NEUROTOXICITY OF MACROPHAGES INFECTED BY HIV1

MARC TARDIEU, CHRISTIANE HERY, and SYLVIANE PEUDENIER
Laboratoire de Neurovirologie et Neuroimmunologie,
Université Paris XI UFR Kremlin Bicêtre

INTRODUCTION

Central nervous system (CNS) lesions are frequently observed during Human Immunodeficiency Virus (HIV) infection. Clinical manifestations are highly variable ranging from no symptom to psycho-motor slowing and to severe cognitive and motor disturbances. The variation in intensities of clinical symptoms is particularly true after materno-fetal transmission of the virus. Thus, the vast majority of infected children have biological evidence of infection of their central nervous system but only 20% of the patients have very severe encephalopathy and absence of brain growth (Blanche et al., 1991). Other children have a normal initial development and brain growth, although some of them develop subsequently a mental retardation.

The pathogenicity of HIV1-induced encephalopathy is still unknown. Recent papers have described a loss of neurons in the brains of adult HIV1-infected patients (Ketzler et al., 1990; Everall et al., 1991). Such a neuronal destruction had been previously described in the brains of very severely affected children (Epstein et al., 1988). On the other hand, viral antigens are frequently found within the brain of patients who died of AIDS, but have a restricted distribution. Signs of viral replication can be observed only in macrophages, whereas astrocytes and neurons harbor very little if any, viral information, as judged by immuno-cytochemistry and in situ hybridization (Wiley et al., 1986; Vazeux et al., 1987; Price et al., 1988). This selective tropism of HIV1 for brain macrophages has been confirmed in vitro (Watkins et al., 1990). Recently, we observed that the infected brain macrophages are not mature microglial cells but either monocytes which could cross the blood-brain barrier or specialized perivascular microglial cells able to phagocytose infected monocytes or even free virus (Peudenier et al., 1991).

The mechanism of neuronal loss during HIV infection is unclear and several hypothesis could be made which will be discussed successively.

COULD A LOW GRADE INFECTION OF NEURONS OR ASTROCYTES BE RESPONSIBLE FOR CELL DEATH?

Because a low grade and persistent viral infection is very difficult to rule out by techniques utilizing post mortem brain sections, several studies have directly tested the susceptibility of cultured neurons and astrocytes to HIV1. Their results remain controversial (Cheng-Mayer et al., 1987; Chiodi et al., 1987; Christofinis et al., 1987;
Dewhurst et al., 1987; Wigdahl et al., 1987; Dewhurst et al., 1988; Harouse et al., 1989; Kunsch et al., 1989; Chesebro et al., 1990). A cytopathic effect was rarely observed and a fully productive persistent infection with HIV1 was difficult to establish, except for some experimental systems using either very high titers of virus or transfection to introduce HIV1 into astrocytic cells. Most studies, moreover, used continuous cell lines of either glial or neuronal origin and did not precisely define the tested cells by antigenic markers. In a recent work, we observed that human neurons and astrocytes in primary cultures from cortex or spinal cord were resistant to HIV1 infection, an observation to be related to the absence of CD4 antigen on their surface and of mRNA CD4 in their cytoplasm (Peudenier et al., 1991; Tardieu et al., 1992). It remains possible that specific clones of HIV1 using an entry mechanism independent of the CD4 antigen, a mode of internalization already suggested, replicated better in neurons and astrocytes than the clones we have tested.

Neurons and astrocytes do not appear to be a frequent target for HIV infection and a persistent low grade infection of these cells do not appear to be the usual mechanism of neural lesions. CNS cells lesion could then depend on the infection of adjacent macrophages which acted either through the secretion of soluble factors active on distant neurons and astrocytes, or directly after adhesion to neurons and astrocytes.

**COULD SOLUBLE FACTORS SECRETED BY HIV1 INFECTED MACROPHAGES INDUCE CNS CELLS LESIONS?**

A role for different soluble factors has been proposed (Brenneman et al., 1988; Dreyer et al., 1990; Kaiser et al., 1990; Giulian et al., 1990; Lipton et al., 1991; Sabatier et al., 1991). Two viral peptides, gp120 and tat, modify the survival of rodent neurons or astrocytes, the former protein acting on calcium channels and the latter on membrane polarization. Another still undefined soluble factor, secreted by infected mononuclear phagocytes (from the permanent U937 cell line), alters the survival of chick and rat neurons (Giulian et al., 1990). This factor differs from viral peptides, cytokines or free radicals and acts by way of N-methyl-D-aspartate receptors and not of calcium channels or membrane polarization.

Since we had the opportunity to test directly human embryonic neurons and astrocytes in primary cultures instead of rodent neural cells, we initially used a similar experimental approach (Tardieu et al., 1992). Supernatants of HIV1-infected U937 cells were harvested at day 4 post infection and transferred on human neural cell cultures on their 10th day post-plating. During five different experiments, cells were observed for up to 21 days, and individual cultures were fixed at different times and stained with anti-neurofilament antibodies. No morphological alteration of neurons and astrocytes was induced by the tested supernatants. During two subsequent experiments, HIV1-infected U937 cells were cultured in a double chamber system with a 0.4 μm porous membrane separating them from the neural cell culture. Here again, no astrocytic or neuronal lesion was observed. Finally, HIV1-infected lymphocytes, which actively replicated the virus and secreted viral products, were co-cultured with neural cells and no cytopathic effect was induced.