Poster Session II

Molecular and Biochemical Approaches to Antiviral Targets

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Soluble T4 (sT4) Mediates Release of gp120 from HIV: Quantitative Electron Microscopy and Biochemical Analysis. Timothy K. Hart[1], Richard Kirsh[2], Dennis M. Lambert[3], Jeff Leary[3], and Peter J. Bugelski[1]. [1]Experimental Pathology, [2]Drug Delivery, and [3]Anti-Infectives, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101, USA.

The human immunodeficiency virus (HIV) is the causative agent for the Acquired Immunodeficiency Syndrome (AIDS). Viral binding and infection is mediated by the interaction of the viral envelope glycoprotein gp120 to the leukocyte surface receptor CD4. A soluble, recombinant form of CD4 (sT4) is being investigated for its ability to block this interaction. This report examines the effect of sT4 on HIV-IIIB infected H9 and CEM cells by quantitative immuno- and cytochemical electron microscopy and biochemical analysis. gp120 was localized as spikes on virions by tannic acid cytochemistry, anti-gp120 immunogold staining and lectin cytochemistry (Con A-HRP-gold). gp41, the transmembrane fusogenic protein, was localized by anti-gp41 immunoperoxidase and immunoferritin cytochemistry. Morphometric analysis of gp120 spikes, immunogold and lectin-gold staining revealed a normal distribution of gp120 on mature virions. Treatment with sT4 caused a significant (25-35 %) decrease of gp120 detection on virions that was dose and time dependant. In addition, anti-gp120 Western Blot analysis of supernatants from treated cells demonstrated a concomitant 300 % increase in free gp120 at 10 µg/ml sT4. Furthermore, the sT4 treatment of HIV infected cells revealed an increase in a gp41 epitope (detectable by a monoclonal antibody) on the surface of virions and cells which was not present at detectable levels on untreated cells. These data suggest that sT4 (CD4) binds to gp120 and potentiates its release from the gp120-gp41 complex on the virion and cell membrane. sT4 mediated gp120 release occurs over the same concentration range as in vitro syncytial inhibition, with maximal gp120 release and syncytial inhibition occuring at 10 µg/ml sT4. The greater increase in free gp120 observed in the media in comparison to the decrease in spike density supports the hypothesis that HIV spikes are multimeric, probably containing 3-4 subunits.