SIXTH INT'L. AIDS CONFERENCE

HIV DISCOVERER IN MYCOPLASMA CONTROVERSY

SAN FRANCISCO—Luc Montagnier, the French discover of human immunodeficiency virus (HIV), is working hard to substantiate his bold and controversial statements made at the Sixth International AIDS conference held here in June. Montagnier relayed his lab's isolation of mycoplasma from the blood of patients with acquired immunodeficiency syndrome (AIDS) and conjectured about mycoplasma's role as a necessary cofactor for an HIV-infected patient's progression to full-blown AIDS. Such a position deviates from his prior held view—and from that of most of the AIDS research establishment—which alleges that HIV alone causes the pathogenesis seen in AIDS patients.

That mycoplasma, which are bacteria-like intracellular parasites without cell walls, could play a role in HIV's *in vitro* T-cell cytopathic effect was published by Montagnier last March in *Research in Virology* (**141**:5, 1990). He and his team showed that tetracycline and some of its derivatives inhibited the killing of single cultured CEM cells and syncytia formation normally seen when these cells are infected with lab strains of HIV-1 and HIV-2. (CEM is a T-lymphoblastoid tumor cell line.) Virus production in the antibiotic-treated cells, however, continued unheeded, "indicating a dissociation between protection against cell death and suppression of virus growth."

The hoopla at the conference was that Montagnier proposed that the enhanced cytopathic effect of HIV by mycoplasma seen *in vitro* may also be seen *in vivo*. Since there is evidence that more cytopathic HIV strains can be isolated from AIDS patients than from asymptomatic carriers, he argued that mycoplasma may play a role in this strain transformation. To show that mycoplasma could exert such an effect *in vivo*, he had to show that the microorganism wasn't only present in HIV-infected cultured cell lines in the laboratory, but that it was also present in the blood of AIDS patients.

Out of 97 AIDS patients, Montagnier reported he found 37 that were positive for mycoplasma by a technique that measures freshly isolated red blood cells' uptake of tritiated uracil, and then subjects the cells to sucrose density gradient centrifugation. Only a prokaryotic organism in association with the cells would take up the label. Upon centrifugation, mycoplasma migrate to a specific density. He also showed that 10 of 54 AIDS patients' lymphocytes were positive for mycoplasma using the labeling method. Of the 37 associated with the red blood cells, 16 were also positive by dot blots. "Actually these figures are now outdated," Montagnier said after the conference, "and we have now higher scores by this technique [dot blot hybridization]."

While critics like Jay Levy of the University of California at San Francisco said that Montagnier's mycoplasma may be just a contaminant, other mycoplasmologists like Joel Baseman of the University of Texas Health Sciences Center (San Antonio) agree that Montagnier used standard microbiological techniques to isolate these strains from blood. "He also isolated *M. genitalium* from blood. It was never done before so it couldn't be a contaminant," he said.

The mechanism of action of the presumed enhanced cytopathic effect by mycoplasma is still unknown. It is also not the only means for HIV to become more cytopathic. "We know that some viral strains in which the *nef* genes are deleted also can replicate faster and give rise to a high cytopathic effect without the presence of mycoplasma," said Montagnier. It is also possible that mycoplasma exert their action on HIV by stimulating lymphoid cells to produce cytokines which are known to enhance viral replication. **—Robin Eisner**

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SOURCE OF AIDS INFECTIVITY STILL A PUZZLE

SAN FRANCISCO—Reports at this year's international conference on AIDS (acquired immunodeficiency syndrome), held here in late June, continued to underscore researchers' attempts to explain, in molecular terms, the pathogenic potential of human immunodeficiency virus (HIV).

One major concept about HIV which has changed is that only CD4-bearing cells can be infected by the virus, the T-4 helper lymphocyte being the prime target for infection. For example, Yasuhiro Takeuchi and colleagues from the Gumma University School of Medicine (Maebashi, Japan) identified an HIV-1 variant highly infectious for fibroblast-like cells (BT cells) from human brain tissue. They produced the variant strain by infecting BT cells with an HIV-1 isolate normally infectious for CD4-bearing cells but not for BT cells: Chimeric recombinant viruses were produced in efforts to elucidate the genetic elements responsible for the change in host-range. A single nucleotide substitution in the coding region for envelope protein at position 931 from a cytosine to a thymine was apparently sufficient to change the virus' tropism. This region defines the type-specific neutralization epitope of the *env* gene.

Similar studies from William O'Brien and colleagues at the University of California (San Francisco) and Z-Q Lui et al. at the University of Kansas (Lawrence) also reported that changes in the *env* protein accounted for a shift in viral tropism. O'Brien's group generated recombinant HIV-1 strains by substituting gene regions from a virus highly infectious for macrophages (HIV-1 JR-FL), originally isolated from an AIDS patient with encephalopathy, into a strain with poor macrophage infectivity (HIV-1 NL 4-3). Peripheral blood mononuclear phagocytes infected with the NL 4-3, which had received genes encoding about 157 amino acids of the highly infectious JR-FL strain envelope protein, produced 100 times more viral DNA than control cells infected with the original, relatively noninfectious, NL 4-3 strain. According to O'Brien, further analysis of the transfected gp120 region accounting for the macrophage tropism did not involve the putative CD4 binding site, the z3 loop region, or the second conserved domain.

Liu's group wished to identify genes accounting for the neural tropism of spinal cord lesion HIV-1 isolates. This HIV-1 isolate (termed 128-A) could not be grown in any T cell lines tested or in a monocytic cell line, but readily propagated in primary macrophage culture. Using the strategy of generating recombinant viruses containing 128-A genes and SF-2 strain genes and examining their infectivity, these investigators showed that only recombinant virus which acquired the 128-A *env* gene had tropism characteristic of the 128-A strain—no other viral genes were required. When 128-A and SF-2 *env* genes were isolated and sequenced they were 93 percent homologous; whatever variation did exist occurred mainly in the hypervariable gp120 region.

One possible reason for the hematopoietic suppression observed in