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IN VITRO PRODUCTIVE INFECTION OF HUMAN MALIGNANT TROPHOBLASTIC CELL LINE JAR WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

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Summary. - Human choriocarcinoma cells of the JAR line, with no demonstrable surface CD4 receptor were infected with human immunodeficiency virus type 1 (HIV-1), strain RF. Primer-directed enzymatic DNA amplification (polymerase chain reaction, PCR) detected the presence of viral DNA when the cultures were investigated at day 5 post-infection (p.i.). The absence of cytopathic changes attributable to virus replication suggested silent infection of these malignant trophoblastic cells. Neither reverse transcriptase (RT) activity nor HIV-specific antigens were found in the culture nutrient medium during JAR cell propagation. However, when the HIV-carrier JAR cells were continously cultured and the cocultivation was initiated with CEM-SS lymphoblastoid cells after two subsequent passages, rescue of infectious virus was observed.

Key words: trophoblast; choriocarcinoma; HIV; PCR

Introduction

Around 30 % of the children born to human immunodeficiency virus (HIV)-infected mothers will carry the virus (Italian Multicentre Study, 1988), which is presumed to be acquired in a proportion of cases in utero (Falloon *et al.*, 1989). What role the trophoblastic cell layer of fetal origin plays in the process of transmission of infection to the offspring is still not completely clarified. Although successful infection of the placental tissue by HIV was described (Maury *et al.*, 1989), the susceptibility of trophoblast cells to the infection as yet has not been experimentally scrutinized. To address whether HIV-1 can directly infect trophoblast cells we studied the *in vitro* susceptibility of the trophoblastic choriocarcinoma cell line JAR to the infection with the RF strain.

Materials and Methods

Cell lines and virus stock. The human choriocarcinoma cell line JAR (HTB 144), the rhabdomyosarcoma cell line TE 671 (HTB 139) (Stratton *et al.,* 1989) and the African green monkey COS-1 cell line (CRL 1650) were obtained from the American Type Culture Collection, Rockville, Md. Biologically cloned CEM-SS cells (Nara *et al.,* 1987), expressing the CD4 receptor in 96 % of the population, which are highly susceptible to HIV were used for cocultivation experiments. The CEM-SS and JAR cells were cultured in RPMI-1640 medium supplemented with 10 % foetal calf serum, glutamine and antibiotics, the other two cell lines were propagated in supplemented Dulbeco's modified MEM. The stock preparation of HIV-1 RF yielded 1.5×10^5 cpm of particle-associated reverse transcriptase (RT) activity (Hoffman *et al.,* 1985) and contained 7.7×10^5 syncytial forming units (SFU) per ml (Nara *et al.,* 1987). The virus presence in the cell free culture nutrient media was investigated by antigen capture ELISA (Nielsen *et al.,* 1987).

Polymerase chain reaction (PCR). A pair of extension primers, 23 and 24 nucleotides long (5'-TGTTGATCAAATATTACAGGGCT and 5'-CCTTTCGAAGCCCTGTCTTATTCT, respectively) were used for enzymatic DNA amplification. They enabled the synthesis of a 1233 bp long fragment extending from nucleotide 7096 to 8328 of the HIV-1 RF proviral DNA as determined for the clone HAT-3 (Database of Human Retroviruses and AIDS 1989, accession M17451, M12508). Probe oligonucleotide (21-mer) was designed to anneal to the plus DNA strand downstream from the position 7821. Amplification reaction was performed on 1 μg of high molecular weight DNA, using 6.3 pmol of primers, 5 U of Taq DNA polymerase (Stratagene) and 1.5 mmol/l Mg^{2+} per 100 μl of total reaction volume. The optimal profile of a single amplification round was used: denaturation at 90 ^{0}C for 30 sec, annealing at 55 ^{0}C for 1 min and extension at 72 ^{0}C for 2 min. The total number of cycles used was 50. A ten μl aliquot of reation mixture was withdrawn and analysed by Southern blot hybridization (Southern, 1975).

Detection of CD4 receptor by immunofluorescence. The expression of cell surface CD4 molecule was assessed with fluorescein-conjugated monoclonal mouse anti-human helper/inducer T-cells antibody (Dako-T4, Dakopatts) according to the manufacturer's instructions. The CEM-SS cells served as a reference for CD4 molecule expression. Evaluation was done by light microscopy employing epifluorescence illumination.

Results and Discussion

The choriocarcinoma JAR cells were infected by HIV-1 RF at the input multiplicity of infection 1 - 5 SFU per target cell. Simultaneously, the TE 671 cells were infected serving as a susceptible cell system lacking the CD4 receptor (Clapham *et al.,* 1989). The COS-1 cells were also included in experiments as a non-susceptible cell system in order to exclude the possibility of passive transfer of residual virus from inoculum during subcultivations. For infection the virus was allowed to adsorb to the cells for 24 hrs, then the inoculum was withdrawn and by thorough washing with PBS, the cell monolayers were incubated at 37 ^{0}C in the appropriate medium until they were first time subcultured. No cytopathic changes in the cultures were apparent after inoculation of the JAR, TE 671 and COS-1 cells by HIV. The HIV antigen capture ELISA and RT assay repeatedly failed to demonstrate a productive HIV infection in the cultured cells.

Virus recovery was attempted from the cell cultures after they had been serially split twice weekly. Extensive trypsinisations of the monolayers were deliberately performed, with the objective to inactivate the virus which could

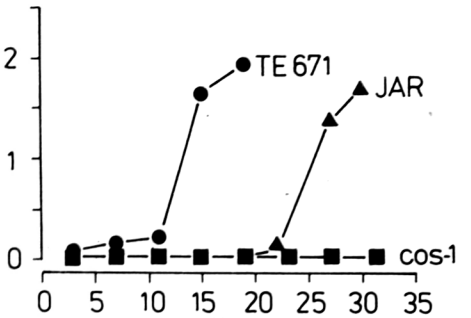

Fig. 1
Subcalture of CEM-SS cells after cocultivation with JAR, TE 671 and COS-1 cells inoculated with HIV-1 RF.
After two serial passages following the inoculation, the cell monolayers were cocultivated for three days with CEM-SS cells. The lymphoblastoid cells were thereafter subcultured separately and assayed for the virus recovery by antigen capture ELISA at indicated intervals. Abscissa: days post-infection; ordinate: absorbance at 492 nm.

have been potentially transferred from the original inoculum (Tateno *et al.*, 1989). The cocultivation experiments were started 24 hrs after seeding of respective cells by adding the suspension of CEM-SS marker cells. After being left in contact with JAR, TE 671 and COS-1 monolayers for 72 hrs, the indicator cells were removed and further subcultured twice a week. The harvested nutrient medium was regularly checked for the presence of viral antigens. The results of a representative cocultivation experiment are presented (Fig. 1). The peak of virus replication was reached considerably earlier in susceptible TE 671 cells than in low-productive JAR cells. No infectious virus was rescued from non-permisive COS-1 cells.

The presence of HIV DNA was investigated in the cells by PCR 5 days p.i. High molecular weight DNA was isolated by digesting the cellular lysates with proteinase K and RNase A and by subsequent phenol-chloroform extraction. The amplification procedure of the abundant template sequences gave rise to the expected band with apparent size 1233 bp, according to mobility in agarose

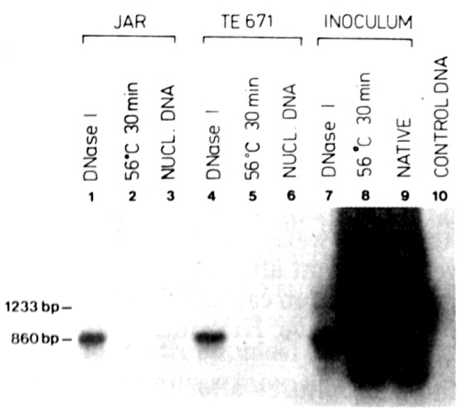

Fig. 2
Primer-directed enzymatic amplification (PCR) of HIV-1 specific DNA in JAR and TE 671 cells and HIV-1 RF inoculum
PCR was performed with the nuclear DNA and with DNA isolated from cells 5 days post-inoculation with native, DNase I-treated or heat-inactivated HIV-1 RF (lanes 1-6) and/or with the inoculum itself (lanes 7-9). Referring to JAR and TE 671 cells, respectively, the lanes 1 and 4 correspond to inoculation with DNase I-treated virus suspension, lanes 2 and 5 to the use of a heat-inactivated inoculum and lanes 3 and 6 to nuclear DNA amplified after inoculation with the virus. The inoculum itself was DNase I-treated (lane 7), heat inactivated (lane 8) or native (lane 9). Lane 10 represents calf thymus DNA.

gel (Fig. 2). Nevertheless, when the amounts of target DNA approached the lower sensitivity limit of the assay a smaller band of approximately 850 bp was coamplified or appeared even as a predominant one. No attempts were made as yet to elucidate the structure of this product, however, additional experiments confirmed that this phenomenon did not compromise the specificity of the assay (data not shown). Interestingly enough, the misspriming events may be linked with particular amplification systems based on the PCR technique (Bell and Ratner, 1989). Preliminary analysis by PCR showed considerable quantity of HIV DNA in the native inoculum (Fig. 2). With the aim to exclude possible misinterpretations, a set of control experiments was implemented. For this purpose the studied cells were inoculated in parallel with heat-inactivated original inoculum (30 min at 56 ^{0}C) or treated before adding to the cells with DNase I in final concentration 2.5μg/ml for 1 hr at ambient temperature. The heating procedure was assumed as sufficient to destroy the virus infectivity (Spire *et al.,* 1985). Results of these experiments are shown in Fig. 2. DNase I treatment of the inoculum removed substantially the amplifiable DNA sequences without afffecting its infectivity titre (data not shown). On the other hand, heat tratment abolished infectivity completely. This proves that the amplification procedure did not process any sequences stemming from the material used for the inoculation which may have contaminated the cell lysates subjected to PCR. It is of interest that only minute signals were obtained upon amplification of isolated nuclear DNA, thus suggesting that the majority of HIV DNA was present in the cystoplasm of infected cells. Overall the presented results witness that HIV DNA sequences found in the JAR and TE 671 cells resulted from reverse transcription of viral RNA following virus penetration. Furthermore, the results of cocultivation experiments show that complete reproduction cycle of HIV was accomplished in JAR cells, with released only low amounts of complete virions.

The experiments indicate that the JAR cells can be infected with HIV in spite of the essential absence of CD4 receptor on their surface as judged from immunofluorescence assay. The low productivity of JAR cell infection, however, suggests the existence of probably multiple factors affecting several stages of the virus replication. Ongoing experiments imply that infection of JAR cells exhibits patterns of persistence, what might be of pathogenetic significance if occurring in vivo (Zachar *et al.,* manuscript in preparation). Virtually, it cannot be excluded that the virus replicating in the mother's organism may reach the trophoblast, infect it and thus increase the probability of HIV transmission to the fetus.

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