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In Vitro Activation of Feline Immunodeficiency Virus in Ramified Microglial Cells from Asymptomatically Infected Cats

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Intravenous infection of cats with feline immunodeficiency virus was used as a model system to study activation of virus replication in brain-resident microglial cells in vitro. Virus release by ramified microglial cells isolated from subclinically infected animals was detectable in cell-free tissue culture supernatant only by reverse transcription and nested PCR of *gag*-specific RNA sequences and not by virion-associated reverse transcriptase activity. In contrast, cocultivation of in vivo-infected microglial cells with mitogen-activated peripheral blood mononuclear cells (PBMC) regularly allows detection of high virus yields in cell-free tissue culture fluid. Besides uptake and multiplication of microglia-derived virus in PBMC, release of virus from microglia is stimulated by cell contact with PBMC. The data suggest that T lymphocytes patrolling the central nervous system could reactivate the semilatent state of lentiviruses in microglial cells in the course of clinically silent central nervous system infection.

Invasion of the central nervous system (CNS) by the human immunodeficiency virus (HIV) can cause serious neurological disease (24). To date, it is well known that HIV enters the CNS early after primary infection and establishes a persistent infection with low release of infectious virus during the subclinical stage of the infection (4, 10, 16). So far, there are no conclusive data which can explain the events regulating viral expression in brain-resident target cells during the subclinical stage of the infection. Nevertheless, for therapeutic and prospective reasons it is desirable to learn more about the viral state in the time period between invasion of the CNS and outbreak of clinically overt neurological disease (21). In this context, it is of particular interest to understand the events that govern the semilatent infection of brain-resident microglial cells, which are known as the main CNS target for lentiviruses.

Most data collected so far on HIV infection of microglial cells come from fetal cells, which probably differ substantially from microglial cells extracted from adult brain with respect to viral replication and cytokine responses (18–20). Therefore, caution should be taken in extrapolating data from fetal cells to events which take place in adult microglial cells infected by immunodeficiency virus. Nevertheless, occasional reports on the interaction of lentiviruses with microglia from nonfetal tissue show that microglial cells, although principally supporting viral replication, usually do not do so *ex vivo* when isolated from neurologically asymptomatic infected hosts (6, 7, 11, 17).

These findings make it clear that during subclinical infection of the CNS, besides a tight control of the virus by immune effector mechanisms (16), intrinsic properties of microglia themselves could account for their low permissiveness for immunodeficiency viruses. Moreover, there are interesting suggestions and findings which open up the prospect that instead of suppressing viral replication, T lymphocytes can enhance virus release from lentivirus-infected cells of the monocyte/macrophage lineage (27, 28). This is in line with recent findings from our laboratory indicating that recovery of infectious feline immunodeficiency virus (FIV) from microglia isolated from adult FIV-infected cats is achievable only by cocultivation with mitogen-activated T lymphoblasts (17). Since early stages and subclinical persistence of lentiviral CNS infection are accompanied by mild inflammatory reactions in the brain, including T-lymphocyte infiltration (16), it seemed likely to us that during clinically silent infection of the CNS, multiple contacts of infected microglia with CNS-patrolling T lymphocytes might result in a microburst of viral replication.

To illuminate the conditions which are necessary to induce virus replication in adult microglial cells, we isolated microglia from cats infected intravenously by FIV and analyzed virus release from these cells under different conditions of culture. Here, we demonstrate that cell-to-cell contact with activated T lymphoblasts effectively induce production of infectious virus from in vivo-infected microglia. These data support the idea that during clinically silent infection of the brain by immunodeficiency viruses, activation of microglia could contribute to viral spread in the CNS and support the selection of CNS-adapted viral variants.

MATERIALS AND METHODS

Animals. Specific-pathogen-free cats (strain Ico:Fec Eur, Tif) 3 months of age were obtained from IFFA Crédo (Lyon, France). They were housed in an air-conditioned animal facility at the Department of Virology, University of Strasbourg, Strasbourg France. Animals were infected intravenously by inoculation of cell-free tissue culture supernatant (TCS) containing 72,000 fluorescence units of reverse transcriptase (RT) activity corresponding to 5,000 50% cat infectious doses (CID_{50}) of FIV strains Wo and $Wo_{microglia}$, respectively, and 2.4×10^8 cpm of RT activity (2.6×10^6 CID_{50}) of strain Villefranche (Vfr). At different time points postinoculation, cats were sacrificed and immediately dissected according to the schedule summarized in Table 1.

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TABLE 1. Infection of cats with different FIV isolates

Animal no.	Virus	dpi
13	Vfr	1
377	Wo	14
653	Wo	28
655	Wo	28
341	Wo	92
378	Wo	183
384	Wo	183
532	$Wo_{microglia}$	28
654	$Wo_{microglia}$	28
283	$Wo_{microglia}$	92
379	$Wo_{microglia}$	183

Virus stocks. The preparation of virus stocks has been previously described (17). They include (i) FIV Vfr (obtained from IFFA Rhône Mérieux); (ii) FIV Petaluma, derived from TCS of chronically infected FL4 cells; (iii) FIV Wo (kind gift of A. Moraillon, École Vétérinaire, Maison d'Alfort, France); and (iv) FIV $Wo_{microglia}$. Strains FIV Vfr and FIV Wo were isolated from peripheral blood mononuclear cells (PBMC), and strain FIV $Wo_{microglia}$ was recovered from microglial cells of infected animals by cocultivation with phytohemagglutinin (PHA)-activated PBMC from healthy donors.

Ramified microglia from adult feline brain. Microglial cells were extracted from adult feline cat brain using an isolation method that was published previously (17). In brief, the brain and brain stem were aseptically removed, meninges were stripped off, and the remaining tissue was mechanically disrupted by mincing through a stainless steel sieve. Following collection, the dissociated material was subjected to enzymatic treatment by 0.78% (wt/vol) collagenase (Serva, Heidelberg, Germany) and 400 U of DNase I (Roche Diagnostics, Mannheim, Germany)/g of tissue at 37°C for 60 min. Digested CNS was sedimented and diluted in Hanks' balanced salt solution (HBSS)-buffered isotonic Percoll at 1,048 g/cm^3 so that the final density reached 1,030 g/cm^3. This suspension was layered over isotonic Percoll at 1,088 g/cm^3 before being covered by HBSS. After a centrifugation step (1,250 × *g*, 20 min, 20°C; acceleration time, 30 s; deceleration time, 5 min), cells were harvested from the 1,030-1,088 g/cm^3 interface and transferred onto a second Percoll gradient consisting of five layers at 1,088, 1,072, 1,060, 1,050, and 1,030 g/cm^3. During subsequent centrifugation (1,250 × *g*, 20 min, 20°C), microglial cells sedimented onto the top of the 1,060 g/cm^3 and 1,072 g/cm^3 interfaces, from which they could be collected. The total number of extracted viable CNS cells was determined by counting trypan blue-negative cells, and 10^6 cells were plated into one well of a 24-well tissue culture dish (Greiner, Nürtingen, Germany) in 0.5 ml of complete growth medium (CGM), i.e., RPMI 1640 (Biochrom, Berlin, Germany) containing 2 mM L-glutamine, 2 mM sodium pyruvate, nonessential amino acids, 100 IU of penicillin/ml, 100 μg of streptomycin (all from Biochrom), and 50 μM 2-mercaptoethanol (Merck, Darmstadt, Germany), supplemented with 5% (vol/vol) fetal calf serum (FCS) (PAA Laboratories, Cölbe, Germany). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 with culture medium renewal every 2 to 3 days.

PBMC and cell lines. PBMC were isolated from pooled citrate-treated venous peripheral blood of at least three different healthy animals by centrifugation over Ficoll-Paque (Amersham Pharmacia, Freiburg, Germany). Subsequently, PBMC were resuspended in CGM containing 10% (vol/vol) FCS and 5 μg of PHA/ml and incubated for 3 days at 37°C and 5% CO_2 before blast cells were recovered by bovine serum albumin (80% [vol/vol] in HBSS) gradient centrifugation (13,000 × *g*, 20 min, 20°C). PHA blasts were cultured in CGM supplemented with 10% (vol/vol) FCS and 200 IU of recombinant human interleukin 2 (IL-2) (R&D Systems, Wiesbaden, Germany)

MYA-1 cells (cell line CRL-2417) were obtained from the American Type Culture Collection (Manassas, Va.) and maintained in CGM supplemented with 10% (vol/vol) FCS and 100 IU of IL-2/ml.

FL4 cells were a generous gift of Jean-Pierre Martin, Department of Virology, University of Strasbourg. They were maintained in CGM supplemented with 10% (vol/vol) FCS.

Coculture of microglial cells and PBMC. Microglial cells (5 × 10^5) from FIV-infected cats were cocultivated either with 5 × 10^5 untreated or mitomycin C (Sigma, Deisenhofen, Germany)-treated (50 μg/ml, 20 min, 37°C) PHA blasts in 24-well cluster plates in a total volume of 1 ml of CGM supplemented with 10% (vol/vol) FCS and 200 IU of IL-2/ml. In the case of membrane-separated cocultures, 5 × 10^5 microglial cells were seeded before tissue culture inserts (0.4-μm pore size; Nunc, Wiesbaden, Germany) were placed in the plates and 5 × 10^5 PHA blasts were added to the upper compartment of the insert. Samples of TCS were tested at intervals for virion-associated RT activity.

Virus recovery by MYA-1 cells. To isolate virus from TCS of FIV-infected microglial cells, aliquots (200 μl) were sampled at different time points after explantation and added in duplicate to 5 × 10^4 MYA-1 cells per well of a 24-well cluster plate. Cultures were maintained in a total volume of 0.5 ml of CGM per well supplemented with 10% (vol/vol) FCS and 100 IU of IL-2/ml. Following 12 days of cultivation, TCS was harvested and subjected to high-speed centrifugation (13,000 × *g*, 5 min). The remaining supernatant was assayed for cell-free RT activity.

Reverse transcription-PCR. For detection of FIV genomes in infected microglial cell cultures, total RNA was extracted from TCS using the QIAmp viral RNA minikit (Qiagen, Hilden, Germany). RNA was DNase I treated (30 Kunitz units, 15 min, 37°C), and 0.4 μg was reverse transcribed (first-strand cDNA synthesis kit; Roche Diagnostics). Subsequently, FIV *gag*-specific nucleic acid sequences were amplified in a nested PCR. The outer primers were 5′-ACACT AGGCCATCTATGAAAG-3′ (*gag* gene positions 974 to 994) and 5′-ATTAA AACACACTGGTCCTGA-3′ (positions 1744 to 1764). The inner primer set used was 5′-CAGAATATGATCGCACACATC-3′ (positions 1262 to 1282) and 5′-AAGAGCTTCTGCCAAGAGTT-3′ (positions 1694 to 1713). The 50-μl PCR mixtures consisted of 5 μl of RT product, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM concentrations of each deoxynucleoside triphosphate, 20 pmol of each outer primer, 0.2 U of inorganic pyrophosphatase (Eurobio, Les Ulis, France), and 2 U of *Taq* polymerase. After the initial denaturation step (2 min, 94°C), amplification was carried out by applying 40 cycles of 94°C for 1 min, 44°C for 1 min, and 72°C for 1.5 min and a final extension of 5 min at 72°C. The second PCR was performed in a 50-μl volume containing 5 μl of the first amplification product and 40 pmol each of the internal primers. PCR was started with denaturation at 92°C for 3 min followed by 40 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 1 min and completed by a 5-min extension at 72°C. PCR was performed on the RoboCycler Gradient 96 system (Stratagene, Amsterdam, The Netherlands). Amplification products (7 μl) were analyzed on 2% agarose gels containing 0.5 μg of ethidium bromide/ml.

Flow cytometry. Microglial cells (2 × 10^5) were double labeled using monoclonal antibodies (MAbs) specific for CD45 (clone WC45a; Serotec, Oxford, United Kingdom) and CD18 (clone MHM23, fluorescein isothiocyanate conjugate; Dako, Glostrup, Denmark). Isotype-matched control antibodies (clone 15H6; SBA, Birmingham, Ala.) served as negative controls. Primary antibodies were detected by R-phycoerythrin-conjugated $F(ab')_2$ fragments of goat anti-mouse immunoglobulin G whole molecule (Dianova, Hamburg, Germany). Samples were assessed on a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany), 10,000 events being acquired in list mode for each sample. Collected data were analyzed with CellQuest software, version 1.0.

Assay for virion-associated RT. Quantitation of retroviral propagation in cell cultures was performed as described in a previous paper (17) using the nonradioactive RT assay (Roche Diagnostics). Briefly, Triton X-100-lysed cell-free TCS samples were mixed with incubation buffer containing digoxigenin-labeled nucleotides and a poly(A) · oligo(dT) template-primer hybrid and incubated for 15 h at 37°C. Newly synthesized DNA was detected by an alkaline phosphatase-conjugated sheep immunoglobulin G fraction specific for digoxigenin and Atto-Phos substrate. Originating fluorescence signals were assessed on a Fluoroskan II fluorometer (Labsystems, Helsinki, Finland) at 444-nm excitation and 555-nm emission.

RESULTS

Release of virus particles from microglia. Microglial cells were isolated from the CNS of adult animals at different times after intravenous infection with FIV. Their purity was monitored by double labeling with MAbs specific for the β2 integrin CD18 and the leukocyte common antigen CD45 (Fig. 1). Usually, more than 88.1% ± 6.5% of extracted cells were $CD18^{low}$ $CD45^{low}$, which identifies these cells as brain-resident parenchymal microglia (17). Immediately after extraction from the CNS, microglia were taken into culture without further external stimuli. TCS was collected at regular intervals and assayed for cell-free virus by determination of virion-associated RT activity. Independently of the time point after intravenous in-

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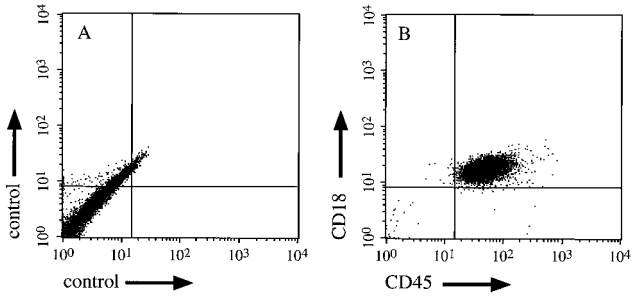

FIG. 1. Phenotype of microglial cells from adult cat brain. Immediately after extraction, CNS cells from cat 13 (1 dpi) were double labeled with either isotype-matched control antibodies (A) or MAbs specific for CD45 and CD18 (B) and subjected to flow cytometry.

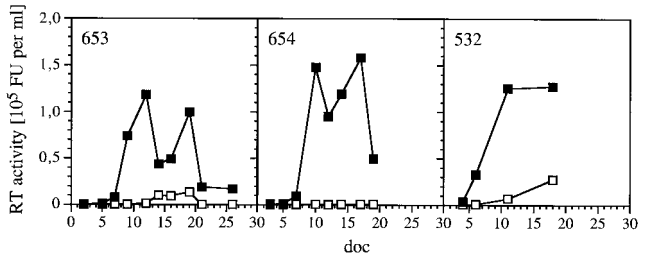

FIG. 3. Evaluation of FIV propagation in microglia-PBMC cocultures. CNS cells of three animals (653, 654, and 532) infected 28 days previously were seeded together with PHA-stimulated PBMC, being separated by a semipermeable membrane (□) or being allowed to develop cell-to-cell contact (■). As a parameter for retroviral production, virion-associated RT activity in TCS samples was regularly determined in duplicate and means thereof were plotted versus days of culture (doc). FU, fluorescence units.

fection of the animals, monocultures of explanted microglia usually do not release enough virus to be detected by virion-associated RT activity in the TCS (Fig. 2A). Nevertheless, this does not exclude spontaneous virus release by these cultures, since at 9 to 10 days of culture it is possible to demonstrate viral RNA in TCS by reverse transcription and nested PCR (Fig. 2B). Thus, release of virus particles from in vivo-infected microglial cells in the course of tissue culture occurs at a level which is beyond the detection limit of an assay for virion-associated RT.

Virus recovery by cocultures of microglia and mitogen-activated PBMC. The subclinical phase of lentiviral CNS infection usually is not associated with complete immunosuppression of the host. It is therefore conceivable that this situation allows interactions between CNS-patrolling T cells and infected microglia, resulting in enhanced virus release. To mimic this process in vitro, microglial cells explanted from in vivo-infected animals were cultured along with mitogen-activated PBMC pooled from at least three healthy donors. As shown in Fig. 2A, large amounts of virus were regularly detectable in these cocultures beginning 6 days past onset of coculture (Fig. 2A). The peak activity of virion-associated RT activity is registered after 10 days of culture, when up to 2×10^5 fluorescence units of RT activity is detectable per ml of cell-free TCS, which corresponds to approximately 1.4×10^4 CID_{50}/ml. Obviously, close proximity between infected microglia and mitogen-activated PBMC enables a more productive viral replication than monocultures of microglial cells.

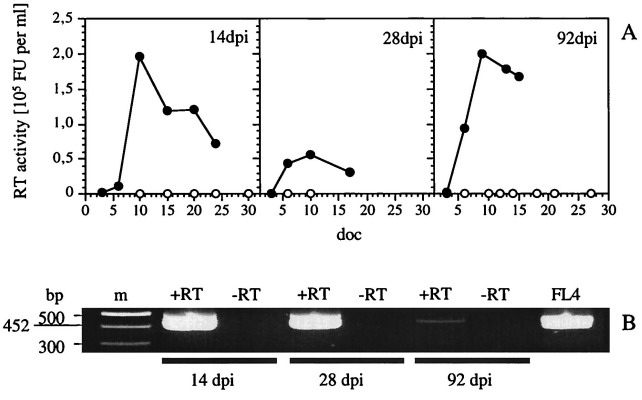

FIG. 2. Detection of retroviral progeny in microglial cell cultures. (A) RT activity in cell-free TCS of microglial monocultures (○) and microglia-PBMC cocultures (●) established from cats 377 (14 dpi), 655 (28 dpi) and 341 (92 dpi). RT activity was determined in duplicates, and means are presented. FU, fluorescence units. (B) Detection of FIV genomes released in the TCS of microglial monocultures explanted from the same cats as in panel A. FIV *gag*-specific RNA sequences were reverse transcribed and amplified in a nested PCR to give a PCR product of 452 bp. To examine DNA contamination, reverse transcription was performed without adding RT (−RT). As a positive control, TCS of FIV *Petaluma* infected FL4 cells was processed under identical conditions. m, DNA molecular size marker.

The data suggested that cell-to-cell contact might facilitate transmission of the virus from microglia to PBMC, resulting in efficient virus replication. Therefore, we cultured in vivo-infected microglia from adult cat brain along with PHA-activated PBMC, which were separated from adherent microglia by a tissue culture insert. The bottom of this insert was formed by a permeable membrane of 0.4-μm pore size, allowing free exchange of culture medium between microglia and PBMC but no cell-to-cell contact. At regular intervals, cell-free virus was monitored in the supernatants of the PBMC in the tissue culture insert by measurement of virion-associated RT.

As can be seen in Fig. 3, cocultures of in vivo-infected microglia and mitogen-activated PBMC in separated compartments only occasionally resulted in the detection of virus in TCSs of PBMC, whereas direct coculture of identical cells always led to very strong virus replication. Moreover, in cases where in membrane-separated cocultures virus was detectable in TCS of PBMC, on average the amount was eight times lower than in a coculture without a separating membrane.

To determine if the smaller amount of recoverable virus in tissue culture inserts is due to an impaired diffusion of cell-free virus across the membrane, we incubated different amounts of cell-free virus in the lower tissue culture dish in combination with mitogen-activated PBMC in the upper tissue culture insert. In these experiments, the FIV strain Petaluma was used instead of strains Wo and $Wo_{microglia}$ because the Petaluma virus can be collected in high titers from chronically infected FL4 cells. However, this should not influence the results, since diffusion of virions is determined by biophysical rather than biological properties of the virus particles. After 10, 14 and 18 days of culture, the level of cell-free virus was determined in the culture medium of the tissue culture insert. Compared to

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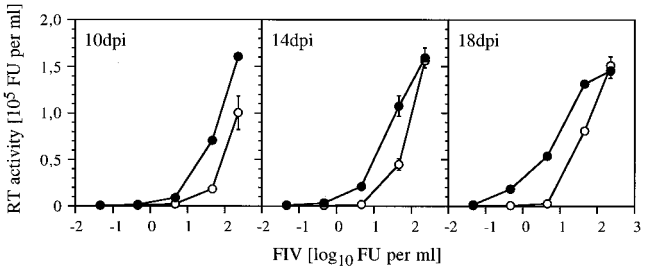

FIG. 4. Analysis of FIV passage through a semipermeable membrane. PHA blast cultures were inoculated with different doses of FIV *Petaluma* either directly (●) or separated by a membrane barrier (○). Retroviral propagation was monitored at the indicated time points by determination of cell-free RT activity in TCS aliquots. Data are the means ± standard deviations of four independent experiments. FU, fluorescence units.

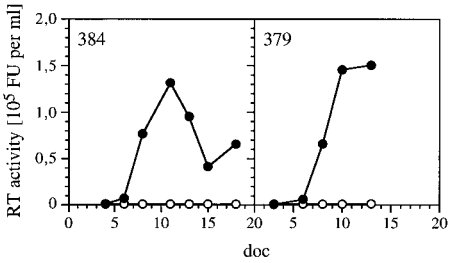

FIG. 5. Examination of FIV release in mitomycin C-treated PBMC-microglia cocultures. Microglial cells extracted from the CNS of cats 384 (183 dpi) and 379 (183 dpi) were cultivated along with untreated (●) and mitomycin C-treated (○) PBMC over a period of 18 days. Regularly, RT activity in TCS samples was recorded in duplicate, and means thereof are shown. FU, fluorescence units; doc, days of culture.

virus added directly to PBMC cultures, separation of cell-free virus and PBMC by the membrane of the tissue culture insert resulted in less virus production only when extremely small amounts of virus (<100 RT units/ml) were put into the lower tissue culture chamber (Fig. 4). Thus, barrier properties of the membrane might impair to a certain extent recovery of microglial virus by PBMC, but this does not sufficiently explain the difference to the situation of direct cocultures, where large amounts of virus can always be detected in cell-free TCSs.

Enhancement of virus release by activation of microglia. A simple and straightforward explanation for the very high virus yield in direct cocultures of infected microglia and mitogen-activated PBMC is the very efficient transmission of microglial virus to PBMC by cell-to-cell contact. Nevertheless, we hypothesized that there might be an additional effect of direct coculture, namely, an enhancement of virus replication in microglial cells by receptor-ligand interactions with PBMC.

To estimate the amount of virus exclusively released by microglial cells after direct contact with PBMC, we set up cocultures of in vivo-infected microglia and mitogen-activated PBMC which were mitomycin C treated before they were added to the microglial cultures. At different times after onset of cultures, 5-μl aliquots of TCS were assayed for virion-associated RT activity. Using microglial cells from animals after different time points after intravenous infection, no virus release was directly detectable in the supernatants (Fig. 5). In contrast, cell-free medium from cocultures of microglial cells with untreated PBMC disclosed high virion-associated RT activity (Fig. 5). This result seemed to exclude a stimulatory effect of cell-to-cell contact on viral release from microglial cell and supported strongly the idea that a very efficient transmission of the virus to replication competent PBMC is the only reason for high virus yields in these cocultures.

Nevertheless, these data did not exclude the possibility that contact between transcriptionally inactive PBMC and virus-infected microglia resulted in virus release beyond the detection limit of the RT assay. Therefore, TCSs from microglial monocultures as well as cocultures were used to inoculate feline MYA-1 T lymphocytes, which are known to be highly susceptible to FIV. Whereas TCS samples from microglial cells cocultivated with mitomycin C-treated PBMC regularly resulted in virus growth, TCSs from microglial monocultures did so only occasionally (Fig. 6). Thus, although not directly detectable, cell-to-cell contact between transcriptionally inactive PBMC and in vivo-infected microglial cells enhances virus release from microglial cells over the level of spontaneously released virus.

DISCUSSION

Information on the state of lentiviruses in parenchymal microglial cells from adult brain is rare. Based mainly on in situ work, it is assumed that lentivirus infection of microglial cells remains at a subclinical level as long as the immune system of the infected host has not collapsed and can suppress viral replication and dissemination (16, 29). However, this assumption has to be made with caution, since neither HIV-infected human brain specimens nor CNS from lentivirus-infected animals allows an unequivocal differentiation between blood-derived monocytes/macrophages and brain-resident microglial cells in situ. Thus, as long as lentivirus-carrying cells in brain tissue are not identified as being true ramified microglia, conclusions on regulation of viral replication in brain-resident microglia remain doubtful.

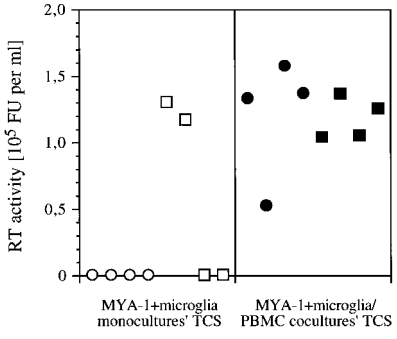

FIG. 6. Virus recovery from TCS of microglial cell cultures by inoculation of MYA-1 cells. Duplicates of microglial cultures set up from cats 378 (183 dpi; ○, ●) and 384 (183 dpi; □, ■) were cultivated either alone or together with mitomycin C-treated PBMC for 4 days before TCS was harvested. Individual TCS samples were used to inoculate two MYA-1 cell cultures each. Following 12 days of cultivation, RT activity in cell-free TCS samples of MYA-1 cells was determined in duplicate, and mean values thereof are presented. FU, fluorescence units.

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Based on previous work from our laboratory, we have successfully adapted a technique to isolate and identify ramified microglial cells from the adult brain of rats to the animal model of FIV infection in cats (17). As with HIV infection of humans, FIV-infected animals eventually develop an immunodeficiency syndrome, including lentivirus-induced encephalopathy with microglial cells as main viral targets in the CNS (12). Using this approach, we demonstrated here for the first time that release of virus particles from microglial cells extracted from asymptomatically infected cats is extremely low in the course of cell culture. This finding fits our previous observation that microglia from FIV-infected animals do not express viral proteins *ex vivo* at all and that it takes a few days of tissue culture for synthesis of *gag*-specific viral proteins to be detectable in less than 1% of all cells (17). Moreover, although FIV-specific DNA is detectable in microglia *ex vivo* as early as 14 days postinfection (dpi), viral RNA is usually not seen (data not shown) unless, as shown here, cells are taken into culture for some days. These data support the idea that besides immunological control mechanisms, intrinsic properties of the feline adult microglial cell and/or the genetic makeup of the infecting agent can account for low virus permissiveness in situ during subclinical brain infection.

Our findings extend in vitro data from others who have shown that monocytes/macrophages can be infected by lentiviruses but do not support replication unless being activated (13, 19). In this context, adherence of the cell to a solid surface, such as the bottom of a tissue culture dish, must already be considered an activation event, because it was shown by Dow et al. (13) that FIV replicates well in adherent monocytes but not in cells where attachment to the plain tissue culture dish was prevented. Comparable data were presented by Janabi et al. (19) for fetal microglia from the human brain. These cells can be infected by HIV, but they do not support viral replication before activation with cytokines, such as gamma interferon. Moreover, serum conditions in the culture medium determine permissiveness of lentiviruses in cells of the myeloid lineage. Withdrawal of serum from microglial cell or monocyte cultures considerably reduces growth of HIV (2, 22).

So far, it is not precisely known at what stage lentiviral infection is restricted in nonactivated monocytes/macrophages. Most likely, both the genetic composition of the virus and that of host cell determine replication efficacy. As shown by Cunningham et al. (9), one-third of HIV isolates are high-replicating viruses in monocytes, one-third are low replicating, and the replication profile of the last third depends on the donor cell. Besides the capability of the virus to use distinct cellular coreceptors, e.g., CCR5, the composition of the viral promoter region in the long terminal repeat (LTR) certainly contributes to high and low permissiveness of HIV for monocytes. Analysis of LTRs from multiple HIV isolates revealed duplication of binding sites for Ras-responsive binding factor 2, which mediates suppressive effects on viral transcription in monocytes and activated T lymphocytes (14).

Our finding that in contrast to monocultures of in vivo-infected microglia, recovery of FIV was always successful when mitogen-activated PBMC were cultured along with microglia allowed two different but not mutually exclusive explanations: (i) transmission of lentiviruses is very effective when cell-to-cell contact is possible, and (ii) mitogen-activated PBMC stimulate virus replication in microglial cells. Obviously, most of the virus recovered from cocultures was amplified by an effective transmission to PBMC. This is evidenced by observations that coculture with a separating membrane between microglia and PBMC was much less successful in picking up virus than direct coculture. Additionally, in TCSs from cocultures of microglia with transcriptionally inactivated PBMC, no virion-associated RT activity was directly detectable. These results are in accordance with those of others, who have repeatedly described cell-to-cell transmission of lentiviruses (8, 26). In particular, monocytes are known to transmit virus from densely packed intracellular viral stores to PBMC by a mechanism which involves cell-to-cell contact but requires no fusion between cells (8).

Despite the failure to directly demonstrate cell-free virus by RT assay in the supernatant of in vivo-infected microglia and transcriptionally inactive PBMC, it is of note that it was possible to recover virus from culture medium by inoculation of feline MYA-1 T lymphocytes with TCSs from these cocultures. This was usually not possible with TCSs from monocultures of microglia coming from the same animal. We assume that in cases where virus could be grown from TCSs of microglial monocultures, the frequency of infected microglial cells in the individual animals was high enough to allow detection of viral particles which were released by activation of the cell through attachment to the culture dish. Thus, one can conclude that besides transmission of virus from microglia to PBMC and subsequent replication, cell-to-cell contact between adherent microglia and PBMC also triggers virus release from in vivo-infected microglia and that this activation generally releases virus more often than attachment-mediated activation.

In a search for possible pathways of signal transduction, which might trigger virus release from microglia by cell-to-cell contact with PBMC, preliminary results from our laboratory imply involvement of the β2-integrin CD18. CD18, as part of the LFA-1 receptor, is expressed on feline adult microglia, and antibody-mediated blocking of the interaction between LFA-1 and ICAM-1 prevents rescue of HIV from nonproductively infected human endothelial cells by $CD4^+$ T lymphocytes (1). Moreover, very recent work presented some evidence that outside-inside signaling by the CD18 molecule increases the transport of JAB-1 to the nucleus, whereby interaction with JUN-1 transcriptional activity is regulated via AP-1 binding sites (5). The AP-1 site is an important binding motif in the 5′ LTR of FIV, which to a certain extent controls viral transcription (31).

Very recent discussions about the etiopathogenesis of HIV-induced dementia (HAD) question the importance of virus-infected ramified microglia as a major pathogenic factor in this disease (15, 23). Data from an HAD patient were presented which revealed tight relationships between bone marrow, monocyte, and white matter CNS sequences of the HIV *env* glycoprotein (23). This has put forward the idea that a continuous influx of HIV-infected bone marrow-derived monocytes into the brain is the decisive pathogenic event in HAD. Based on this assumption, compartmentalization of HIV in long-living ramified microglia for many years after acute peripheral infection would play a negligible role in the development of CNS disease. Clearly, our data presented in this paper challenge this hypothesis.

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It is well acknowledged that lentiviruses enter the CNS in the course of acute peripheral infection of the host, and scarce data from the following subclinical stage of the infection show that a mild inflammatory reaction is present in asymptomatically infected patients, accompanied by activation of CNS-resident cells, which certainly include microglia (3, 16). In view of these and our data, we assume that this situation stimulates from time to time a very low level of virus replication in microglial cells and probably also in latently infected astrocytes, as has been suggested by Messam and Major (25). As long as the infected patient is not severely immunocompromised, these microbursts are of minor clinical relevance, since release of infectious virus can be controlled by a local immune system response, especially in the form of virus-specific antibody-secreting cells. As we have shown recently, a clinically silent infection of the simian CNS by simian immunodeficiency virus is indeed associated with a strong intraparenchymal accumulation of antibody-secreting cells with specificity for the viral *env* protein (30). However, multiple episodes of T-lymphocyte-enhanced viral replication in microglia might add to the development of CNS-adapted virus. In case of breakdown of immunological competence, this virus could gain a growth advantage in brain tissue and contribute to heavy virus replication detectable in brain tissue of patients with overt AIDS.

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