HIV-1 MT4 cell line.

Virus inoculation

Primary PBMC culture for were co-cultured with HIV-1 MT4 cell line in BSL (Bio Safety Level 3) Laboratory Institute of Tropical Diseases Airlangga University, Surabaya, Indonesia.

After 7 days, the supernatant was stored to determine whether the PBMC was infected with HIV-1 MT4 by ELISA. Detection of p24 antigen (this protein is a component of the viral capsid) using RETROtek HIV-1 p24 Antigen of ZeptraMetrix (ZMC catalog #: 0801111) and the measurement of IFN alpha using Ray Bio® Human IFN alpha Elisa Kit.

Measurement of Expression levels of p24 and IFN α

ELISA kit containing microwell coated with a monoclonal antibody specific for p24 HIV-1 or IFN alpha. Virus antigen contained in the supernatant will be captured specifically by the antibodies coated on the well. The antigen has been bound by the antibodies then react with anti-HIV-1 antibody or anti-IFN alpha antibody which has been conjugated with biotin. By incubating with streptavidin-peroxidase enzymes, will form a color change as a result of binding the enzyme to the substrate. Results are calculated changes its optical density to determine the amount of HIV p24 antigen and IFN alpha contained in the supernatant.

Result

Measurements of the HIV-1 p24 protein in cultured PBMC cell cultures

On day 7 after the inoculation of the HIV-1MT4 virus in PBMC cell cultures, measured levels of p24 protein, which is a protein on the HIV viral capsid that could be detected by ELISA. Protein examination is intended to determine whether the PBMC cell culture has been infected or not. The protein concentration is expressed in units of pg / ml of fluid by calculating the value of the absorbance at a wavelength of 450 nm. By linear regression formula $y = 0,005x$ where y: absorbance values and x: the concentration of protein, so he found the concentration of p24 according to the table.

The linear value obtained where the rate of absorbance is proportional to the concentration of the protein in supernatant.

Relationship of virus levels with levels of Interferon α

From the 10 samples tested, the relationship HIV-1 p24 protein antigen levels which describe the levels of virus in cell cultures and the levels of Interferon α which describe a natural immune response of the cell, as in the diagram.

Correlation test is intended to determine whether the two variables have a significant relationship, if it has a significant relationship, how the direction of the relationship and how strong the relationship.

Seen from the diagram above, the levels of HIV-1 p24 antigen relatively constant in all samples, while varying levels of IFN alpha. The correlation coefficient = 0.354, is a weak correlation level. 0-0.5 is a weak correlation, and 0.5-1 is a strong correlation. Significance value = 0.178 ($p > \alpha$), it was concluded that there was no significant relationship between the concentration of protein antigens of HIV-1 p24 in cell cultures with concentrations of IFN alpha.

Discussion

HIV infection in PBMC cell culture

To detect whether a cell culture PBMC been infected with HIV-1, can be detected by elisa, but also can be detected by PCR (Polymerase Chain Reaction). ELISA principle is to detect p24 antigen, which is a protein in the capsid HIV-1 virus. The binding of p24 antigen contained in the sample by using anti-p24 monoclonal antibody that has been coated on the bottom of the well. The components in the sample that are not bound to the antibody is rinsed with washing, and the bound antigen detected with other specific antibodies against p24 that has been coated with an enzyme (horseradish peroxidase or alkaline phosphatase). While the principle of PCR is the detection of viral nucleic acid, RNA virus which has been copied and duplicated by enzymatic reactions. Compared with PCR, antigen detection by ELISA has a lower sensitivity¹⁰.

P24 antigen test with ELISA has some limitations, such as when an antibody to p24 has been formed and neutralization within the cell will occur, it will lead to false negative results, the second problem is if specific antibodies such factors as *natural -rheumatoid antibodies-* which is a tracer antibody, will causing an increase in antibody binding and causing false positive results¹⁰.

P24 is an important structural component of the viral particle and it is estimated that as many as 2,000-4,000 molecules per virion. p24 antigen will be positive 7 days after cultured . If the p24 antigen tests carried out on blood samples from patients, when the patient's body already has antibodies against the antigen p24, p24 antigen antibodies will neutralize that the levels become low or undetectable, so it can be said to give a false negative result¹⁰. Although in this study the samples examined is PBMC cell cultures in which the formation of antibodies against p24 antigen derived from the HIV virus, as mentioned that the in vitro conditions did not occur as the formation of antibodies in vivo conditions.

Interferon α as an immune response against viral infections

Type 1 interferon produced from cell cultures infected T lymphocytes mumps virus have been investigated by Nakayama in 1983. It is said that interferon α and γ is the immune response generated by the lymphocyte cell cultures infected with the mumps virus¹¹.

Interferon Type 1 has an important role as a defense and natural immune response to an acute viral infection, but in persistent viral infections, exposure to interferon type 1 can reduce the protection and immunity against the virus¹².

In this study, the infection occurs at day 7, and interferon α also observed at 7 days post-inoculation with the virus, of which this phase is still included in the acute phase of infection. Limitations of the study in cell cultures is the age of the cells in culture were limited, in which adult cells can no longer be differentiated and proliferate on a limited basis because they can not divide anymore.

Interferon α can modulate the function of natural and adaptive immune responses, activates monocytes, Antigen Presenting Cells, dendritic cells, macrophages, Natural Killer cells (NK), T lymphocytes and B lymphocytes In early infection, interferon α will increase the expression of molecules of the Major Histocompatibility Complex (MHC) class 1 and II on the surface of monocytes, stimulates the proliferation and activation of NK cells and dendritic cells, activating T helper 1 and induce differentiation of B cells into plasma cells. Interferon α also induces restriction factors that impede the integration of virus and viral shedding after replication in infected cells^{12,13}.

This research can not observe the activation of IFN α function of each cell, as PBMC consists of several types of cells such as monocytes, lymphocytes and dendritic cells, as well as in cultured cells in vitro were indistinguishable and only able to proliferate on a limited basis.

In in vitro conditions, exposure to interferon in PBMC cultures were able to regulate the expression of pro-apoptotic molecules in lymphocytes, inducing apoptosis of lymphocytes T. In patients with HIV, interferon is responsible for the loss of a large number of CD4 T lymphocytes and mediates Fas-mediated apoptosis. Apoptosis in infected T lymphocytes can not be observed in this study, because the process is more easily observed in in vivo conditions involving a complex signal molecules^{12,14}.

Relationships levels of interferon α with p24 levels of virus in cell cultures PBMCs

Primary infection is the stage where there is a balance between viral replication and immune response, which depends on the strain of virus and host genetic factors. Interferon α is interferon type 1 which has a strong antiviral effect at the beginning of infection, either directly or through the activation of the immune system⁶. Interferon α is not only to suppress viral replication through increased immune response against the HIV virus, but also help clean the reservoir virus⁵.

Interferon α has antiviral effects against HIV infection by inhibiting several phase in viral replication, and increased levels of interferon- α is accompanied by a decrease in HIV-1 DNA in Peripheral Blood mononuclear cells (PBMC) of patients⁵.

This study found no significant association between the levels of HIV-1 p24 antigen with levels of interferon- α which is produced by cells in PBMC cultures infected HIV. It was likely caused by a less precise time of measurement of interferon, so that the levels are not optimal. Mentioned that the peak level of interferon- α is produced in 3 days (72 hours) post-infection¹⁵, whereas interferon level examination in this study performed at 7 days post-inoculation with the virus, which made possible the levels have declined.

Another factor that causes the protein level of HIV-1 p24 antigen remained high, interferon α is not the only natural host cell mechanisms in inhibiting viral replication. When compared to the in vivo conditions in which the immune response can occur more complex and involves many cells, in vitro conditions is limited to one type of cell.

Mentioned that not only interferon α , but interferon- γ also has a role in the pathogenesis of HIV, where γ interferon along with other proinflammatory cytokines involved in immune activation, but interferon- γ did not have antiviral activity against the HIV virus in primary culture. The increase of interferon- γ found in HIV-infected individuals can enhance cytotoxic T lymphocytes⁹.

The level of Interferon α in Peripheral Blood Mononuclear Cells (PBMCs) infected HIV-1 obtained at the following results

Table 1 Concentration of p24 protein in cultured PBMC cells were infected with the HIV-1MT4 (note : A1 = absorbance)

sample	A1	Concentration (pg/ml)
1	1,263	252,6
2	1,272	254,4
3	1,393	278,6
4	1,373	274,6
5	1,363	272,6
6	1,378	275,6
7	1,357	271,4
8	1,384	276,8
9	1,354	270,8
10	1,37	274,0

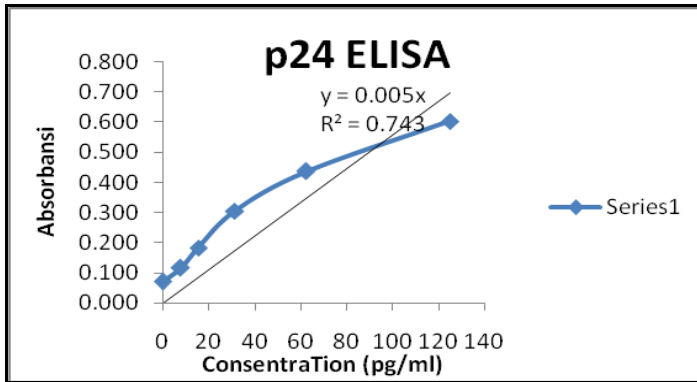


Figure 1. The linear curve between the absorbance with concentration levels of HIV p24 protein in cultured PBMC cells (black line: from the standard solution, the blue line: from the sample)

Table 2 Results of interferon α concentrations in PBMC cell cultures infected with the HIV virus (Description: A1 = absorbance)

Sample	A1	Concentration (pg/ml)
1	0,094	15,7
2	0,225	37,5
3	0,747	124,5
4	0,584	97,3
5	0,653	108,8
6	0,45	75,0
7	0,444	74,0
8	1,095	182,5
9	1,11	185
10	1,099	183,1667

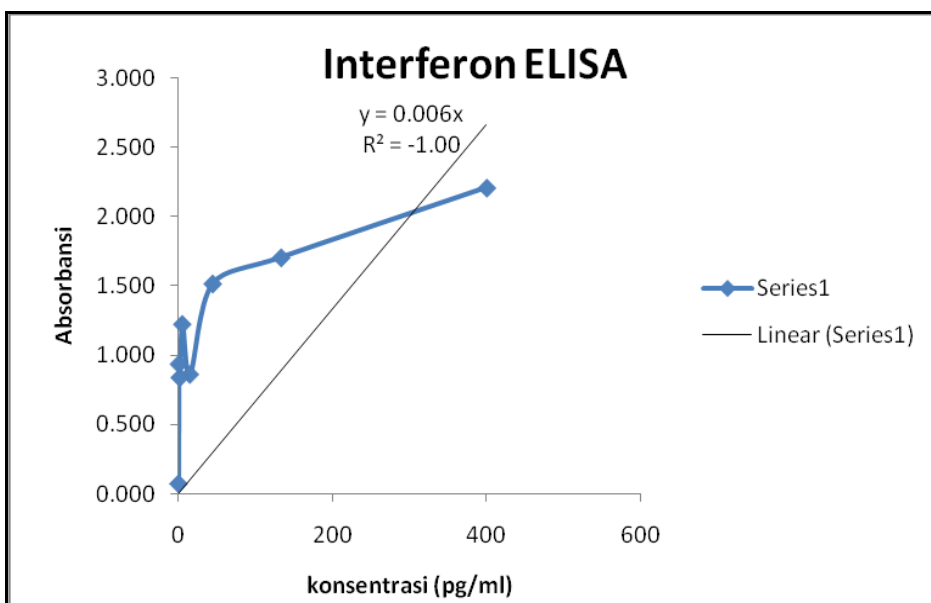


Figure 2 The linear curve between the absorbance concentration levels of interferon α in cultured of PBMC infected HIV-1 (black line: from the standard solution, the blue line: from the sample).

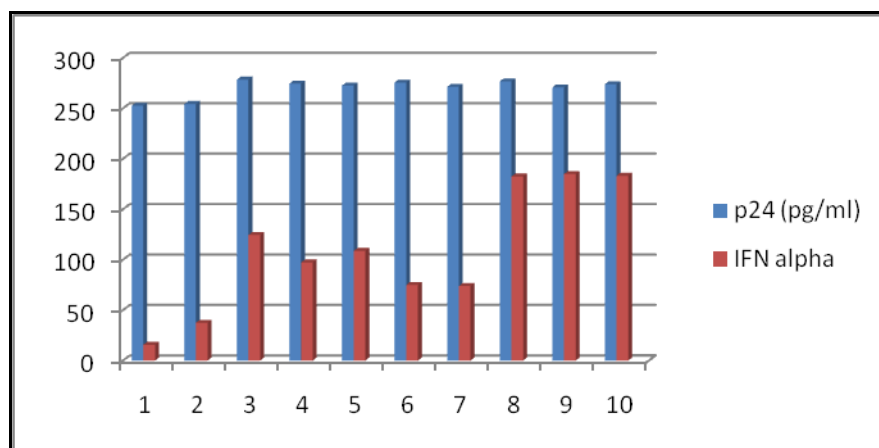


Figure 3. Correlation between the levels of HIV-1 p24 antigen and Interferon α in PBMC culture infected HIV-1.

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