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Flow Cytometry as an Aid in Antiviral Research

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Infection of the cells by Epstein-Barr virus (EBV), cytomegalovirus (CMV) or human immunodeficiency virus (HIV), can be readily monitored by using polyclonal or monoclonal antibodies (mAbs) against specific viral antigens, followed by flow cytometry. This methodology is particularly valuable when the virus-infected cells do not show cytopathic changes. Using flow cytometry one can easily establish whether a given cell line or type is susceptible to virus infection. Flow cytometry also allows an accurate determination of the percentage of virus-infected cells, as well as the time of appearance of the viral antigens. This, in turn, is helpful in deciphering at which stage the viral replicative cycle may be blocked by antiviral agents. Using specific staining techniques for cell surface markers and cellular DNA, followed by flow cytometry, we have demonstrated that syncytium formation between HIV-infected and uninfected human T-cell lines leads to a selective destruction of the uninfected T-cells. This process can be prevented by polyanionic compounds such as sulfated polymers and aurintricarboxylic acid. These compounds inhibit HIV adsorption to $CD4^+$ T-cells. They interfere with CD4 expression (aurintricarboxylic acid) or gp120 expression (sulfated polymers). Thus, flow cytometry-based procedures have proven very helpful in elucidating the mode of action of antiviral compounds.

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A Sensitive Method for the Determination of Anti-Viral Agents and Drug Content in Drug-Neoglycoprotein Conjugates.

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Rapid and sensitive HPLC analyses for the determination of the anti-viral agents AZT and its monophosphate derivative AZTMP, PMEA and araAMP are developed. The analyses are relatively easy to perform: they require a simple potassium phosphate buffer supplemented with tetrabutylammoniumsulphate. Combination of this eluent with a μ-Bondapak C18 reversed phase column results in a separation based on ionpair formation. Detection limits of the compounds AZT, AZTMP, PMEA and araAMP are 1.0, 10.0, 5.0 and 2.0 ng/ml, retention times 12, 15, 6 and 7 min, respectively. In order to investigate whether neoglycoproteins (ngp's) can potentially act as carriers for targeting of the drugs to certain cell types in the body, various ngp's were synthesized. Conjugation of the anti-viral drugs to the ngp's was performed using ECDI mediated coupling. Analysis of the amount of drug bound to the protein usually is carried out by spectrophotometric determination. Because of substantial influence of ECDI, reacted with the protein, on the spectral characteristics of the product, we developed an acid hydrolysis/HPLC assay, based on HPLC analysis after acid hydrolysis of covalently bound nucleoside.