Molecular analysis of the early replication cycle of Human immunodeficiency virus

Ph.D thesis

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Introduction

The deadliest disease attacking the human race for more than 30 years is AIDS (Acquired Immunodeficiency Syndrome). It has caused an estimated 39 million deaths and approximately 3 million new infections are registered every year. HIV (human immunodeficiency virus) is the causing agent of AIDS.

A retrovirus causing cytopathic effect was isolated from samples of patients with chronic lymphadenopathy and patients with immunodeficiency syndrome. This happened in 1983, two years after the symptoms of AIDS were first published. At a conference in 1984 Robert Gallo and Luc Montagnier agreed that HTLV-III, isolated by Dr Gallo, and LAV, isolated by Dr Montagnier, are the same virus and are likely to be the causing agent of AIDS. In 1986 the International Commitee on the Taxonomy of Viruses officialy accepted that the virus causing AIDS is HIV.

With antiretroviral therapy the life expectancy of HIV infected people is almost the same as the ones without the infection. HIV replicates rapidly and it is essential to get more exact information about the always changing properties of the virus. The generation time is approximatly 2.5 days, 10^{10}-10^{12} new virions can form each day. The highest degree of mutability can be found in the hypervariable regions, such as the *env* gene coding the surface proteins. One of the enzymes responsible for replication is the reverse transcriptase. In each of the replication cycles a transcriptional mistake is made at approximately every 2000 basis pair. Antigenic shift or drift can also happen during the replication cycle. Further replication errors can occur with the host cell's RNA polimerase II activity transcribing the proviral sequence. The route of infection, such as vertical, parenteral or sexual transmission, has a significant role in the genetic diversity and the development of antiretroviral resistancy. Epidemiological heterogenity forms by migration, global prostitution and sex-tourism. Countries in which stigmatisation, discrimination, the denial of the presence of drug abuse and prostitution - the parenteral and sexual forms of transmissions - makes the control of transmission difficult.

The mutations developed in the *pol* and *pr* genes can be detected with the help of genotyping assays. The increased number of mutations make the virus resistant against combined antiretroviral therapies. This helps the faster development of AIDS which results in clinical and public health consequences.

Cofactors are needed in addition to the CD4 receptor for HIV to infect a host cell. Isolates propagating in the macrophage/monocyte cell lines are the monocytotrop (M tropic) HIV strains. The coreceptor needed for the M tropic HIV is the CCR5. The CXCR4 coreceptor is significant for the early replication cycle of the T tropic HIV strains infecting T cells. Certain HIV strains can enter the host cell by changing their surface protein structural properties. They can use different coreceptors than CCR5 or CXCR4 such as CCR2, CCR3, CCR8, CCR9, STRL (Bonzo), Gpr 15 (Bob), Gpr 1, APJ or ChemR23.

Studies *in vitro* and *in vivo* of infection with HIV-1 proved cell proliferation effect on T-lymphocytes. Cellular elements, such as the lipid raft located in the cell membrane, are influenced by complex cellular cascade processes. Increased number of stabilizing -SH groups will be present in the protein molecules. Receptors and coreceptors occure in these cell surface lipid rafts. The -SH groups present in the cell surface may have antioxidant activity. Signal transduction mechanism acting on cell growth may also play a role in apoptosis. HIV enters and exits the host cell at the lipid raft.

Polythiolated oligonucleotides used in our studies are influencing the redox processes of the cells. The compounds are interacting with the -SH (thiol) groups on the cell surface and the -SH groups of the gp120 located on the envelop of the HIV.

The effect of the compounds used in our study may inhibit HIV entry into the host cell by interfering with the reducing effect of the PDI and thioredoxin in the CD4 receptors and in the gp120 molecule's -SH groups. Further research and development of the polythiolated oligonucleotides used in our study may result in these compounds becoming an active part of anti-HIV therapeutic agents as an entry inhibitor.

Objectives

The aim of our study was to have a more comprehensive understanding of the steps of HIV infections early replication cycle using molecular analysis. These processes are selectively influencing the entry mechanism of the virus with various tropisms. In our research we are looking for answers to expand our knowledge of the following:

- Will any informative and quantitative results be provided by reproducing HIV infection using lentiviral vectors in inhibition experiments? The experiments will be carried out with the test compounds of antiretroviral activity based on the HIV pseudovirion's co-receptor preference. Examinations of the function of the chemokine receptors are necessary for the productive HIV infection.
- Will the early replication cycle of HIV's Env-mediated fusion change through the testing of new alternative mechanisms? These mechanisms are based on modifications of disulfide bonds on the cell surface, which may influence redox changes.
- Synthetically produced polythiolated oligonucleotides are to be tested for antiretroviral HIV entry inhibitory effect. Human retroviruses have a high mutation rate and easily develop resistance against the current therapies used in enzyme inhibitors (NNRTI, NRTI, PI). Could *entry inhibitors* be the primary therapeutic agents of the future?
- How can the susceptibility to the infection and the antiretroviral therapy effect the functional integrity of the receptors that influence the early replication cycle of HIV and the mutations in the genes encoding them?
- Hungary is located in the center of Europe's migration point. Can circulating subtypes of HIV from certain geographical areas be present in therapy-naïve HIV infected patients in Hungary?

Methods

Cell lines applied

Cell lines expressing CD4 receptors used in our experiments are human H9 and MT-2 T cell lines and HeLaCD4-LTR-β-gal and HEK293T monolayer cell lines. The origin of these cell lines are NIH AIDS Research and Reference Reagent Program (Bethesda, MD). The P4-CCR5 cells are originated from HeLaCD4-LTR-β-gal cells. These transfected cells carry the *lacZ* gene between LTR sequences and are expressing the CCR5 coreceptor on their cell surface. The 3T3.T4.CCR5 and 3T3.T4.CXCR4 mouse fibroblast and P4-CCR5 human fibroblast cells are from Dr Christian Jassoy (Institute of Virology, University of Leipzig, Faculty of Medicine, Leipzig, Germany).

The cell lines were maintained in DMEM culture medium with 10% FCS (fetal calf serum) and 0,1% Penicillin/Streptomycin (Sigma-Aldrich). For the H9 and MT-2 cell lines we used RPMI-1640 culture medium, 10% FCS (fetal calf serum) and 0,1% Penicillin/Streptomycin complemented with 2 mM L-glutamine (Sigma-Aldrich).

HIV-1_{IIIB} virus strain

HIV-1_{IIIB} virus strain (R. Weiss, London University) was propagated in permanent human H9 T-lymphocyte cell line (NIH AIDS Research and Reference Reagent Program, Bethesda, MD). Infection titer was determined by infectivity assays on MT-2 human T-lymphocyte cell line. Supernatants with titers of 2.1×10^5 IU/ml were filtered through a 0.45 μm pore size filter (Merck Millipore).

Production of HIV pseudovirions

The chimera vectors have been created by modifying a pcDNA3.1 (Addgene) plasmid. The HIV surface coding *env* genes originated from clinical isolates or laboratory strains were inserted into the plasmids. The plasmids are carrying pEnvM-ad, CCR5-tropic, pEnvHXB2 CXCR4-tropic and pEnvM-ad/HXB2 dual-tropic surface glycoprotein coding genes.

Plasmids carrying HIV *gag-pol* genes are inserted between LTR sequences. In addition these plasmids are carrying reporter genes and antibiotic resistance marker genes between the LTR sequences. The reporter genes in the plasmids are the luciferase enzyme's gene in the pGJ3-luciferase plasmid, the enhanced green fluorescent protein's gene in pGJ3-eGFP plasmid or the *E. coli lacZ* gene in pGJ3-lacZ plasmid (Origin of the plasmids: Institute of Virology, University of Leipzig, Faculty of Medicine, Leipzig, Germany).

The selection marker gene in the vector constructions is the ampicillin resistance gene which helps to detect the plasmids propagated in DH5α *E. coli* bacterial cells. Isolation of the plasmids was according to the protocol of Qiagen Plasmid Midi Kit (Qiagen).

HEK293T cells were used for transfection with plasmid pairs, plasmids carrying HIV *gag-pol* with reporter genes and plasmids carrying the *env* gene. The assembled virions appeared in the supernatant. Since the additional HIV genes are missing and the *env* gene is not inserted between LTR sequences the pseudovirions are replication incompetent. Using them in our experiment they are able to infect host cells but are not capable of replicating. The method to detect the infectivity was selected on the basis of the reporter genes.

Infecting cells with HIV-1_{IIIB} virus strain or with HIV pseudovirions

Cells were seeded in 24 well plate 5-8 x 10^4 cells/well in 1 ml culture medium in final volume or in 96 well plate 3-5 x 10^4 cells/well in 125 µl culture medium in final volume. The culture medium was removed 24 hours after seeding. Various dilutions (5-40 µl) of HIV-1_{IIIB} stock or HIV pseudovirions were used for infecting the cell lines. The volume of culture medium used on the 24 well plate was 150 µl and on the 96 well plate was 25 µl. The 20 µg/ml DEAE-dextran mediated viral infection's incubation was carried out for 2 hours at 37 °C after the incubation culture medium was added. On the 24 well plate the final volume with culture medium was 1 ml and on the 96 well plate was 125 µl. We detected the results of the infections after 48 hours of additional incubation.

In our experiments polythiolated oligonucleotids were used to test their antiviral activity. We applied the compounds half an hour prior to, at the time of or half an hour after viral infection in various dilutions (0,5-20 μg/ml)

Detecting the antiretroviral effect of the polythiolated oligonucleotids used in our experiments

Cell-virus based biological assays such as Syncytium inhibition and induction assay, Quantitative HIV-1 p24 antigen assay, MAGI assay (multinuclear activation of galactosidase inhibition) and quantitative Luciferase assay were used for quantitative analysis. XTT assay was used to detect the cytotoxicity of the compounds.

Thiolated pirimidin compounds used in our sudies as HIV entry inhibitors

The 4-tio-uridilate (UD29) was created with H_2S modification of citidin 5'-monophosphate. The basic compound was further altered chemically (UD29-new, UD30, UD30-new, UD31, MOD-94 and MOD-2012). With these compounds we analysed their cytotoxocity and their HIV entry inhibition effect. These polythiolated oligonucleotides may inhibit the viral entry by redox modifications in the -SH groups of the molecules. The polythiolated oligonucleotides were provided by Dr. János Aradi (University of Debrecen)

Syncytium inhibition and induction assay

Multinucleated giant cells (or baloon cells) are formed by cell fusions which can lead to cell death. The early replication cycle of HIV and the syncytium formation has a lot in common. The examination of syncytium forming effect of biologically active HIV-1_{IIIB} and HIV pseudovirions were carried out on human H9 and MT-2 T cell lines.

Syncytium inhibition effect of polythiolated oligonucleotides (0,5-40 μg/ml) of various concentrations were tested. The compounds were applied 30 minutes prior to, at the time of or 30 minutes after viral infection of the cells. Uninfected cells, both treated and untreated, were used as control. An inverted microscope was used to detect the results (Motic AE31).

Quantitative HIV-1 p24 antigen assay

The p24 is coded by the HIV *gag* gene. The quantitative HIV-1 p24 antigen assay is a modified ELISA technology. The intensity of fluorescence used for detection is in ratio with the p24 antigen in the samples. The detection is carried out at 450 nanometer. The results are printed out after an automatic evaluation by the machine using a calibration curve from the memory (VIDAS® HIV p24 II (p24), BioMérieux). Presence of the HIV-1_{IIIB} p24 protein was detected from the supernatants of human H9 and MT-2 T cells.

Detecting infectivity with the help of reporter genes

The expression of the reporter genes such as the luciferase, the enhanced green fluorescent protein (eGFP) or the β-galactosidase enzyme can be detected quantitatively. The β-galactosidase enzyme activity was measured with MAGI assay (Multinuclear activation of galactosidase inhibition) on HeLaCD4-LTR-βgal and P4-CCR5 cells. The luciferase activity is detected by measuring fluorescence with luminometer (Victor 3 Multilabel plate reader, Perkin Elmer) on 3T3.T4.CCR5, 3T3.T4.CXCR4 and P4-CCR5 cell lines. The presence of eGFP can be detected with UV light which will show the infected green cells. The detections were carried out 24 and 48 hours after infecton.

Multinuclear activation of galactosidase inhibition (MAGI) assay

Virus titration was carried out on HeLaCD4-LTR-βgal cells following the protocol. The infected cells nucleus was colored blue and then quatitative measurment was carried out with the help of an inverted microscope. The HeLaCD4-LTR-βgal cells are transfected with the coding gene of β-galactosidase. The gene expression is activated by the HIV Tat protein. The reagent, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), will dye the infected cells blue. Inverted microscopy was used for detection. The MAGI assay is a sensitive endpoint titrating method for HIV-1 or HIV-2 isolates. Hill analysis was used to evaluate the infectivity percentage with the

quantitative result of the infected cells.

Polythiolated oligonucleotides (0,5-40 μg/ml) of various concentrations were tested. The compounds were applied 30 minutes prior to, at the time of or 30 minutes after viral infection of the cells. Uninfected cells, both treated and untreated, were used as control. Hill analysis was used to determine IC_{50} and IC_{90}.

Quantitative Luciferase assay

3T3.T4.CCR5, 3T3.T4.CXCR4 and P4-CCR5 cell lines were used. DMEM culture medium with 0,1% Penicillin/Streptomycin and 10% FCS (Sigma-Aldrich) was used. Various concentrations of M-ad, HXB2/M-ad and HXB2 HIV pseudovirions were used for infection (5-10-20 μl). The luciferase assay was carried out following the protocol. Luminometer was used for detection (Victor3, Perkin Elmer). The polythiolated oligonucleotides (0,5-40 μg/ml) of various concentrations were tested. The compounds were applied 30 minutes prior to, at the time of or 30 minutes after viral infection of the cells. Cells without treatment and cells infected with various concentrations of HIV pseudovirus were used as control. Measuring RLU (relative luminescence unit) was according to the protocol.

XTT assay for detecting cytotoxicity

The mitochondrial dehydrogenase activity of the live cells can be detected with the XTT *in vitro* Toxicology Assay Kit (Sigma-Aldrich) using spectrophotometer. The cytotoxic effect of the polythiolated oligonucleotides were tested on human P4-CCR5, HeLaCD4-LTR-βgal monolayer and MT-2 and H9 T cell lines with or without infected with various concentrations of HIV-1_{IIIB} strain, or with M-ad, HXB2/M-ad, HXB2 HIV pseudovirions (5-10-20 μl). Infected and uninfected cells without treatment of polytiolated oligonucleotides were used as control. The absorbancy was measured by spectophotometer using 450 and 690 nanometer. The mithochondrial dehydrogenase of the live cells are reducing the tetrazolium ring of the XTT reagent (2,3-bis/2-Mthoxy-4-nitro5-sulphophenyl/-2H-terazolium-5-carboxyanilide inner salt). Yellow formazan derivate forms as a result. Hill analysis was used to determine IC_{50} and $IC_{90.}$ Further analysis was carried out with the Student probe.

Antiretroviral resistance found in therapy-naïve HIV-1 isolates in Hungary

In our study mutations responsible for therapeutic antiretroviral resistance were detected in the HIV *pol* gene of therapy-naïve HIV infected patients. The samples tested were from Hungarians who got their infection from foreign partners. These partners were from Africa, Asia and other European countries.

Serum samples

Genotyping was carried out on HIV-1 samples of Hungarian patients who acquired their infection from foreign partners. ELISA and other immunohistochemical methods were used (Uniform HIV-1 Ag/Ab, Vidas HIV-1 Ag/Ab, Capillus Trinity HIV1/2) to screen the samples. Confirmation of the results was with Western blot (HIV-1 Diagnostic Biotechnology). Seropositive results were 14.300-1.630.000 HIV copy/ml. Detection happened by Roche Amplicor HIV-1 Monitor Assay. Analysis of the RT and PR coding regions was with Trugene HIV-1 Genotyping Kit and OpenGene DNA Sequencing System (Siemens).

Antiretroviral resistance is caused by the mutations in the open reading sequences coding proteins with therapeutic targets.

Applying Trugene HIV-1 Genotyping Kit

Sequence analysis of the HIV-1 protease (4-99 codons) and the reverse transcriptase (38-248 codon) enzyme coding gene's was carried out by Trugene HIV-1 Genotyping Kit. RNA was extracted from the virus found in the plasma samples (≥ 1000 copy). The cDNA was amplified with reverse transcriptase PCR. Detection of the unpurified CLIP sequence was carried out using 6% polyacrylamide-urea-Tris-borate-EDTA gele electrophoresis (MicroCel™, Long-Reader Tower) for 50 minutes in 2000 V. Detection was carried out with UV light.

Sequence analysis

The OpenGene DNA Sequencing System evaluates the results in real time. The

pattern of the electropherogram results help the evaluation.

Mutations of clinical importance are detected in the protease and reverse transcriptase enzyme coding regions with the help of algorithmic software.

Detection of HIV-1 subtypes

The subtyping of the isolates was completed by comparing the results with the reference sequences in the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu).

Results

Detection of the plasmid vectors

The Qiagen Plasmid Midi Kit protocol was used for isolating the plasmids propagated in competent DHα *E.coli* bacterial cells. BamHI and EcoRI restriction endonucleases were used for digestion. The detection of the plasmids was carried out by gel electrophoresis.

Detection of HIV pseudovirions

Viral sequences integrated into the HEK293T cells helped the expression of plasmid vectors. The HIV pseudovirions form and are collected from the supernatant of the HEK293T cells. Using HIV pseudovirions to infect cell lines the level of infectivity can be quantitatively detected. The integrated and expressed pseudovirus were detected by the luciferase assay using luminometer. The UV microscope was used for detection of eGFP activation.

Figure 1 shows the result of the infectivity of HIV pseudovirions of different tropisms. The result of the luciferase assay was detected 48 hours after infection.

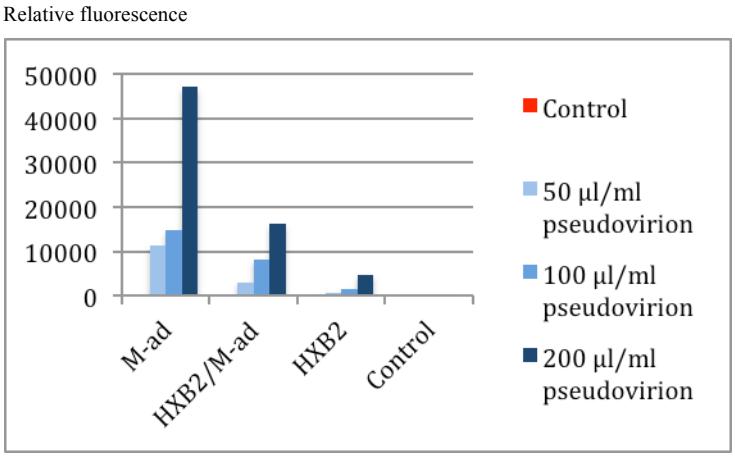


Figure 1. **Results of the Luciferase assay.** P4-CCR5 cells are carrying the CCR5 coreceptor on their surface. The type M-ad, R5-tropic HIV pseudovirions entered the cell line in the highest number.

Integrated and expressed HIV pseudovirions with eGFP reporter gene were quantitativly evaluated with a UV microscope. The infected cells were green.

Antiretroviral effect of Thiolated pirimidin compounds used in our sudies

The best results occurred when the compounds were applied 30 minutes prior to the infection of the cells. The polythiolated oligonucleotides are interfering with the redox events of the cell surface inhibiting the entry of the HIV-1_{IIIB} and the HIV pseudovirions into the host cell.

The results of syncytium inhibition assay and quantitative p24 antigen assay on human MT-2 and H9 T-lymphocytes

Syncytium inducing effect of HIV pseudovirions and biologically active HIV-1_{IIIB} was tested on human H9 and MT-2 T-lymphocyte cell lines. The inhibition of syncytium formation effect was significant when using polythiolated oligonucleotides on human H9 and MT-2 cells 30 minutes prior to the viral infection. Quantitative measurments were carried out from the supernatant of the p24 antigene of HIV-1_{IIIB} infected cells using p24 antigene ELISA (MiniVIDAS®, BioMérieux, France).

The results of Multinuclear activation of galactosidase inhibition (MAGI) assay

In our studies the MAGI assay, a cell-based method using human HeLaCD4-LTR-βgal and P4-CCR5 monolayer cell lines, was applied to detect the antiviral activity of polythiolated oligonucleotides. Hill analysis was used to determine IC_{50} and IC_{90} (Chart 1, Chart 2).

Chart 1. **Antiviral effect of the UD29 polythiolated oligonucleotid**

	IC_{50}[a]	*TC_{50}[b]*	*TI[c]*
Cell fusion	*11.7 µg/ml*	*>200*	*>17*
Viral infection	*4.75 µg/ml*	*>200 >42*	*n/a*

[a]50% HIV infection inhibition
[b]50% viability of the cells
[c]*In vitro* therapeutic index
[d]Result of the MAGI assay

Chart 2. **Antiviral effect of the UD29 polythiolated oligonucleotid from the results of MAGI assay**

UD29 concentration µg/ml	*Inhibitory % -30 min*	*at viral infection*	*+30 min*
1	*2*	*2*	*<1*
2.5	*40*	*19*	*2*
5	*46*	*26*	*5*
10	*81*	*32*	*7*
20	*85*	*39*	*8*
40	*91*	*48*	*9*
IC_{50}	*4.75 µg/ml*	*>40 µg/ml*	*>40 µg/ml*
IC_{90}	*39.7 µg/ml*	*>40 µg/ml*	*>40 µg/ml*

UD29 polythiolated oligonucleotide (1-40 µg/ml) of various concentrations were applied 30 minutes prior to the viral infection of the cells. HIV infected cell titers are represented by the ratio of the blue stained cells (Figure 2.).

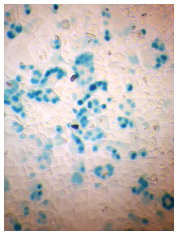
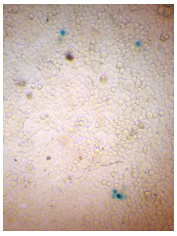
a.)  b.) 

Figure 2. **Multinuclear activation of galactosidase inhibition (MAGI) assay on the HeLaCD4-LTR-βgal monolayer cell line**

a.) HIV-1_{IIIB} infected HeLaCD4-LTR-βgal cells stained blue. b.) The effect of UD29 polythiolated oligonucleotid used in 5 µg/ml concentration 30 minutes prior to HIV-1_{IIIB} infection. Detection was carried out 48 hours after infection. Significant inhibition effect can be seen.

Result of the quantitative Luciferase assay

The luciferase assay is a sensitive and fast chemiluminescence based detection method. The results of our experiments were quantitatively measured using relative fluorescence. Our results confirmed the results of the syncytium inhibition assay, the p24 antigen assay and the MAGI assay. The best results showed at 5 µg/ml. The value was observed in the UD29 and all of its derivates (Figure 3.).

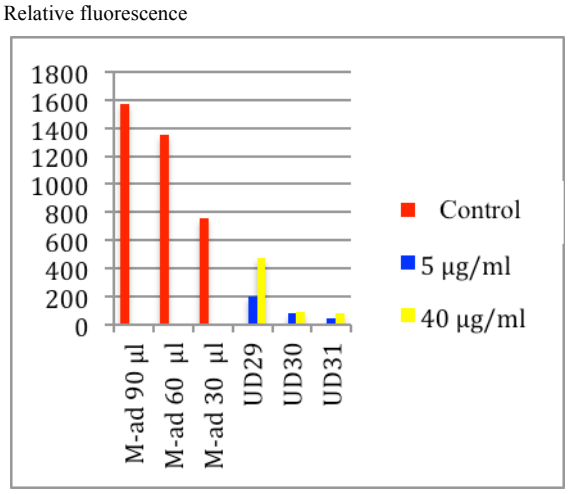


Figure 3. **HIV pseudovirion entry inhibitory effect of the UD29, UD30 and UD31 polythiolated oligonucleotides**

Polythiolated compound: 5 µg/ml, 40 µg/ml; Human cell line: P4-CCR5; Seeded cells: $7x10^4$ cells/well - confluency 80% at infection; HIV pseudovirion: HXB2/M-ad (20 µl is used at infection).

The inhibitory effect of the UD30-new, which is the newest developed UD29 derivate, gave the best inhibition results at the administered value of 1,87 µM/ml.

Result of the XTT assay

The applied 5 µg/ml concentration of the UD29, UD30, UD31 on infected human H9 cells showed some toxic effects. In dose-dependent results mild toxicity was observed after 24 and 48 hour incubation periods. Some derivates, such as UD29-new and MOD2012 showed 30% toxic effects and MOD-94 did not show any toxic effect at

the 24 hour test. The UD30-new compound applied on the infected cells showed higher mithocondrial dehidrogenase activity after 24 and 48 hour incubation periods.

The toxic effects of the polythiolated oligonucleotides were more pronounced on the T cell line than on the HeLaCD4-LTR-βgal and P4-CCR5 monolayer cells. The toxicity values were virus concentration- and dose-dependant.

Detecting antiretroviral resistance found in therapy-naïve HIV-1 isolates in Hungary

HIV CRFs were examined from the samples of Hungarians who acquired the infection from partners of various countries of origin. Line Probe Assay (Inno-LiPA) applying in situ DNA hybridization and the Standford AIDS database with the Trugene HIV-1 Genotyping Kit and OpenGene Sequencing System (Siemens) was used.

Detected HIV-1 subtypes were the following: A, B (25%), C (10.7%), F1 (7.2%), G (3.6%), J and K (3.6%). The dominant recombinants were: CRF02_AG (28.5%), CRF06_cpx (17.8%) and CRF11_cpx (3.6%).

72 codons were examined, 64 of them were resistance codons. Multidrug resistance was also detected in the study. 67.8% was detected between heterosexuals. This was the most frequent route of transmission. 21.4% was the result in MSM population, 7.2% IDU (intravenous drug users) and 3.6% nosocomial infections. 15% of viruses were resistant to antiretroviral treatment. Multidrug resistant strains were also found. 11% of the viruses were resistant to two kinds of antiretroviral agents, while 7% of them were resistant to three kinds of drugs. The HIV CRF detected in one of the patients showed resistance against 14 kinds of antiretroviral agents.

Conclusions

The aim of our research was the molecular analysis of the early replication mechanism of HIV. The most important new findings and results are the following:

- *In vitro* studies of the early replication cycle of HIV could be carried out with the help of lymphocytes and transfected cell line based model methods.
- Replication defective HIV pseudovirions were created using transfected plasmids. The HIV pseudovirions were able to infect cell lines expressing the relevant coreceptors on the surface. The infectivity rate was measured by the reporter genes. The coreceptor characteristics necessary for productive infection could be studied.
- New data was obtained during the molecular analysis of the early replication cycle of human immunodeficiency virus. In our *in vitro* sudies polythiolated oligonucleotides were used to get more details of the thio-redox processes of the cell's surface which have an important role of the primer HIV infection.
- Polythiolated oligonucleotides used in our studies provided the maximum inhibitory effect by applying them 30 minutes prior to viral infection. The effect was significally reduced when administered after the viral infection.
- HIV-1_{IIIB} clinical isolates and HIV pseudovirions entry inhibiton by the polythiolated oligonucleotides were time- and dose-dependent. The virus and compound concentrations and the passage number of the cell lines also influenced the viral entry.
- Their entry inhibitory effect is a result of the redox modifications of the cell surface and the viral envelop proteins.
- The number of cell surface -SH groups are increasing during cell proliferation. *In vivo* and *in vitro* HIV-1 infection is activating the proliferation of the T cells. Viral entry inhibition into the host cell may be carried out by influencing redox processes. The compounds used in our studies are interfering with the disulfide bonds in the CD4 and gp120 molecules inhibiting viral entry.
- During our in vitro study the cytotoxic effect of the applied polythiolated oligonucleotides were observed. The cytotoxicity was more significant on the

uninfected cells compared to the infected. The effect was dose dependent and the viral concentrations influenced the level of toxicity.

- The polythiolated oligonucleotides used in our study are developed and modified in Hungary. We proved their effectivity in HIV antiviral activity. As an entry inhibitor they act at the first steps of the early cycle of viral replication, while other antiretroviral agents act intracellularly. Perspectivly this compound family could be further developed for the purpose of a more effective therapy in AIDS.
- The results we obtained gave us a better understanding of the molecular mechanism of HIV entry.
- Hungary is located in the N-S and E-W migration cross road of Europe. Continuing earlier studies, isolates of therapy-naïve primer HIV infections were genotyped for antiretroviral resistance. HIV isolates obtained from Hungarian patients who acquired their primer infection from partners of other countries of origin were monitored. Appearance of non-B subtypes, CRFs and multiresistant strains in Hungary were confirmed in 71% of the samples. The results indicate that we should be on high alert for clinical and public health consequences.

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