VOLUME 2 ABSTRACTS

FINAL PROGRAM AND ABSTRACTS

FRIDAY 22 JUNE 1990

SIXTH INTERNATIONAL CONFERENCE ON AIDS

SAN FRANCISCO CALIFORNIA, USA

TRACK A: BASIC SCIENCE
POSTER SESSION

F.A.336 AZT-RESISTANT HIV DETECTION BY RNA-RNA HYBRIDIZATION

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Objective: To develop a sensitive and specific in-vitro assay which reliably detects HIV strains resistant to antiviral agents.
Methods: PHA-stimulated PBMC's and MT-2 cells were acutely infected with viral supernatants from: 1) wild-type laboratory HIV strain, H9/HTLV-IIIB, 2) HIV co-culture with PBMC's from an HIV-infected individual (EP113089) who had been taking AZT for greater than one year and had clinical deterioration. After a two hour acute infection, cells were washed twice and grown in culture for three days (MT-2) or seven days (PBMC) in the presence of increasing concentrations of AZT or ddI. At harvesting, cells were counted and standardized, RNA was solubilized in guanidine thiocyanate and placed in -20C for storage. RNA from 200,000 cells was subjected to RNA-RNA hybridization (Gillespie et al. Molecular and Cellular Probes 1989: 3;73-86). Beads were placed on a nitrocellulose membrane and subjected to autoradiography at -70C for twenty-four hours. Autoradiographs were quantified by densitometry.
Results:

	ID_{50} (uM AZT)		ID_{50} (uM ddI)		
	PBMC	MT-2	PBMC	MT-2	
H9/HTLV-IIIB	0.01	0.05	2.2	20.0	
EP113089	0.30	p	11.0	p	p=pending

Conclusions: 1) Compared to wild type H9/HIV IIIB, patient HIV strain EP113089 appears to be thirty-fold resistant to AZT and cross resistant by five-fold to ddI. 2) Laboratory evidence of HIV resistance may correlate with disease progression. 3) Our results with RNA-RNA hybridization compare favorably with data reported by Larder et al. (Science 1989: 243:1731). 4) MT-2 cell line has the advantages of eliminating the need for PBMC's and requiring a shorter incubation to produce quantifiable HIV RNA.

F.A.337 QUANTITATION OF HIV-1 RNA IN THE SERUM OF ARC AND AIDS PATIENTS USING THE POLYMERASE CHAIN REACTION(PCR)

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Objective: The amount of infectious HIV-1 present in serum may be a potential marker in HIV related disease. A method that detects and quantifies HIV-1 viral RNA in serum is presented.
Methods: To detect HIV-1 RNA, sera was extracted by a guanadinium thiocyanate method, reverse transcribed with MLV reverse transcriptase and amplified by the polymerase chain reaction using a gag gene primer pair(SK38/39) including a biotin labelled upstream primer. The biotinylated PCR product was liquid hybridized to a horseradish peroxidase conjugated probe, bound to avidin, and quantitated from the optical density of a colorimetric reaction.
Results: Reverse transcription and amplification of known amounts of gag gene RNA and infectious HIV_{mn} virus RNA alone yielded a log-linear relationship between optical density and 10^3 and 10^8 copies of gag RNA and 10^1 and 10^5 $TCID_{50}$ of virus respectively. In HIV infected patients who were not receiving therapy, serum HIV-1 RNA was detected in 0/5 asymptomatic, 4/5 ARC and 4/5 AIDS patients with copy numbers ranging from 10^3-10^5/200ul of serum. In a small number of AIDS patient sera, co-cultivation with donor lymphocytes demonstrated a correlation between infectious titer and copy number by PCR. Ultracentrifugation of patient sera revealed detectable signal in pellets, but not supernatant. Similarly, signal from sera could be adsorbed to rCD4 bound to sepharose beads, indicating that signal is attributable to viral RNA in intact viral particles. In addition, extracted material was directly amplified for the presence of viral DNA and gave no detectable signal.
Conclusions: We have demonstrated that HIV-1 viral RNA can be detected and quantitated in patient serum over a four log range by a nonisotopic enzyme-linked affinity assay. The RNA detected in patient sera is from infectious viral particles as evidenced by CD4 binding, sedimentation and culture. Quantitation of HIV-1 viral RNA in serum by PCR may be a useful marker for disease progression or monitoring antiviral therapy.

F.A.338 SENSITIVE DETECTION OF HIV DNA IN T4 LYMPHOCYTES OF INFECTED INDIVIDUALS BY POLYMERASE CHAIN REACTION

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Objective: To determine the percentage of T4 lymphocytes containing HIV DNA in infected individuals.
Methods: Peripheral blood lymphocytes (PBL) isolated by Ficoll-Hypaque gradient centrifugation were sorted into T4 cells by fluorescence-activated cell sorting (FACS) with Mab anti-Leu-3A. Crude cell lysates of 10^5, 10^3, and 10^1 T4 cells and of HIV-infected cell lines (ACH2, 8E5) were subjected to standard and booster polymerase chain reaction (PCR) with SK38 and SK39 primers. The specific HIV sequences amplified were analyzed by polyacrylamide gel electrophoresis after hybridization with ^{32}P-end-labeled SK19 probe.
Results: With standard PCR (35 cycles) using 0.5uM primers, HIV DNA was detected in 10 ACH2 or 8E5 cells and in 10^3-10^4 T cells. Sensitivity was increased 10-100 fold by booster PCR (15 cycles with 10nM primers) preceding the standard PCR. With the booster PCR, HIV DNA could be detected in as low as 1 ACH2 (or 8E5) cell and in 10 T4 lymphocytes of patients at different stages of disease progression.
Conclusion: 1) The amount of proviral DNA contained in certain T4 cell populations may be quantitated by standard PCR with control cell lines containing 1 copy of viral genome per cell (such as 8E5 and ACH2). 2) The detection of HIV DNA in 10 T4 cells with booster PCR, suggests that at least 10% of circulating T4 cells were infected with HIV in some infected individuals at all stages of HIV-related diseases. 3) These data

F.A.339 COMPARISON OF MARKERS FOR EARLY DETECTION OF HIV-1 REPLICATION IN T-CELL CULTURES.

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Objective: To compare the relative sensitivity and range of signal strength of HIV-1 markers used to detect early virus replication in T-cell cultures.
Methods: H9 cells were exposed to cell-free $HTLV\text{-}III_B$ ($5x10^3$ TCIU/$4x10^6$ cells) for 1h, washed, and cultured for 19d. H9 cells and culture fluids were cryopreserved at various times after infection. Samples were tested for infectivity and TAT expression in a 3 day culture assay using an HIV-1 LTR-CAT transfected T-cell line (H938, Science 219:184, 1988). DNA from lysed cells was analyzed by PCR for HIV-1 gag and env sequences. Supernatants and cell lysates were tested for p24 antigen expression by capture immunoassay (Coulter) and reverse transcriptase (RT) activity by a rapid microassay.
Results: Data showed that from 2h through 24h, HIV-1 infection of H9 cells could only be detected by PCR for gag sequences. All markers significantly increased 2-5d after infection and followed nearly parallel changes throughout the study, except that infectivity was not detected in supernatants by 3 day assay. Marker signals increased 2-500-fold over uninfected controls.
Conclusions: Among the markers studied, HIV infected H9 cells were detected first by DNA PCR. However, after 24h, virus replication was signalled comparably by DNA PCR, antigen expression, RT