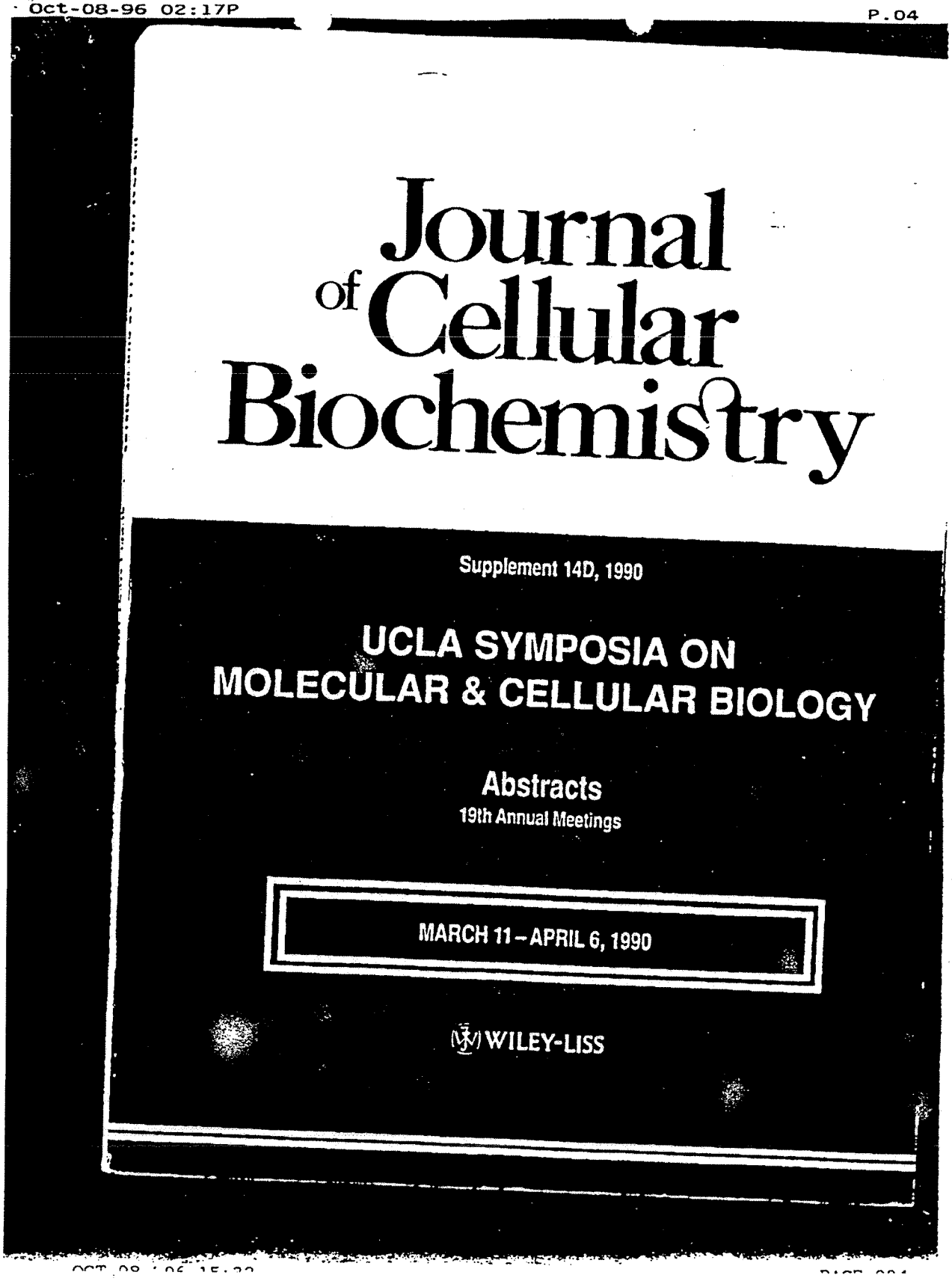

HIV and AIDS: Pathogenesis, Therapy and Vaccine

L 516 HIV-1 ENTRY INTO GLIAL CELLS IS NOT MEDIATED BY CD4 BUT IS EFFICIENT
J.M. Harouse, M. A. Laughlin, B. Godfrey, H. Friedman, F. Gonzalez-Scarano

A number of CD4-negative glial cell lines can be infected with HIV-1. This infection cannot be blocked with anti-CD4 monoclonals like Leu3a or OKT4a, nor by the use of soluble CD4 preparations and it is normally only detectable by co-cultivation of the infected glial culture with highly susceptible CD4 positive cells. It has not been determined whether glial infection proceeds via a specific alternate receptor or by non-specific viral fusion at the plasma membrane. To characterize glial infection (U373) we have taken two approaches: (1) we transfected a glial cell line with a CD4 construct and determined the characteristics of HIV-1 infection in it and (2) we used a viral internalization assay based on the intracellular detection of $p24^{gag}$ after a short period of incubation of virus and cells. The results indicate that glial cells produce low levels of virus after infection even when they express a functional CD4 molecule. This would suggest that latency is determined by factors other than the efficiency of entry. Furthermore, the results of internalization assays indicate that there is significant viral uptake into intracellular compartments of glial cells after 30 min exposure at 36°C. Preliminary results indicate that this viral entry is mediated by a protease-sensitive molecule at the cell surface.

L 517 A PUTATIVE HIV TM RECEPTOR ON THE CELL SURFACE IS IDENTIFIED THROUGH THE USE OF A SYNTHETIC PEPTIDE. Lee A. Henderson, Nasar M. Qureshi, David H. Coy and Robert F. Garry, Departments of Pathology, Medicine and Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112.

A specific TM sequence, denoted CS3, inhibits T cell activation in vitro and antibody specific to CS3 is associated with the absence of AIDS related disease in HIV seropositive patients. CS3, when conjugated to human serum albumin (HSA) and labelled with fluorescein, bound specifically to $CD4^+$ cell lines and human T cells, B cells and mononuclear cells. Crosslinking of CS3-HSA to its receptor on RH9 cells revealed a putative subunit size of approximately 44 Kd. Incubation of RH9 cells, a CD4 cell line, with CS3-HSA prior to addition of HIV prevented HIV mediated cell lysis. These results suggest that the interaction of the CS3 region of HIV TM with a specific cell surface receptor may be required for HIV mediated cell lysis. The biological response to CS3 was also investigated to extended prior observations. Incubation of PBMC with CS3-HSA for 24-72 hrs prior to activation with mitogen (PHA) resulted in a progressive decline in the ability of mitogen to stimulate incorporation of ^{3}H-thymidine. Furthermore, even at low doses of CS3 (10 ng/ml), CS3-HSA initially enhanced anti-CD3 induced intracellular calcium mobilization and ^{3}H-thymidine incorporation, but the peak response of proliferation was significantly reduced. The biological significance of interaction of HIV TM with its receptor portends several avenues of approach for therapeutic treatment and vaccine development.

L 518 QUANTITATION OF HIV-1 RNA IN SERUM AND CORRELATION WITH DISEASE STATUS USING THE POLYMERASE CHAIN REACTION. Mark Holodniy, David A. Katzenstein, Sohini Sengupta, Alice Wang*, Clayton Casipit*, David H. Schwartz, Mike Konrad*, Eric Groves* and Thomas C. Merigan, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, CA 94305, *Cetus Corporation, Emeryville, CA 94608.

The amount of 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. 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.

Reverse transcription and amplification of known amounts of gag gene RNA and infectious HIV_{IIIB} virus yielded a log-linear relationship between optical density and 10^4 and 10^9 copies of gag RNA and $TCID_{50}$ of virus respectively. No HIV viral RNA was detected in the serum of 5 seronegative healthy controls. 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. Ultracentrifugation of patient sera revealed detectable signal in pellets,but not supernatant, indicating that signal is attributable to viral RNA. In addition, extracted material was directly amplified for the presence of viral DNA and gave no detectable signal.

We have demonstrated that HIV-1 viral RNA can be detected and quantitated in patient serum over a four log range. An RNA gag gene sequence was used to quantitate viral copy number. In addition, a nonisotopic enzyme-linked affinity assay in a microtiter plate system allows easy PCR product detection and quantitation. Quantitation of HIV-1 viral RNA in serum by PCR may be a useful marker for disease progression or monitoring antiviral therapy.