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Chain Termination Mapping: A Novel Approach Utilizing Ganciclovir (DHPG) to Identify the Origin of Replication of the Human Cytomegalovirus (HCMV).

F. M. Hamzeh, P. S. Lietman, G. S. Hayward, W. Gibson, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

Traditional techniques, such as transient-transfection assays, have failed to identify the origin of HCMV DNA replication. We have shown that DHPG treatment of Towne strain HCMV-infected cells leads to the intranuclear accumulation of short and incomplete HCMV DNA fragments. These DNA fragments are neither packaged nor released into the culture medium. We hypothesized that DHPG leads to the accumulation of subgenomic viral DNA fragments that are close the origin or origins of HCMV DNA replication. Southern-blot and dot-blot analyses of these short viral DNA fragments that are produced in the presence of DHPG, using probes prepared from the slowly sedimenting viral DNA (small size DNA) and from cloned subgenomic viral DNA, shows that these short DNA fragments represent an amplification of sequences from only one small region of the HCMV genome. These amplified sequences map between 0.36 and 0.40 in the middle of the long unique (U_L) segment of the viral genome. These findings strongly suggest that the origin of replication of HCMV is within a 3 kb segment in the BamHI K fragment. This work represents the first identification of an origin of HCMV DNA replication and demonstrates the potential for employing this novel approach, utilizing antiviral agents, to study the molecular mechanisms of DNA replication of complex viruses.

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Targeting of Anti-Viral Drugs to HIV Infected T4-Lymphocytes: Anti-HIV Activity of Neoglycoprotein-AZTMP Conjugates In Vitro.

G. Molema[1], R.W. Jansen[1], R. Pauwels[2], E. de Clercq[2], D.K.F. Meijer[1]

[1] Department of Pharmacology & Therapeutics, University Centre for Pharmacy, Groningen, The Netherlands.
[2] Division of Microbiology, Department of Human Biology, Rega Institute for Medical Research, Leuven, Belgium.

The delivery of the anti-HIV agent AZT, in its 5'-monophosphate form, (in)to human T-lymphocyte MT4-cells in vitro through covalent coupling to neoglycoproteins (ngp's) was investigated. The rationale for the design of the ngp carriers is based on the existence of sugar recognizing lectins on T-lymphocytes. Using a phenyl-linkage between sugar and Human Serum Albumin (HSA), various mannose-, fucose-, galactose- and glucose-containing ngp's were synthesized. The intrinsic anti-viral activity was tested in vitro in HIV-1 and HIV-2 infected MT4-cells. Only $Man_{40}HSA$ shows pronounced anti-HIV-1 activity itself. After conjugation with AZTMP, the mannose- as well as the fucose- and galactose-containing conjugates exhibited a pronounced activity. Conjugates of glucose-HSA and HSA displayed ten times less activity in spite of the fact that drug loading was considerably higher compared with the other conjugates. In the series of mannose-ngp's, the $Man_{23}HSA$-AZTMP conjugate was shown to be more than five times as active compared to AZTMP itself.