Acta Biochimica Polonica

Vol. 43 No. 1/1996

175–182

QUARTERLY

Dedicated to Professor David Shugar on his 80th birthday

Transfer of antivirals to skin Langerhans cells — A novel approach to anti-HIV treatment by "Antiviral Peplotion"

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Key words: synthetic antivirals, transepidermal transport, HIV-1; AIDS, antiviral peplotion, HLA class I, HLA class II, Langerhans cells, skin keratinocytes

The analysis of the history of the research on antivirals especially the treatment of HIV-1-infected individuals with antivirals which were developed prior to the current AIDS epidemic led to suggest a different approach to the targeting of antivirals in the AIDS patients. Since HIV-1 replication in infected individuals occurs in the lymph nodes, it is suggested that modified anti-HIV-1 drugs should be applied to Langerhans cells in the skin. The Langerhans cells can serve as the carries of the antiviral drugs attached to their surfaces due to their ability to migrate from the skin through the lymph vessels and to home to the lymph node. At that site Langerhans cells interact with T cells. Transfer of the anti-HIV-1 drugs to infected $CD4^+$ T cells in the lymph node will reduce virus replication in the lymph nodes and will reduce the cytotoxic systemic effects of the antiviral drug. Such an antiviral treatment requires the development of efficient methods of drug delivery through the skin.

The 1963 research on messenger RNA (mRNA) in poliovirus-infected HeLa cells with Jim Darnell, Sheldon Penman, Klaus Scherrer and Alex Rich at MIT Department of Biology led to the discovery that poliovirus genomic RNA serves as mRNA in infected cells and to the discovery of polyribosomes as the cellular machinery for the synthesis of proteins [1, 2]. Experiments on vaccinia virus-infected HeLa cells, carried out with Bill (W.K.) Joklik, led to the first experimental demonstration that mRNA is transcribed from the viral DNA and associates with cytoplasmic ribosomes [3]. These studies contributed to the knowledge of trascription of mRNA from DNA which was inferred by Jacob & Monod [4] from experiments done by Volkin & Astrachan [5]. The discovery that mRNA and polyribosomes constitute the protein-synthesizing machinery of all cells and their parasites, together with former discoveries on DNA- and RNA-encoded genes, opened the era of molecular virology. The finding that actinomycin D, an inhibitor of nuclear mRNA synthesis, does not inhibit replication of poliovirus RNA indicated that it is possible to separate molecular processes in the host cells from molecular events in virus replication. These and other studies led to my interest in chemical molecules with antiviral properties.

My conceptual approach to antiviral compounds was stated in my review "Antiviral drugs, mode of action and chemotherapy of viral infections of man" [6] published in 1976:

Abbreviations: CTL, cytolytic T lymphocytes; DC, dendric cells; LC, Langerhans cells; RT, reverse transcriptase; TK, thymidine kinase; AZT, 3'-azido-3'-deoxythymidine; ddA, 2',3'-dideoxyadenosine; ddC, 2',3'-dideoxycytidine; FITC, fluorescein isothiocyanate; HSV, herpes simplex virus.

"Research on mammalian viruses during the past fifteen years made it clear that since viruses are obligate parasites of cells, the development of antiviral agents would depend on utilization of the molecular and biochemical processes which occur during the replication of the virus. Only some of these processes are specified by the viral genome and they closely resemble similar processes and molecules which function in the host cells. Therefore compounds which affect the replication of viruses may also affect the metabolic process in uninfected cells" [6]. Twenty years ago I also wrote "An understanding of the molecular processes which are specified by the virus and which differ from cellular functions is necessary for the development of antiviral drugs which would act on specific targets induced by the virus and which would have absolutely no effect on the host cell". The 1976 review attempted to correlate the molecular events with the mode of virus replication.

In "Antivirals 1980 — An update" [7] I noted that "although developments in the production of viral vaccines during the latter half of the century have eliminated many of the diseases caused by viruses, a few virus pathogens of humans which require antiviral chemotherapy do remain" and "the search for new antivirals effective in humans requires, basically, the understanding of the tissue responses to both the virus infection and the drug used for its treatment. The ideal approach to virus chemotherapy calls for a drug which inhibits a specific virus-coded molecular event, as well as a drug that can inhibit the tissue response to infection, used either separately or in combination as the need arises" [7].

SYNTHETIC ANTIVIRALS — FROM THE SYNTHESIS OF 5-IODO-2'-DEOXYURIDINE TO ACYCLOGUANOSINE

The foundation for synthetic antivirals was laid in 1959 by W.H. Prusoff [8] who noted that replacement of the methyl group in position 5 of thymidine with halogen did not much change van der Waals radii and the phosphorylated derivatives of IdUTP mimic dTTP in incorporation into viral DNA during infection of cells. Hermann [9] reported that the compound inhibited the replication of several DNA-containing viruses in cultured cells, and the study of H.E. Kaufman *et al.* [10] on the clinical cure of herpes simplex keratitis by 5-iodo-2'-deoxyuridine (IUDR) demonstrated the curative effect of IUDR in inhibiting HSV infection in the human eye. These studies demonstrated the usefulness and the potential of a synthetic antiviral based on chemically modified nucleotides, the essential building blocks of viral DNA genome. The pioneering study of W.H. Prusoff [8] opened a new approach to the synthesis of antivirals based on modifications in the four deoxyribonucleosides and the four ribonucleosides. A systematic program of synthesis and testing of purines and pyrimidines, and their analogs, as inhibitors of nucleic acid synthesis has been pursued in G. Elion's laboratory for many years [11]. A modification reported by Schaeffer *et al.* [12] in the sugar moiety resulting in an acyclic side chain by the removal of two carbon atoms led to the synthesis of acycloguanosine (acyclovir) which was proved by G. Elion and collaborators [11] to be extremely active against HSV-1 and HSV-2, surpassing in potency any of the previously known antiherpetic agents.

The discovery of acyclovir revealed a new principle for antivirals since this antiviral compound has the properties of a prodrug which can only be activated (phosphorylated) by the HSV enzyme thymidine kinase (TK) and not by the cellular thymidine kinase. Due to the selective phosphorylation of the prodrug by the viral TK, the advantage of acyclovir is its selective inhibition of HSV DNA synthesis in infected cells with no effect on uninfected cells. On the other hand, TK-deficient HSV mutants are resistant to acyclovir.

In the book on antiviral drugs which I edited in 1984 [13] I noted that "most synthetic and natural antivirals have toxic side effects making it impossible to use them in chemotherapy. However, studies on the group of antivirals activated by the HSV-1 thymidine kinase have great potential for future developments in antiviral chemotherapy".

The anti-herpesvirus group of antivirals with an acyclo sugar molecule set a new milestone on the road to obtaining antivirals lacking toxicity and capable of curing human viral diseases for which effective vaccines are not available. Indeed, much of the enthusiasm for the development of new antiviral drugs was

quenched in the 1970's and part of the 1980's by the realization that vaccination programs using killed or live poliomyelitis and smallpox virus vaccines, respectively, efficiently halted poliomyelitis and smallpox epidemics, and were thus a deterrent to antiviral research. The success in the development of virus vaccines, which prevented virus diseases in children and adults, also suggested that research and development on virus vaccines should have priority over that on antivirals.

This concept, which was the guideline for many years, was challenged by the emergence and spread of HIV-1, leading to AIDS, against which no vaccine has been developed in the fifteen years since HIV-1 made its appearance in the population of homosexual men on the west coast of the U.S.A., as well as in patients from Central Africa. In addition, neither vaccines nor antivirals have become available to cure AIDS and human diseases caused by dengue fever virus and respiratory syncytial virus.

WHY HAVE ANTI-HIV-1 DRUGS BASED ON NOVEL CONCEPTS OF ANTIVIRALS NOT BEEN DEVELOPED IN THE LAST FIFTEEN YEARS?

At the beginning of molecular virology some 40 years ago we assumed that an understanding of virus genes coding for viral structural and nonstructural proteins and an understanding of the function of each of the viral proteins during the virus growth cycle in infected cells would make it possible to identify molecular viral targets for the action natural or synthetic antivirals which would selectively inhibit virus replication with high activity against the virus and with no or minimal effect on uninfected cells in the infected human. While the molecular information on HIV was quickly attained, the antivirals currently used to treat HIV-1 infected individuals are 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyadenosine (ddA), 2',3'-dideoxycytidine (ddC) and phosphonoformic acid, all of which were developed prior to the spread of HIV-1 in the world. Anti-HIV-1 prodrugs have not yet been developed.

In addition, due to the high rate of mutations and nucleotide exchanges during the replication of HIV cDNA by the RNA-dependent DNA polymerase reverse transcriptase (RT) resistance quickly arises in HIV-1-infected individuals. It should be stated that since it was realized that HIV-1 is a virus disease that shortens the life span of each HIV-infected individual, a marked effort to synthesize new anti-HIV-1 antivirals to inhibit protease, RT, as well as, inhibitors of the synthesis of the viral dsDNA, has been made and we hope that new anti-HIV-1 antivirals are underway.

In this presentation I wish to suggest an approach to targeting the available antivirals to the lymph nodes, the tissue site where HIV-1 replication in HIV-1-infected individuals takes place, by directing the antiviral drugs to skin Langerhans cells, which may serve as carriers of the antiviral drugs to the HIV infected lymphocytes. It appears that in order to develop novel antivirals to cure HIV-1 infection, the uniqueness of HIV-1 infection in humans must be taken into account.

THE ENIGMA OF HIV-1

The review of the studies on HIV-1 molecular biology revealed the ability of the virus to use cellular processes to ensure its synthesis and the transport of the viral molecules to the plasma membrane, the site of virion assembly. The unique adaptability of the virus to host immune system cells suggests that the virus evolved to find the optimal conditions for its existence in specific cell types of the human host. The amino-acid sequence in the viral glycoprotein enables binding to the cellular CD4 glycoprotein on the surface of the $CD4^+$T cells, macrophages and dendritic cells, thus ensuring its uptake by the cell and the synthesis of the DNA version of its RNA genome for integration into the host cell DNA genome. This may indicate that the virus evolved to evade the host defense mechanism by either *in vivo* selection of escape mutants or by entering into a latent state in certain cell types with the ability to reactivate at a later time. The intensive research on HIV-1 aims at understanding the behavior of the virus in the infected host and its replicative cycle in infected cells *in vitro* and *in vivo*. This research was carried out with the hope that this knowledge will help in devising immunological and antiviral approaches to prevent the spread of HIV-1 infections in the seronegative population and to cure HIV-1 infected individuals. The attempts to develop therapeutic vac-

cines to stimulate the synthesis of antiviral glycoprotein antibodies in immunized animals serving as models for human immunization have not provided the expected results [14]. Since the development of anti HIV-1 vaccines (as indicated by Haynes [14]) is a national priority in the U.S.A., and since the conceptual approach to the vaccine development is based on the successful development of an inactivated poliovirus vaccine by Dr Jonas Salk, it is of interest that Dr Salk and collaborators indicated [15] that "a prophylactic vaccine against human immunodeficiency virus (HIV) infection represents the best hope for controlling the continuing and devastating worldwide AIDS epidemic". The authors suggested that "a vaccination strategy which will induce a stable Th1 predominant memory state will favor the induction of a strong cytolytic T lymphocytes (CTL) response upon subsequent exposure to live HIV even if such responses are not induced by the primary immunization" [15]. In addition, the authors suggested induction of a "CTL response at the primary immunization by using appropriate adjuvants, synthetic peptides or vectors" [15]. In developing the CTL response to protect against infection with HIV-1 it should be remembered that Phillips *et al.* [16] reported on genetic variation in HIV *gag* CTL epitopes in HIV-seropositive donors with loss of CTL recognition. This is one way by which HIV escapes immune surveillance.

Albert Sabin maintained the view that since the transmission of HIV-1 from person to person is carried out by transmission of infected white blood cells, protective vaccination may be ineffective and will not eliminate the latently-infected cells. Sabin suggested that "the main challenge is to find a way to kill cells with chromosomally integrated HIV cDNA without harming normal cells" [17, 18].

The development of an HIV vaccine which will induce both antiviral antibodies and $CD8^+$ cytotoxic T cells in vaccinated healthy individuals [15] may eliminate both the released virus and HIV-1 infected cells, which present HIV-1 peptides by HLA class I molecules on the cell membrane of infected cells. Such a vaccine will not eliminate HIV-infected cells that do not replicate HIV and do not present viral peptides on HLA class I molecules. However, if such latent HIV-1-infected cells present HLA haplotypes different from those of the host, such infected cells may be eliminated if the foreign infecting cells will generate allograft reaction and will be rejected by the host. Under these conditions the infected cells will not be able to produce virus progeny. It was shown by Stott [19] that HIV virions carry the HLA molecules of the host cell in which the virus replicated and that immunization of monkeys with uninfected human cells produced cytotoxic sera and prevented the infection of the monkeys by SIV replicating in the human cells, suggesting that anti-HLA antibodies may be important in prevention of infection by HIV-1-infected cells. These studies highlight the importance of understanding the role of foreign HLA class I and class II antigens in the protection against HIV. The HLA haplotypes of individuals in a population determine the peptide motifs of HIV-1 peptide presentation by HLA class I molecules to $CD8^+$ cytotoxic T cells. HLA class II haplotypes will determine the properties of the viral peptides presented to $CD4^+$ T helper cells. Combined immunological approaches to eliminate the incoming HIV-infected cells in individuals of a particular population by generating rejection of the HIV-infected cells carrying foreign HLA antigens, as well as generation of $CD8^+$ cytotoxic T cells by synthetic peptides with motifs to fit the HLA class I molecules of the immunized individuals and antigenic viral proteins to induce anti-viral antibodies and memory T cells may provide the initial defense against infection.

SKIN LANGERHANS CELLS AND THEIR PROPERTIES — WILL IT BE POSSIBLE TO DIRECT ANTI-HIV ANTIVIRAL DRUGS BY TRANSEPIDERMAL TRANSPORT?

The human skin is comprised of the epidermis containing the keratinocytes, and the dermis below the stratum granulosum. Additional cell types in the epidermis are Langerhans cells (LC), members of the dendritic cell (DC) system. The LC in the skin are generated in the bone marrow from stem cells defined as dendritic/LC colony-forming units [20]. It was also found that macrophages and dendritic/LC share a common bone marrow stem cell [20]. The release of DC to the blood and their migration to the skin, first to the dermis and then to

the epidermis by chemotaxis, was recently discussed by Knight *et al.* [21] and Bergstresser *et al.* [22]. LC are distributed among the keratinocytes forming a regular (1:32) single layer network above the stratum granulosum. The involvement of LC in the regulation of proliferation of the skin keratinocytes has been suggested [23].

Response of LC to haptens: uptake of haptens and migration to the afferent lymph nodes

Hapten painting of the mouse skin was reported to stimulate LC migration from the skin epidermis through the draining lymph nodes in the dermis to the regional lymph node [24]. Painting of human skin with dinitrochlorobenzene led to an increase in $CD1^+$ cells in the afferent lymph nodes draining the site [21]. Painting of mouse footpad skin with the contact sensitizer fluorescein isothiocyanate (FITC) and preparation of the epidermal sheets for observation under the UV microscope revealed that FITC was present only in association with LC, while the keratinocytes were not stained by this hapten. Shortly after FITC treatment, fluorescein-labeled DC were found in the afferent lymph node cells (Becker, Y., Sprecher, E. & David, D., unpublished). This observation is in agreement with Macatonia *et al.* [25] and Knight *et al.* [21] who studied the effect of a second exposure of mice to FITC one month after an initial exposure. It was found that a second exposure to FITC caused significantly lower numbers of DC carrying the antigen to the lymph node, as well as reduced T-cell proliferation.

The direct route of LC migration from the skin into the dermis was established in an organ culture system. Larsen *et al.* [26] reported that the majority of the migrating leukocytes were Ia^+ (HLA class II^+) LC and the remainder of the cells were comprised of Thy-1^+, $CD3^+$, $CD4^-$, $CD8^-$, γ/δ receptor$^+$ epidermal dendritic T cells that clustered with LC and a small population of macrophages. It was suggested that the maturation of LC commences in the epidermis and continues during LC migration.

Introduction of bioreactive peptides into the skin

Banerjee & Ritschel reported [27] on the influence of pH, peptide concentration, hair shaving and surfactant on the *in vitro* transdermal permeation of vasopressin. They reported that a higher transdermal permeation of vasopressin was noted at pH 5.0 at a concentration of 49.5 mg/ml. Also it was noted that treating the skin by stripping off 25 times with cellophane tape caused a 70-fold increase of transdermal permeation as compared to untreated skin. Cellophane tape stripping off the skin is known to inactivate skin LCs, indicating that the skin LCs are a barrier to transdermal transport of peptide through the skin, which is the purpose of drug delivery through the skin into the skin dermal vascular network. For the purpose of transepidermal delivery of immunoreactive peptides to the skin LC, the method reported by Banerjee & Ritschel [27] may suffice.

Choi *et al.* [28] investigated the effects of the nonionic surfactant, *n*-decylmethyl sulfoxide (NDMS), and pH on the permeation of the pentapeptide enkephalin through hairless mouse skin. It was reported that permeation of the enkephalin peptide (Tyr-Gly-Gly-Phe-Leu) was rapidly stopped by cleavage at the Tyr-Gly bond by a peptidase. At pH 5.0 the metabolic activity was significantly reduced and a substantial flux of Tyr-Gly-Gly-Phe-Leu was observed. The authors concluded that a combination of a skin permeation enhancer, pH adjustment and inhibitors of the proteolytic activity in the skin can increase transdermal delivery of peptides.

Transepidermal transport of HLA class I-related synthetic antiviral peptides targeted to the skin Langerhans cells

Based on the available knowledge on the transport of bioreactive peptides through the skin and the role of LC in their movement from the epidermis to the dermis, I suggested that "HIV-1 peplotion vaccine" may be useful for protection against HIV-1 [29]. It is possible to suggest that under appropriate conditions the antiviral compounds like synthetic peptides and drugs may be directed to the Langerhans cells in the skin. LC, being professional antigen-presenting cells, will carry the antiviral peptides and transport them to the lymph nodes in infected individuals. The "Peplotion antiviral" concept should consider the following parameters:

– 1) The optimal conditions for transepidermal transport of the antiviral peptides and drugs in the human skin need to be developed since the skin is a physical barrier preventing water

loss and withstanding mechanical, chemical and microbial assaults. To perform these functions the entire layer of the skin (the epidermis) undergoes keratinization [30].

-2) The attachment of the antiviral drugs to a carrier molecule that can be directed to the skin Langerhans cells which will serve as drug carriers to the HIV-1-infected lymph nodes and will allow the release of the antiviral drug from the LC or, alternatively, transmit the drugs to HIV-1-infected lymphocytes in the lymph nodes.

-3) Design of the viral nonapeptides which will fit the peptide binding grooves of HLA class I molecules on LCs as inducers of antiviral CTL response to reduce the number of infected CD4+ lymphocytes.

-4) Use of antiviral peptides which bind to HIV-1 receptors on CD4+ T cells and LCs for transfer into lymph nodes. Such peptides (e.g., soluble CD4+) may interact with CD4 molecules on LC and be released when LC reach the infected lymph nodes. The released peptides may interfere with HIV-1 infection of CD4+ T cells in the lymph nodes.

-5) The "Peplotion" approach may also use the ability of LCs to present antiviral peptides to HLA class II molecules to interact with longer antigenic viral peptides which are presented to CD4+ T helper cells and induce specific neutralizing antibodies. Such antiviral antibodies may be useful in immunizing against those virus infections against which antiviral antibodies protect. The concept of inducing skin LCs to prime T cells may be expanded to the use of gut dendritic cells and lung dendritic cells to prime CTLs or CD4+ T cells in the gut or lung, respectively. Such an approach may provide enhancement of the immune responses in different organs of the body and may lead to more successful elimination of virus infections in humans and economically important animals.

It may be that the introduction of anti-HIV-1 antivirals, which are toxic upon intravenous administration, to LCs would have lower toxicity when introduced directly into HIV-1-infected lymph nodes.

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