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Infection of choroid plexus cells by human T cell leukaemia virus type I

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Very little is known about the factors that determine the outcome of infection by human T cell leukaemia virus type I (HTLV-I) and the neurotropism of this virus is still a controversial point. In transgenic mice, the HTLV-I LTR is active mainly in the central nervous system (CNS), in parenchyma as well as in ependymal and choroid plexus cells. The latter are of particular interest and could represent the way of entry of the virus into the CNS. In this study we show that primary cultures of sheep choroid plexus can be infected with HTLV-I, leading to characteristic multinucleated syncytial cells containing virus RNA and proteins. HTLV-I p24 Gag protein was detected in the culture medium and the presence of virus particles was observed by electron microscopy 40 days after infection. At this time post-infection HTLV-I could be transmitted to human cord blood cells.

Human T cell leukaemia virus type I (HTLV-I) causes a variety of diseases, including adult T cell leukaemia/lymphoma and a non-neoplastic inflammatory neurological syndrome called either tropical spastic paraparesis or HTLV-I-associated myelopathy (TSP/HAM) (Gessain *et al.*, 1985; Osame *et al.*, 1986; Poiesz *et al.*, 1980; Yoshida *et al.*, 1982). Virus tropism is a central point in studies of the pathogenesis of HTLV-I-associated diseases. In the peripheral blood, the main virus targets are the $CD4^+CD8^-CD45RO^+$ lymphocytes (Richardson *et al.*, 1990). The neurotropism of HTLV-I has been difficult to study because of the rarity of autopsy material from cases of TSP/HAM, the low level of HTLV-I expression in tissues and the lack of an animal model. However, some observations suggest the presence of the virus in the CNS of TSP/HAM patients. Infected T cells (Hirose *et al.*, 1986; Jacobson *et al.*, 1988), HTLV-I-specific IgG, IgA, IgM and cytotoxic T cells (Gupta *et al.*, 1988; Jacobson *et al.*, 1990) have been found in the cerebrospinal fluid. Provirus DNA has been observed by PCR in the CNS of TSP/HAM patients (Bhigjee *et al.*, 1991; Kira *et al.*, 1992; Kubota *et al.*, 1994). However, from these experiments it is impossible to know whether the provirus DNA was present in peripheral blood lymphocytes or in neural cells. Hara *et al.* (1994), using *in situ* PCR amplification, detected provirus DNA in the nucleus of infiltrating lymphocytes in the spinal cords of TSP/HAM patients, but not in neural cells. In contrast, Lehky *et al.* (1995) reported the detection of a few glial fibrillary acidic protein-positive cells containing *tax* mRNA in the spinal cord of three TSP/HAM patients indicating that astrocytes could be infected. HTLV-I could invade the CNS by infecting the choroid plexus, as described in several retrovirus infections (Georgsson, 1994). In a previous study we investigated, with a transient transfection assay, the activity of several HTLV-I promoters isolated from patients with different HTLV-I-associated diseases (Gonzalez-Dunia *et al.*, 1992, 1993). All eight LTRs tested were active in sheep choroid plexus (SCP) cells.

In the present work, we show that SCP cells can be productively infected *in vitro* with HTLV-I. Because human choroid plexus cells were not available, primary cultures were established with SCP aseptically removed from the lateral ventricles of brains of recently slaughtered lambs (Hooghe-Peters *et al.*, 1979). The SCP were rinsed in PBS, trypsinized with mild agitation and cultivated with Leibowitz medium supplemented with 10% foetal calf serum (FCS), glutamine and antibiotics. A monolayer of cells with the appearance of fibroblasts was present in the culture after a few days and the cells were routinely propagated for up to 10 passages. After four passages *in vitro*, SCP cells were cocultivated with irradiated C91PL (as a source of virus) or irradiated JJhan T cells (as a negative control). The irradiation dose (100 Gy) was lethal for these cells (100% of the cells were dead by day 4 post-irradiation as shown by Trypan blue staining). SCP cell monolayers were cocultivated for 3 h at 37 °C with the irradiated cells at a 1:1 ratio and extensively washed three times with serum-free medium. The medium was also changed 24 h after infection. Cultures were maintained in Leibowitz medium with 1% FCS and aliquots of medium were collected. Large syncytia were observed after coculture of SCP cells with irradiated C91PL cells (Fig. 1*a*, *b*). Syncytia appeared 1 week after infection and were never observed in SCP cells coculti-

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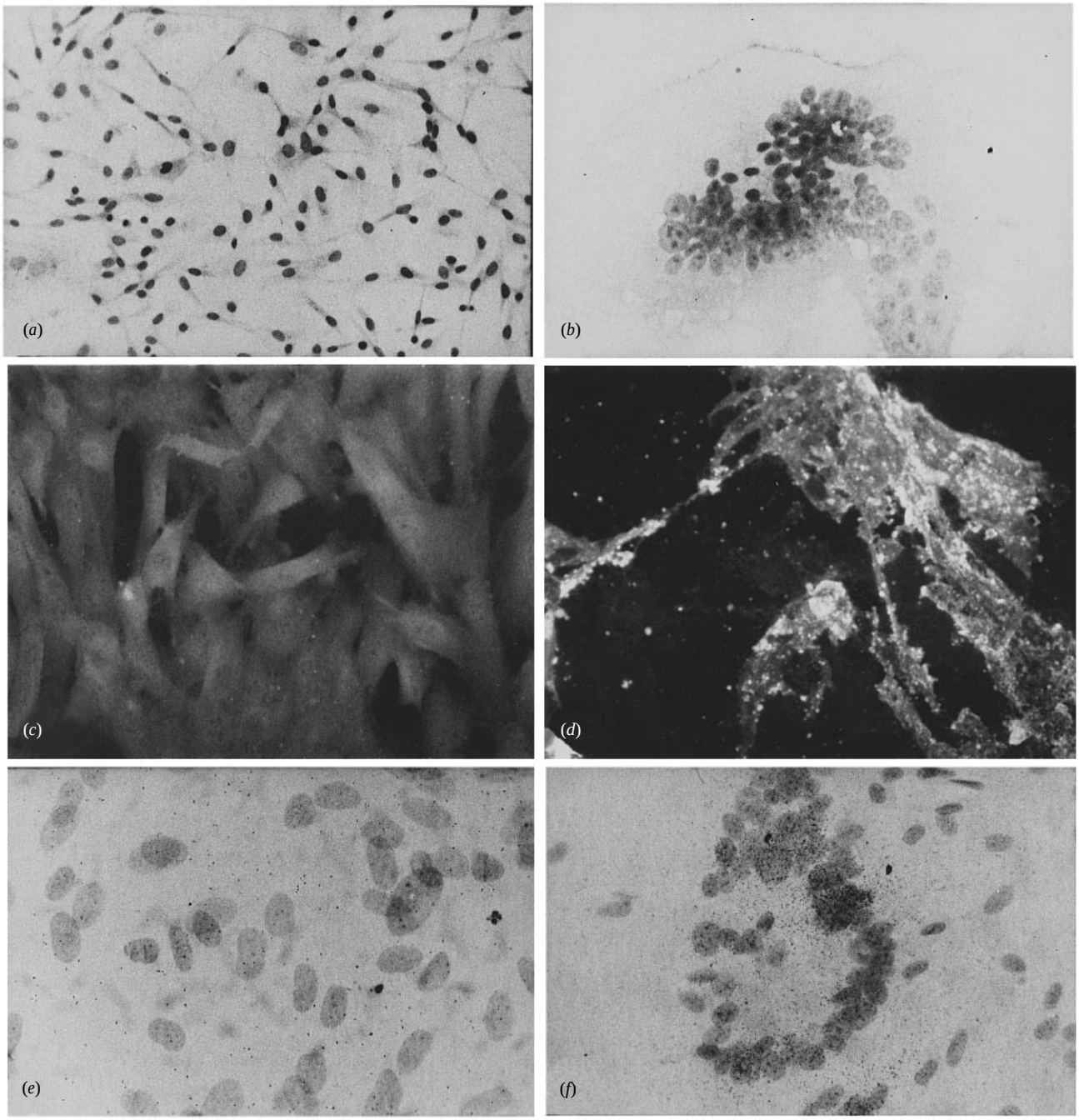

Fig. 1. Infection of primary cultures of SCP cells. (*a*, *b*) Syncytia formation. SCP cells were cocultivated either with JJhan HTLV-I-negative human T cells (*a*) or with irradiated C91PL (*b*). Syncytia formation appeared approximately 1 week after infection. (*c*, *d*) Detection of HTLV-I antigens by indirect immunofluorescence, performed 15 days following coculture. Staining was seen in syncytia of SCP cocultivated with C91PL (*d*) but never in SCP cells cocultivated with JJhan cells (*c*). (*e*, *f*) *In situ* hybridization, performed 15 days post-infection with an anti-*tax* riboprobe. A positive signal was observed in syncytia of infected cells (*f*). No signal was seen in SCP cells cocultivated with JJhan cells (*e*).

vated with JJhan cells. We did not observe immortalization of the infected SCP cells, which died with vacuolated cytoplasm and picnotic nuclei 50 to 60 days post-infection.

HTLV-I p24 was detected in the culture medium using an HTLV-I p24 antigen capture assay (Coulter). A peak in p24 antigen production was detected 18 days post-infection indicating that HTLV-I particles were produced. Antigen was detected up to 40 days after infection (Fig. 2).

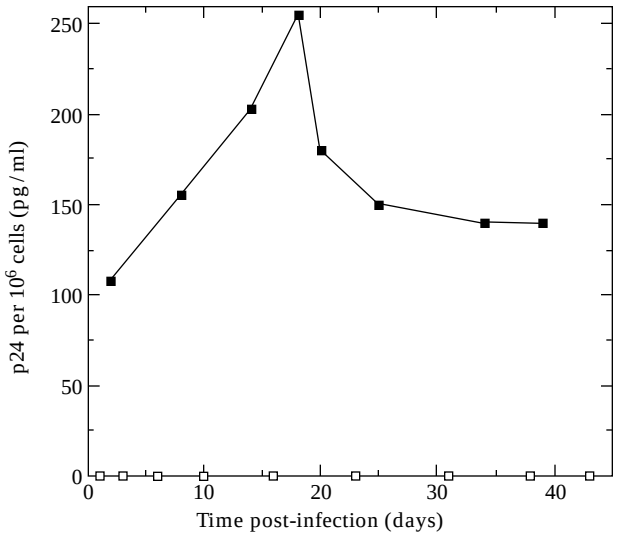

Fig. 2. p24 production by infected SCP cells. Detection of p24 in the medium of SCP cells cocultivated either with irradiated C91PL cells (■) or JJhan T cells (□). The cell culture was maintained up to 40 days after infection.

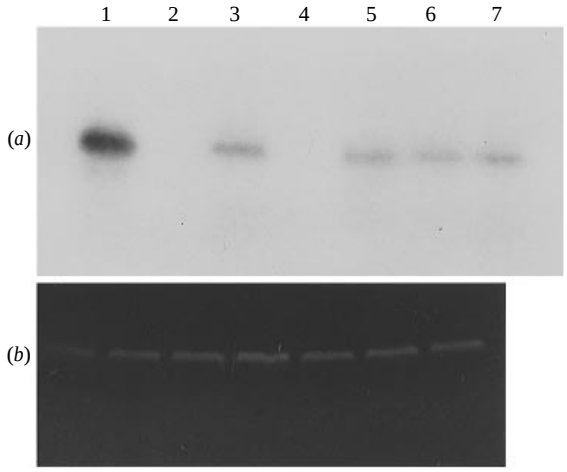

Fig. 3. Detection of HTLV-I *tax–rex* mRNA in infected cells. (*a*) RNA subjected to RT–PCR was analysed by agarose gel electrophoresis and hybridization with a specific *tax* oligonucleotide probe. Lane 1, C91PL cells; lane 2, SCP cells cocultivated with JJhan cells; lane 3, SCP cells 30 days after infection by cocultivation with C91PL cells; lane 4, uninfected human cord blood cells on day 20 of culture; lanes 5–7, human cord blood cells cocultivated with irradiated, infected SCP cells, harvested 20, 36 and 50 days after cocultivation, respectively. (*b*) RT–PCR amplification of GAPDH RNA in the same RNA samples used for (*a*).

HTLV-I-specific antigens were detected in the infected SCP cells by indirect immunofluorescence. The cells were fixed for 20 min with 4% buffered paraformaldehyde at room temperature, rinsed and incubated with a 1:100 dilution of serum from a seropositive patient (kindly provided by A. Gessain). A biotinylated anti-human IgG (Cappell) was used as secondary antibody, followed by incubation with streptavidin–FITC (Amersham). Fig. 1(*d*) shows a representative example of intracytoplasmic staining, mostly on syncytia. Cells cocultivated with irradiated JJhan T cells were consistently negative in parallel experiments (Fig. 1*c*).

In situ hybridization was performed using a ^{35}S-labelled antisense riboprobe complementary to the *tax* region of HTLV-I (sp. act. 2×10^9 d.p.m./μg). The infected cells were grown on glass coverslips; the protocol used has been described previously (Brahic & Ozden, 1992). Exposure time was 5 days at 4 °C. Fig. 1(*f*) shows positive signals 15 days post-infection, a time at which numerous syncytia were observed. No hybridization was seen in control experiments (SCP cells cocultivated with JJhan T cells, Fig. 1*e*).

Detection of HTLV-I *tax–rex* mRNA by RT–PCR was performed on total RNA prepared by the method of Chomczynski & Sacchi (1987). The RNA was reverse-transcribed using random hexamers and the cDNA was used for PCR amplification with a pair of primers surrounding the second splice site of *tax–rex* mRNA. This amplification yielded a 509 bp fragment. We also performed PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene-specific primers to verify the efficacy of reverse transcription. The sequences of the primers used were: 5′ TCTGGAGA-CAGGGTTGGGA 3′ and 5′ CGCTAGTTCTGCCCAGTG-GA 3′ for *tax* amplification; and 5′ CCATGGAGAAGGC-TGGGG 3′ and 5′ CAAAGTTGTCATGGATGACC 3′ for GAPDH gene amplification. Fig. 3 shows the results obtained after 30 cycles of *tax–rex* mRNA amplification by RT–PCR. HTLV-I-infected SCP cells expressed a correctly spliced *tax* gene product at 30 days after infection indicating that the virus can infect and replicate in these cells. HTLV-I RNA transcripts were not amplified in SCP cells cocultivated with JJhan cells.

Primary sheep fibroblast cultures derived from skin and lung were infected using the protocol described for the SCP cells. Syncytia were observed in these experiments; however, *tax* mRNA and the Gag antigen of HTLV-I were not detected 3 weeks post-infection, suggesting a non-productive infection of these cells.

Thin sections of SCP cell monolayers were analysed by electron microscopy 40 days after infection. Virus particles were observed within the cytoplasm and in the extracellular spaces. The cells which contained these particles had a clearly identified apical pole consistent with polarized cuboidal choroid plexus cells (data not shown). Virus particles were not detected in uninfected SCP cells.

We tested if infected SCP cells could be used as a source of virus to infect human cord blood cells. At 40 days post-infection, the SCP cells were irradiated and cocultivated with human umbilical T cells. These cells were purified in a Ficoll gradient and cultivated for 3 days with 2 μg per 10^6 cells of phytohaemagglutinin and then with 20 U/ml of interleukin 2. Cells were examined up to 50 days after infection. The infection and virus replication were estimated by *in situ* hybridization and RT–PCR reaction. HTLV-I mRNA corresponding to the *tax–rex* gene was detected by both

techniques. The results obtained after *tax–rex* amplification are shown in Fig. 3.

Studies of retrovirus infection by visna virus, human immunodeficiency virus and Moloney murine leukaemia virus indicated that these viruses may invade the CNS by infecting choroid plexus cells (Falangola *et al.*, 1995; Georgsson, 1994; Stoica *et al.*, 1993). In this study, we provide evidence that HTLV-I can infect and replicate in a primary culture of SCP cells. It is not known if choroid plexus cells divide *in vivo* or if HTLV-I can infect non-dividing cells. However, the fact that MoMLV, which infects only dividing cells, infects choroid plexus cells *in vivo* suggests that these cells divide. Although the relevance of these data obtained with non-human cells should be assessed carefully, if the choroid plexi are indeed permissive for HTLV-I replication in man, they could represent an important site of virus entry into the cerebrospinal fluid and from there to the CNS parenchyma.

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