Department of physics, chemistry and biology

Bachelor's thesis

Effects of different conditions of HIV-1 on plasmacytoid dendritic cells in maturation and function

Author
Susana Häggqvist

Performed at the Department of Molecular Virology, IKE
May 2008

LITH-IFM-EX--08/1976—SE

TEKNISKA HÖGSKOLAN
LINKÖPINGS UNIVERSITET

Department of physics, chemistry and biology
Linköping University
SE-581 83 Linköping, Sweden

Department of physics, chemistry and biology

Bachelor's thesis

Effects of different conditions of HIV-1 on plasmacytoid dendritic cells in maturation and function

Author
Susana Häggqvist

Performed at the Department of Molecular Virology, IKE
May 2008

Supervisor
Marie Larsson

Examiner
Helena Herbertsson

TEKNISKA HÖGSKOLAN
LINKÖPINGS UNIVERSITET

Abstract

Plasmacytoid dendritic cells (PDCs) are one cellular target of HIV-1 and respond to the virus by producing type I interferons and chemokines. PDCs exposed to HIV-1 strongly upregulate the expression of maturation markers such as CD83, CD80, CD86 and CCR7, which will turn them into professional antigen presenting cells with the ability to stimulate naïve $CD4^{+}$T cells. When HIV-1 binds to the CD4 receptor and a co-receptor (CCR5 or CXCR4) on PDCs, the cell takes up the virus by endocytosis. In response to this, PDCs will become activated and express maturation markers on their surface that make them able to stimulate T cells to trigger an immune response. In this thesis, studies have been performed with different forms of HIV-1, i.e. opsonized virions covered in complement and antibodies since these forms are supposed to be more similar to how HIV appears in the body. According to our results there is no significant difference in PDC maturation between the free and opsonized HIV-1.

Key words: Plasmacytoid dendritic cells, Interferon-α, HIV-1, complement and immune complexed opsonized.

Table of Contents

INTRODUCTION

Dendritic cells

We have a mononuclear cell specialized in recognizing when the body is under attack from pathogens, i.e. the dendritic cell (DC) **(Fig. 1).** This cell is located in all our tissues and blood to sense when we are infected [Guo, Z. *et al.* 2005*,* McKenna, Kelli. *et al.* 2005]. The DC play an important roll in initiating immune responses and is the link between the innate and the adaptive immunity [Guo, Z. *et al.* 2005*,* McKenna, Kelli. *et al.* 2005]. The DCs are now known to influence many different classes of lymphocytes and the type of T cell response induced. The maturation of the DCs is an important stage which happens in response to infection and inflammation [Steinman, RM. 2003].
DCs are derived from hemopoietic bone marrow progenitor cells **(Fig.2)**. These cells initially develop into immature DCs and circulate in blood or are located in tissue until they become activated. The activation occurs when the DCs take up and process a foreign antigen and migrate to the lymph nodes and spleen where it present antigen peptides for the T-lymphocytes (T cells) and stimulate an immune response against the antigen. Since DCs are the only antigen-presenting cells able to stimulate naïve T cells, they classifies as the professional antigen-presenting cells. DCs present an antigen on the cell surface via major histocompatibility complex (MHC) class I or II molecules. This MHC-antigen complex is required for the T cells to recognize an antigen. Stimulation of T cells is an important part of the immune response and is necessary for activation of other immune cells [Janeway Jr, CA. et al. 2005].
When a DC takes up a pathogen by endocytosis, the pathogen breaks down into small peptides in the endosome. These peptides are presented on the cell surface through the MHC class II molecules. Antigen expressed on MHC class II molecules stimulates $CD4^+$T cells. If the pathogen enters the cytosol is the antigen bound to MHC class I molecules and activates the $CD8^+$ T cells. Immature DCs have a low expression of the MHC molecules and an efficient uptake of antigens.

Mature DCs upregulate their expression of the MHC molecules but reduce their ability to pick up antigen and this make them very efficient when activating the T cells [Agger, R. et al. 2006].

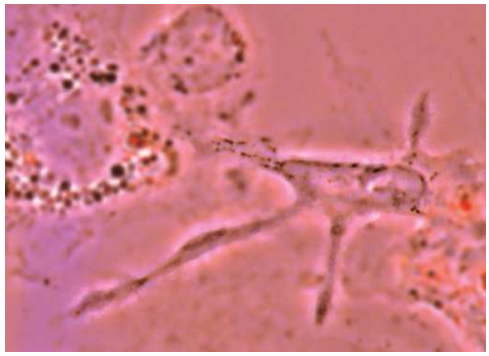

Figure 1. Dendritic cell
A light microscope picture of a dendritic cell.
Immuno Biology 6th edition, Charles A. Janeway, Jr

T cells

T cells develop from lymphoid progenitors in the bone marrow and these progenitors migrate to the thymus where the maturation into naïve T cells occurs. **(Fig. 2)** T cells are key players in cell-mediated adaptive immunity [Janeway Jr, CA. et al. 2005]. Naïve T cells express CD4 or CD8 receptors on their surface dependent on their function. $CD4^+$T cells, called T-helper cells, are responsible for activation and regulation of other cells in the immune system such as B cells and $CD8^+$T cells by direct interaction and by the cytokines they produce. The $CD8^+$T cells, called cytotoxic T cells, control viral infections by killing infected cells before the release of new virus can occur and eliminate cancer cells [Agger, R. et al. 2006]. Since T cells do not recognize free antigens they require antigen presentation by antigen presenting cells (APCs), in this case DCs. A T cell receptor (TCR) on the T cells interact with the antigen

peptide displayed by the MHC molecules on the APC allowing the transfer of necessary signals for T cell activation [Janeway Jr, CA. et al. 2005].

Lymphocyte development

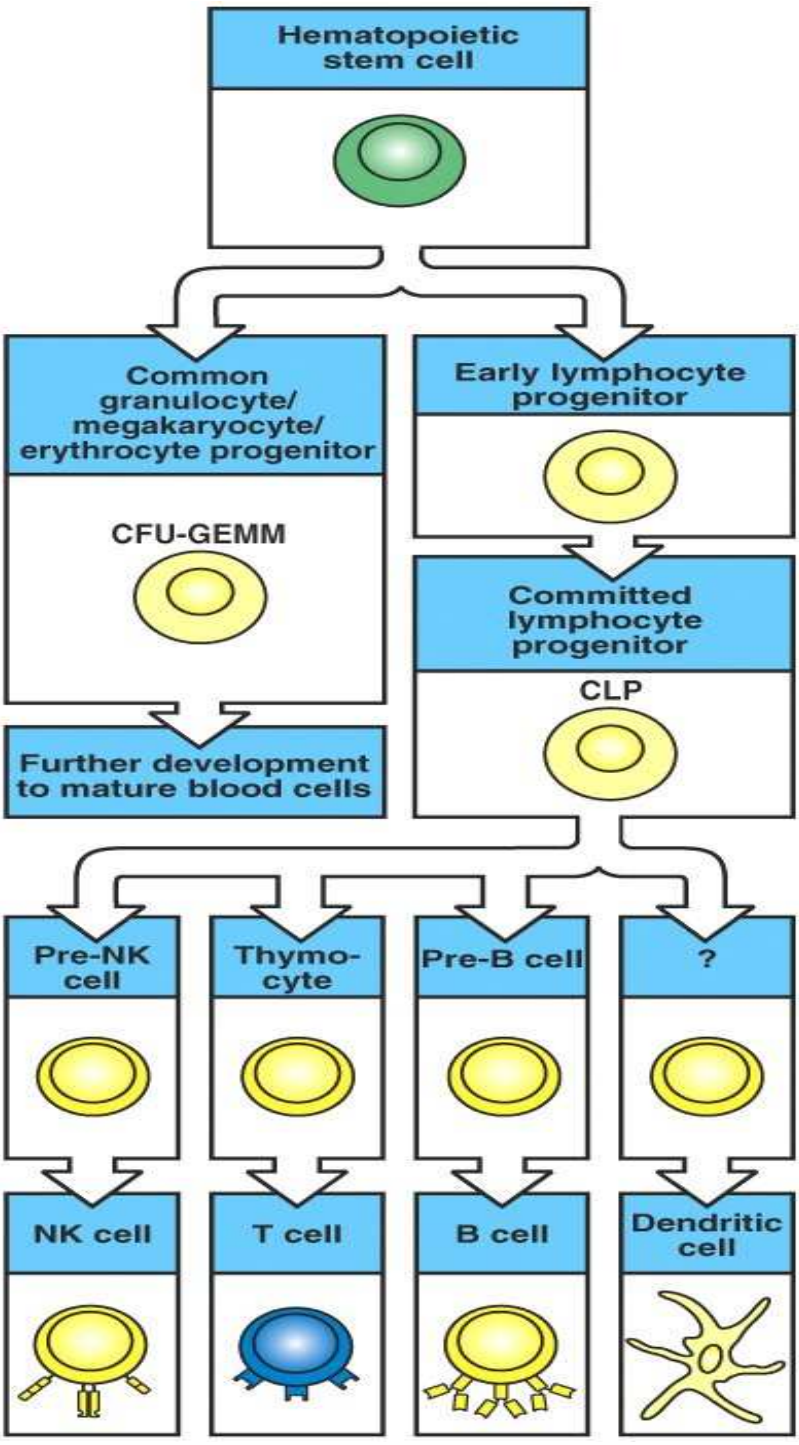

Figure 2. Development of T cells and dendritic cells from hematopoietic stem cell
Immuno Biology, 6th edition, Charles A. Janeway, Jr. © 2005 by Garland Science publishing

Plasmacytoid dendritic cells

There are two different types of DCs acting in the blood, myeloid and plasmacytoid DCs, distinguished by their phenotype and function [Steinman, RM. 2003, Beignon, A-S. et al. 2005]. In this case we only looked at plasmacytoid dendritic cells. Plasmacytoid dendritic cells (PDCs) have a round morphology with an eccentric nucleus and abundant endoplasmic reticulum like a plasma cell, thus plasmacytoid [Asselin-Paturel C. et al. 2005]. These cells express CD4 and CD123 but lack expression of myeloid markers like CD11b, CD11c, CD13 and CD33 [Fonteneau, J-F. et al. 2004].

A PDC has the significant function to produce type I interferon, IFN-α/β, in case of virus invasion. The secretion of IFN-α/β is an important action for the immune system since it leads to virus resistance and inhibition of virus replication in infected cells.

PDCs preferentially express toll like receptor (TLR)7 and TLR9. TLR7 and TLR9 are intracellular endosomal receptors that allow PDCs to respond to single stranded RNA and DNA viruses.

According to this receptor location, the cells need to take up a virus by endocytosis for maturation and production of type I IFNs [Asselin-Paturel C. et al. 2005].

Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus (HIV) is a retrovirus and a member of the lentivirus family. The HIV is built-up by a capsid, including two copies of single-stranded RNA, reverse transcriptase and other viral proteins. **(Fig. 3)**

The capsid consists of p24, a protein from the gag gene in the HIV genome. This protein is detected in HIV positive individuals and is used to measure the viral load in the body. A frequent used method for these measurements is ELISA (Enzyme-linked immunosorbent assay, described on page 7). Outside the capsid is another protein layer called the matrix followed by a phospholipid bilayer envelope as a final shell. Bound to the envelope, there are two noncovalently associated glycoproteins (gp41 and gp120), creating spikes on the viral surface. [Abbas, A. et al. 2000]

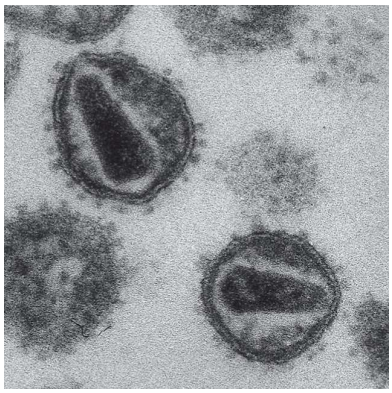

Figure 3. **HIV-1**
HIV virions visualized by electron microscopy
Immuno Biology, 6th edition, Charles A. Janeway, Jr. © 2005 by Garland Science publishing

Two related types of HIV has been identified, HIV-1 and HIV-2. HIV-1 is by far the most common cause of the acquired immunodeficiency syndrome (AIDS), a condition when the immune system fail, which makes it too hard for the body to fight off infections [Abbas, A. et al. 2000]. The most common ways of HIV transmission are sexual intercourse, use of contaminated needles, and through the placenta from the mother to her developing baby. Infections with HIV are characterized by extended clinical latency, continuous virus mutation, neuropathology, and infection of $CD4^+$ lymphocytes.

There is a long but variable period of latency when HIV exists as a provirus integrated in host's genome without any viral transcription. When the level of $CD4^+$ cells in blood has decreased to less than 200 cells/µl, due to infection and death of these cells by the virus, the infected individuals are diagnosed to have AIDS. In about 50% of HIV positive individuals the progression to AIDS not occurs until after 10 years or more of infection [Male, D. et al. 2006].

Today there are approximately 40 million HIV-positive people in the world (2006). Over 90% of them live in developing countries, mainly in sub-Saharan Africa. Today's spread take place to 80% by the heterosexual route. Over 25 million people have died since the first cases were described and identified in the early 80's [Male, D. et al. 2006]. Even though the most HIV positive people live in Africa, at the moment the biggest rise in infections is in east Europe. So far there is no cure or vaccination available but rather good antiretroviral therapy, which delays the course of the disease. Unfortunately, this treatment has a lot of secondary effects [SMI, 2006-05-10].

Mechanism of HIV entering a cell

The viruses fix on the target cells by binding the gp120 subunit to the CD4 receptor and a co-receptor on the cell surface. Then, HIV particles enter the target cell through fusion of the envelope with the cellular plasma membrane [Abbas, A. et al. 2000]. The fusion is catalyzed by the gp41 subunit [Wyma, DJ. et al. 2004]. Once inside, the viral genome enters the cytoplasm and since HIV is a retrovirus, the genome requires a transformation into double stranded DNA (provirus) to be able to integrate with the cell genome. The reverse transcription of the HIV's RNA genome is done by the enzyme reverse transcriptase which is also carried in the capsid. Incorporated in the genome of the host cell, the provirus is copied by the host cells own protein synthesis machinery and new virions are leaving the cell [Abbas, A. et al. 2000]. **(Fig. 4)**

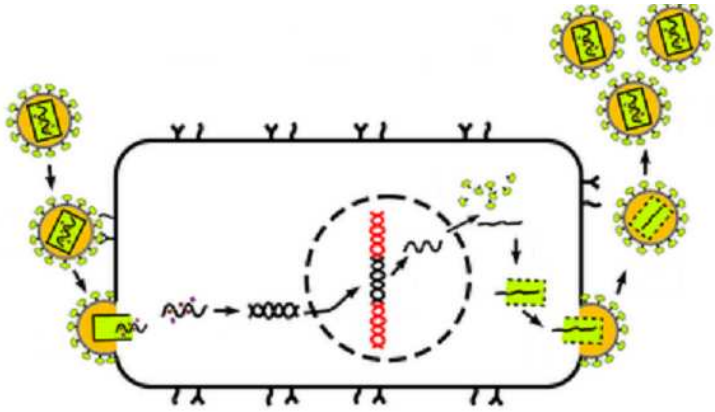

Figure 4. HIV-1 infection
HIV enters into a cell by fusion followed by production of a provirus that integrates into the host genome. The provirus is transcribed and new virus produced.
Adapted from:
http://images.google.se/imgres

HIV-1 and PDCs

PDCs are cellular targets for HIV-1 and respond to the virus by producing type I Interferon (IFN), i.e. alpha and beta, which is an important pathway for the immune response [Fonteneau, J-F. et al. 2004]. When HIV-1 comes in contact with the PDC, via the interactions between CD4 receptor and co-receptor CCR5 or CXCR4 on the PDC cell surface and gp120 on the virus envelope, do this result in endocytosis of the virus [Wagner, EK. et al. 2004].
When the virus enters by endocytosis, the PDCs secrets IFN-α/β and upregulates maturation markers such as CD83, CD80, CD86 and CCR7 and migrates to the lymph nodes. Once in lymphoid tissues, PDCs may pass HIV-1 to CD4 T cells via direct cell-cell contact. T cells are highly susceptible for HIV-1 infection and this gives a depletion in CD4 T-cells, which leads to dysfunction of the immune system [Fonteneau, J-F. et al. 2004].

Aim

The aim of this project is to find out how different forms of HIV-1 (complement opsonized, immune complexed and complement opsonized and immune complexed) affect the PDCs in

functions such as maturation and secretion of type I IFN. The purpose is to look at HIV-1 in the same way as it appears when infecting the body and compare to the studies done with free HIV. T cell activation by DCs, cultured with HIV, was also studied to observe the potential of infected PDCs to stimulate T cells which is an important function of the immune system.

METHODS AND EXPERIMENTAL PROCEDURE

Preparation of peripheral blood mononuclear cells from buffy coats

Buffy coats were obtained from the blood bank in Huddinge. The peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation on Ficoll-Paque™ (GE Healthcare). ~25 ml blood was mixed with RPMI media to total volume at ~40ml. 0.2% EDTA was added to the media to block aggregation of cells. The cells of interest were extracted from the Buffy coat using Ficoll-Paque, a hydrophilic polysaccharide that separates cells from blood in regard to their density. To receive a good separation, the blood was carefully layered above 10ml of Ficoll-Paque in a tube and centrifuged at 2200 rotation per minute (RPM) at room temperature for 22 minutes without brake. After separation, red blood cells were located in the bottom of the tube under the Ficoll-Paque and the PBMC's were located in a thin layer above. The PBMCs were collected and washed with RPMI medium by spinning for 10 minutes at 4°C four times with different speeds. First spin at 1800 followed by 1500, 1100 and finally 900RPM. The remaining PBMCs were counted in a microscope using a Bürker chamber.

Positive Selection of cells by magnetic bead separation

Figure 5. Magnetic bead separation on a MidiMACS™ Separator from Miltenyi Biotec.

The cells of interest, the plasmacytoid dendritic cells (PDCs), were sorted out with a micro bead kit MACS® from Miltenyi Biotec.
To separate PDCs from the mononuclear cells, the cells were spun down in MACS® buffer and then reconstituted with 80µl buffer/10 $x10^6$ cells and 10µl magnetically labeled antibodies/$10x10^6$ cells and incubated in refrigerator for 15 minutes.
After incubation, buffer was added to the cells and then centrifuged at 1700RPM for 12 minutes at 4°C. The supernatant was removed and the cell pellet resuspended in 2ml buffer and loaded onto the column placed in a magnetic field. The LS column was previously washed with 5 ml buffer.
The magnetic bead coupled anti-BDCA-4 antibodies bind to the surface on the PDCs via BDCA-4 receptor, a protein specifically expressed by PDCs. When the cell suspension is loaded onto a column placed in a magnetic field, the PDCs bound to the antibodies will remain in the column while the other cells flow through [MACS®, 2008], i.e. a positive selection. The column was washed 3 times with 5 ml buffer before eluting the PDCs from the column. To collect the PDCs, the column was removed from the magnetic field and loaded with buffer and the PDCs were flushed out with a plunger.

Staining of PDCs

The positively selected cells were stained with antibodies for CD123, to prove that the cells were PDCs and to give an estimate of their purity and with antibody for CD83, a maturation marker, to make sure the fresh PDCs are immature. The samples were analyzed by flow cytometry.

Incubation with HIV-1

The virus was opsonized with complement (fresh serum) and/or IgG and incubated for one hour before adding them to the cells. Opsonization is a process when a particle or a foreign microorganism is coated with proteins that make them more susceptible to phagocytosis. PDCs were exposed to free HIV-1, complement opsonized HIV-1, immune complexed HIV-1 with IgG, and both complement and immune complexed opsonized HIV-1. Negative controls were PDCs alone, and positive control was PDCs exposed for TLR7/8 ligand, known to activate PDCs. The HIV-1 concentrations were 750ng/ml.

HIV-1 Strain	PDC exposure					
$HIV\text{-}1_{MN}$	Free	CO	IgG	CO & IgG	Neg. Control	Pos. Control
$HIV\text{-}1_{BaL}$	Free	CO	IgG	CO & IgG	Neg. Control	Pos. Control

CO = complement opsonized
IgG = Immune complexed
CO & IgG = complement immune complexed opsonized
Neg. control = only pDC's
Pos. control = TLR7/8 ligand
$HIV\text{-}1_{MN}$ = CXCR4 tropic strain of HIV-1
$HIV\text{-}1_{BaL}$ = CCR5 tropic strain of HIV-1
The cells were incubated for 16 hours in 37ºC before analysis by flow cytometry.

Flow cytometry

Examinations of the cells were done by flow cytometry. This technique measures the properties of single cells suspended in a stream of fluid. Light from a laser beam hits the cell as they pass through the flow chamber and the way the light bounce of each cell gives information about the cells phenotypic properties [OSU, 2008 (1)]. The scatter on the x-axis (forward scatter) shows the size of the cells and the scatter on the y-axis (side scatter) depends on the amount of granules. Each type of cell in the immune system has a unique combination of forward and side scatter measurements which means you could count the number of cells of each type [OSU, 2008 (2)].

ELISA

ELISA is a frequently used method in detecting HIV-1 infection as well as other virus infections. [Abbas, AK. et al. 2000, Janeway Jr., CA. et al. 2005] In this case, a modification of ELISA called sandwich ELISA, was used to detect secreted INF-α. A 96-well plate is coated with antibodies recognizing human IFN-α. IFN-α in the samples is captured by the solid bound antibodies and unbound material are washed away. A tracer (a second antibody) is added to the wells and binds to the IFN-α. After excess tracer is removed, a tracer antibody, with a covalently coupled enzyme, is applied to the wells. This conjugate reacts specifically with the tracer bound onto the detected IFN-α. Nonbound conjugate is washed away and a substrate is added. A color change can be spectrophotometrically measured and is proportional to the amount of INF-α. The concentration of IFN-α can be determined with a standard curve.

Supernatant was collected from the PDCs incubated with different HIV-1 forms and added (100µl/well) to the plate. Standard samples with decreasing amount of IFN-α were added in 2

x 8 wells. The plate was incubated for 2 hours at 37ºC. The wells were washed with 200µl wash buffer 3 times and 100µl biotinylated antibodies (tracer) were added before the plate was incubated in room temperature for 1 hour. The wells were washed again same way as before and 100µl streptavidin-peroxidase conjugate were added and the plate was incubated for 1 more hour in room temperature. After the last wash (same as previous) 100µl tetramethylbenzidine (TMB) were added to start the enzyme-substrate reaction. (The plate was wrapped in aluminium foil to avoid exposure to strong light.) The enzyme reaction was stopped after 30 minutes by addition of 100µl citric acid and the absorbance at 450nm was measured. The ELISA kit for human IFN-α kit was manufactured by Hycult Biotechnology, Netherlands.

T cells

As T cell source was the non adherent cell fraction from PBMCs. PBMC's were added to 8 tissue culture plates, ($40x10^6$ cells/plate), for 2 hours to deplete monocytes and DCs which will adhere to the plastic plates. The non adherent cells were harvested and washed before use.

Allogeneic T cell activation by PDCs activated by different forms of HIV-1

PBMC's were purified from Buffy coats. T cells and PDCs from different donors were used in the mixed lymphocyte reaction (MLR). PDCs were collected by positive selection as described above and exposed to different forms of HIV (complement opsonized HIV-1, immune complexed HIV-1 with IgG, and both complement and immune complexed opsonized HIV-1) and incubated over night. The HIV-1 concentration was 750ng/ml. The different PDC groups were added at different doses to a flat bottom 96 well plate together with 200 000 T cells/well. After five days, the MLR were pulsed with ^{3}H-Thymidine and incubated over night to allow incorporation of ^{3}H-Thymidine in dividing cells which gives a measure of T cell proliferation. The cells were harvested on nitrocellulose filters and analyzed with a β-counter.

RESULTS

Opsonized HIV-1 and free HIV-1 induce PDC maturation

The PDCs were purified from PBMCs by positive selection. The separation was done by BDCA-4 magnetic beads. The fresh cells were stained with PE conjugated CD123 and APC conjugated CD83 and analyzed by flow cytometry to determine purity. The $CD123^{+}$cell populations obtained by this method varied in purity between 75-85% and had an immature phenotype. (Like the ones shown in **Fig. 6**). The purified PDCs were cultured with free HIV-1, complement opsonized HIV-1, immune complexed HIV-1 with IgG, and both complement and immune complexed opsonized HIV-1 over night. Negative controls were PDCs alone, and positive control was PDCs incubated with TLR7/8 ligand. The effects HIV-1 exposure had on the PDCs were assessed by flow cytometry staining for the maturation marker CD83 **(Fig. 7)**. The complement, IgG, or complement IgG opsonized HIV-1 was almost as efficient as the free HIV-1 in inducing the PDC maturation as measured by CD83 expression. The differences was not statistical significant. **(Fig. 8)**

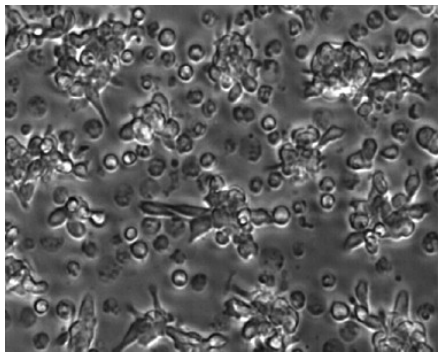

Figure 6. PDCs cultured with HIV-1_{MN}. Fonteneau, J-F. et al. Journal of Virology, Vol 78, 2004.

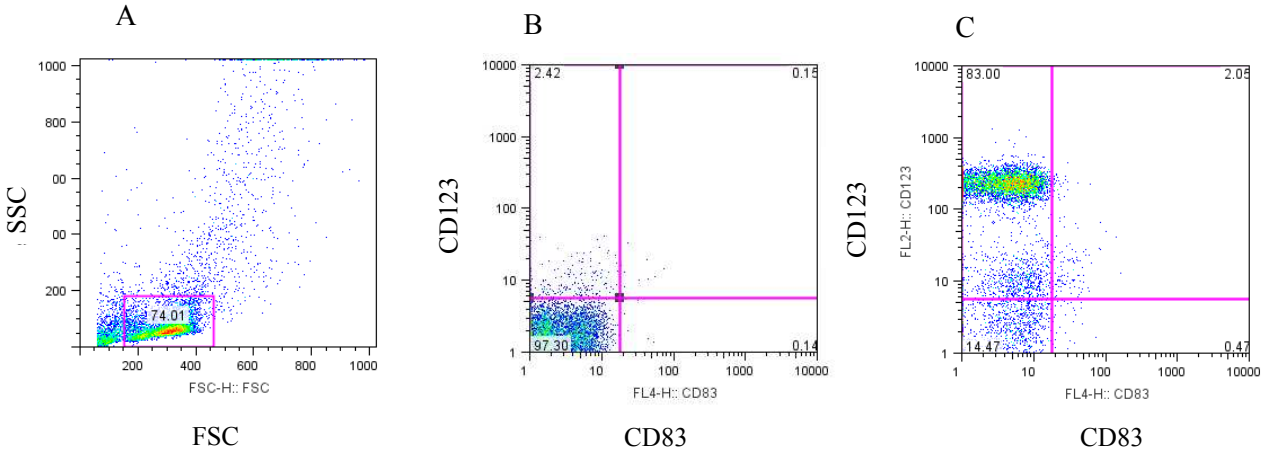

Figure 7. Fresh PDC phenotype. Selection of PDCs from PBMCs was done by BDCA-4 magnetic bead positive selection. The purity of the PDCs determined by staining the cells with PE conjugated CD123 mab and APC conjugated CD83 mab and acquired on flow cytometry. A: Flow graph for cell size (FSC) and granularity (SCC) for the purified cells.B: Negativ control. C: The $CD123^{+}$cell population with purity of 80% and almost no expression of the maturation marker CD83.

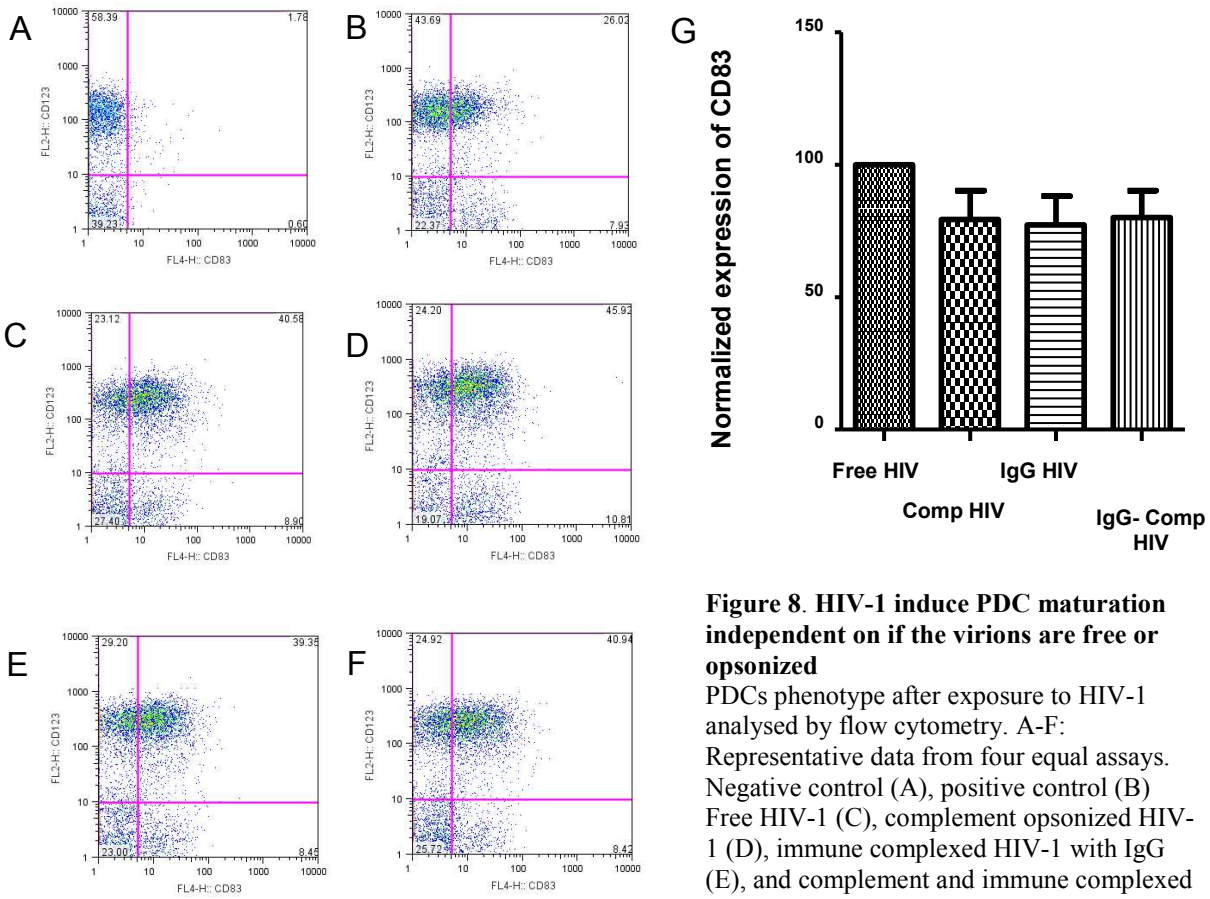

Figure 8. HIV-1 induce PDC maturation independent on if the virions are free or opsonized
PDCs phenotype after exposure to HIV-1 analysed by flow cytometry. A-F: Representative data from four equal assays. Negative control (A), positive control (B) Free HIV-1 (C), complement opsonized HIV-1 (D), immune complexed HIV-1 with IgG (E), and complement and immune complexed opsonized HIV-1 (F). The Normalized expresson of CD83 maturation marker from four different experiments (G).

T cell activation by PDC phenotype cultured with different HIV forms

T cells from PBMCs were exposed to PDCs cultured with different forms of HIV-1. The cultures were pulsed with ^{3}H-Thymidine to determine PDCs ability to activate T cells proliferation. HIV-1 activated PDCs are more efficient than untreated PDCs. Preliminary data shows that the increased proliferation was due to HIV-1 induced PDC maturation. **(Fig. 9)**

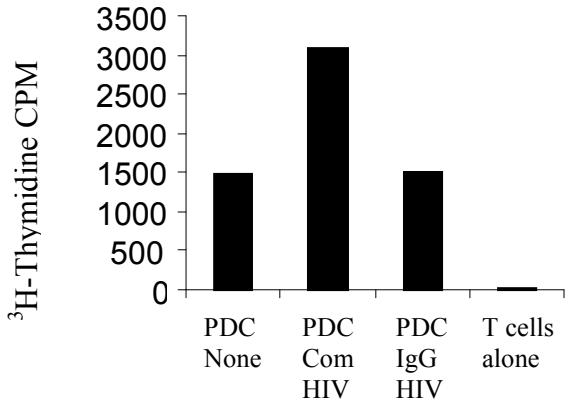

Figure 9. HIV exposed PDCs induce efficient T cell proliferation. Cocultures of T cells and PDCs pulsed with ^{3}H-Thymidine after 5 days of culture. The assay was harvested and analysed with a β-counter. (CPM=counts per minutes)

PDC effector function

Supernatants from PDCs cultured with HIV-1 were analysed by the sandwich ELISA principle for detecting secreted IFN-α from the PDCs infected with different form of HIV-1. The amount of IFN-α was spectrophotometrically measured. HIV-1 induce high levels of IFN- α, independent on if the virus was opsonized or free, compared to our positive control. **(Fig. 10)** Unfortunately, the amounts of IFN-α were higher than expected and we could not draw any conclusions between the free and opsonized HIV-1s efficiency to induce IFN-α production by the PDCs. Future analysis will be performed with samples diluted before use.

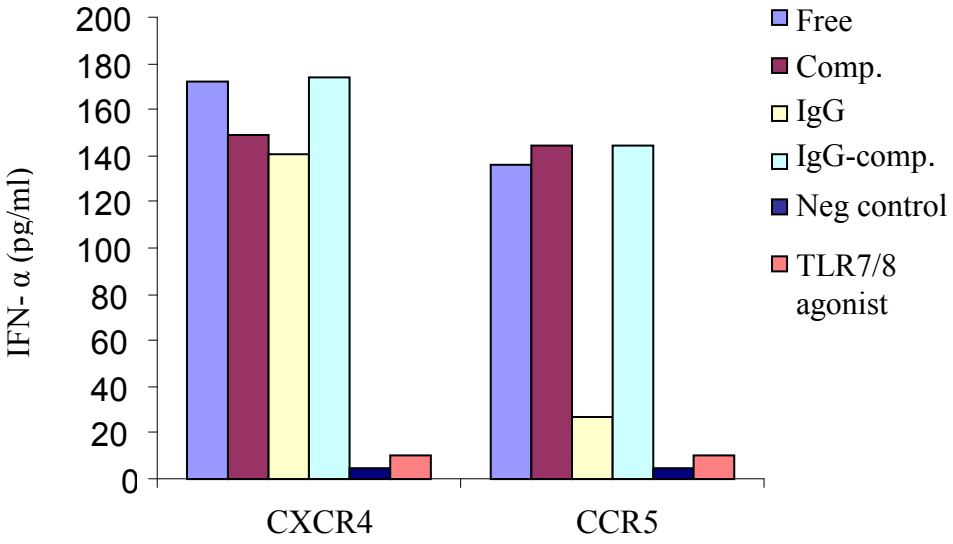

Figure 10. HIV exposed PDCs produce high levels of IFN-α.
Amount of produced IFN-α from PDCs infected with different form of HIV. The bar graph shows a representation from different experiments.

DISCUSSION

PDCs have an important roll in innate immunity against virus as the major IFN-α producer. HIV has a negative effect on PDCs and other cells in the immune system. In response to HIV-1, PDCs produce large amount of IFN-α to avoid further virus infection themselves and in other cells. After HIV-1 exposure, they upregulate costimulatory molecules, MHC molecules and maturation markers [Fonteneau, J-F. et al. 2004]. When HIV-1 enters the body it is already opsonized with complement and antibodies from the body fluids. [Stiober, H. et al. 2001.] In this thesis, we have compared 3 different forms of HIV-1, e.g. complement opsonized, immune complexed, and complement and immune complex opsonized HIV-1 that exist in vivo to free HIV-1. To examine the different opsonized HIV-1 is of importance as they do not have the same properties and exert different effects on the immune system compared to the free HIV-1. In our study there was no major differnce between the opsonized virus ability to induce PDC maturation, measured by CD83 expression compared to the free HIV-1. The small differences in maturation between free and opsonized HIV-1 may depend on the pathway utilized by the cell to bind and internalize the virus [Stiober, H. et al. 2001.], which differ between the free and the opsonized HIV-1. As the signaling receptors which regulate PDC maturation after HIV-1 exposure are located in the endosomes, endocytosis of the virus is required for expression of the maturation markers on PDCs [Fonteneau, J-F. et al. 2004] and the amount of virions endocytosed and reaching this endosomes may differ between the free vs. the opsonized HIV-1. The total amount of HIV-1 internalized can be measured with p24 ELISA (which is planned) but this method does not give the intracellular location. The ability to efficiently activate T cells is connected to the DC maturation status and we found that HIV-1 exposed PDC was better than the PDC alone, which correlate to the HIV induced maturation.

We also looked at the level of type I IFN produced by the PDC after HIV-1 exposure as this cell is know to secrete high levels as a antiviral defense and depend on other signaling pathways than the one required for the PDC maturation. HIV-1 exposure induced very high levels of IFN-α secretion by PDCs compared to TLR7/8 ligand activation even if the level of maturation between them was similar.

In conclusion, the HIV-1 forms existing in vivo are as efficient as free virus to induce maturation and IFN-α secretion. Further experiments will tell if the opsonized virus is taken up with the same efficiency as free and if there are differences in their ability to stimulate T cells.

ACKNOWLEDGEMENTS

Many thanks to my supervisor Marie Larsson for her kindness and for giving me the opportunity to do my bachelor's thesis at the department for Molecular Virology, even though I did not have any earlier experience in virology.
I would also like to thank Karlhans for giving me lots of practical and theoretical help and for always being kind, patient and enthusiastic.
Thanks to Petter, for helping me to get in touch with Marie and IKE.
Thanks to Martin, for your support and belief in me, and for listening to my issues even though you probably did not understand a thing.
Finally, to the other nice people at the department of Molecular Virology, thank you so much for your great hospitality. I have really enjoyed my time on floor 13.

REFERENCES

Abbas, Abul K., Lichman Andrew H., Pober Jordan S. ”Cellular and Molecular Immunology, 4th ed.” W.B. Saunders Company, 2000, New York, USA.

Agger, Rolf., Andersen, Vagn., Leslie, Graham., Aasted, Bent ”Immunologi” Narayana Press, 2006, Denmark.

Asselin-Paturel, Carine and Trinchieri, Giorgio. (2005) “Production of type I interferons: plasmacytoid dendritic cells and beyond” J. Exp. Med., Vol. 202, No. 4, p. 461–465.

Beignon, Anne-Sophie., McKenna, Kelli., Skoberne, Moica., Manches, Olivier., DaSilva, Ida., Kavanagh, Daniel G., Larsson, Marie., Gorelick, Robert J., Lifson, Jeffrey D. and Bhardwaj, Nina. (2005) “Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor– viral RNA interactions” J. Clin. Invest. Vol. 115, No. 11, p. 3265-3275.

Fonteneau, Jean-François., Larsson, Marie., Beignon, Anne-Sophie., McKenna, Kelli., Dasilva, Ida., Amara, Ali., Liu, Yong-Jun., Lifson, Jeffrey D., Littman, Dan R. and Bhardwaj, Nina. (2004) “Human Immunodeficiency Virus Type 1 Activates Plasmacytoid Dendritic Cells and Concomitantly Induces the Bystander Maturation of Myeloid Dendritic Cells” Journal of virology, Vol. 78, No. 10, p. 5223-5232.

Google images, “HIV life cycle” URL: http://images.google.se/imgres?imgurl=http://lh4.google.com/budcare/RoCMGdoixLI/AAAAAAAAAAH4/qUVwZz6ZQLQ/s400/HIV%2520life%2520cycle-large.png&imgrefurl=http://hivcare.blogspot.com/2007/07/hiv-budding-from-cell.html&h=257&w=400&sz=79&hl=sv&start=1&um=1&tbnid=LJFBZ7uhddGihM:&tbnh=80&tbnw=124&prev=/images%3Fq%3DHIV%2B%252Blife%2Bcycle%2Blarge%26um%3D1%26hl%3Dsv%26rlz%3D1T4ADBR_enSE240SE270 Access; 2008-05-20

Guo, Zhenhong., Zhang, Minghui., Tang, Hua. And Cao, Xuetao. (2005) “ Fas signal links innate and adaptive immunity by promoting dendritic-cell secretion of CC and CXC chemokines ” Blood, Vol. 106, No. 6, p. 2033-2041.

Janeway, Jr., Charles A., Travers, Paul., Walport, Mark and Shlomchik, Mark J. “Immuno Biology, -the immune system in health and disease, 6th ed.” Garland Science, 2005, New York.

Kwong, Peter D., Wyatt, Richard., Robinson, James., Sweet, Raymond W., Sodroski, Joseph., and Hendrickson, Wayne A. (1998) “Structure of an HIVgp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody” Nature, Vol 393, p. 648-659.

Loré, Karin., Smed-Sörensen, Anna., Vasudevan, Jayanand., Mascola, John R. and Koup, Richard A. (2005) "Myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific $CD4^+$ T cells " JEM, Vol. 201, No. 12, p.2023-2033

MACS®, Magnetic cell sorting instruction manual, order no. 30-090-532, Miltenyi Biotec, Auburn, USA, 2008

McKenna, Kelli., Beignon, Ann-Sophie., Bhardwaj, Nina. (2005) "Plasmacytoid Dendritic Cells: Linking Innate and Adaptive Immunity" Journal of Virology, Vol. 79. No. 1, p. 17-27.

Male, David., Brostoff, Jonathan., Roth, David B., Roitt, Ivan. "Immunology, 7th ed." Elsevier Limited, 2006, UK.

OSU, Oregon State University, Environmental Health Sciences Center, 2008, "Flow cytometry", (1)
URL:http://www.unsolvedmysteries.oregonstate.edu/flow_cytometry_06.shtml, Access; 2008-05-12

OSU, Oregon State University, Environmental Health Sciences Center, 2008, "Flow cytometry", (2)
URL:http://www.unsolvedmysteries.oregonstate.edu/flow_cytometry_07.shtml, Access; 2008-05-12

SMI, Swedish Institute of Infection Disease Control, "HIV infection", 2006-05-10.
URL:http://www.smittskyddsinstitutet.se/sjukdomar/hivinfektion/ Access; 2008-05-13

Steinman, Ralph M. (2003) "Some interfaces of dendritic cell biology" APMIS, Vol. 111 (7-8), p. 675-697.

Stoiber, Heribert., Kacani, L., Speth, C., Würzner, R. and Dierich, M. P. (2001) "The supportive role of complement in HIV pathogenesis" Immunological Reviews, Vol. 180, Issue 1, p. 168-176.

Wagner Edward K. and Hewlett, Martinez J. "Basic Virology, 2nd ed." Blackwell Science Ltd, 2004, Oxford, UK.

Wyma, Donald J., Jiang, Jiyang., Shi, Jiong., Zhou, Jing., Lineberger, Janet E., Miller, Michael D. and Aiken, Cristopher. (2004) "Coupling of Human Immunodeficiency Virus Type 1 Fusion to Virion Maturation: a Novel Role of the gp41 Cytoplasmic Tail" J Virol., Vol. 78, No. 7, p. 3429–3435.