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CD4-Independent Infection of Human Neural Cells by Human Immunodeficiency Virus Type 1

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A number of studies have indicated that central nervous system-derived cells can be infected with human immunodeficiency virus type 1 (HIV-1). To determine whether CD4, the receptor for HIV-1 in lymphoid cells, was responsible for infection of neural cells, we characterized infectable human central nervous system tumor lines and primary fetal neural cells and did not detect either CD4 protein or mRNA. We then attempted to block infection with anti-CD4 antibodies known to block infection of lymphoid cells; we noted no effect on any of these cultured cells. The results indicate that CD4 is not the receptor for HIV-1 infection of the glioblastoma line U373-MG, medulloblastoma line MED 217, or primary human fetal neural cells.

In addition to its function as a recognition molecule for the immune system, the CD4 molecule is the major receptor for the infection of T cells and some cells of the monocyte-macrophage lineage by human immunodeficiency virus type 1 (HIV-1), the cause of acquired immunodeficiency syndrome (7, 8, 17, 19, 23–28). Various studies indicate that the cellular tropism of HIV is dependent on the expression of CD4 on the surfaces of target cells, and there is considerable experimental evidence demonstrating that the large HIV-1 glycoprotein gp120 is responsible for mediating binding of the virus to this membrane protein (21, 22, 27, 36). In addition to the immune system, the central and peripheral nervous systems are important sites of HIV-induced pathology, and several characteristic syndromes have been attributed to direct and indirect HIV-1 infection of the brain (12, 18, 29). However, the role of CD4 in central nervous system (CNS) infection is far from clear (11). On the one hand, in situ hybridization and antigen detection studies indicate that the most commonly infected cells in the brains of individuals with HIV-induced neurologic syndromes are macrophages (13, 20, 33, 35), which express CD4 on their surfaces (37). However, scattered astrocytic, neuronal, and endothelial cell infection has been reported as well (13, 30, 35). Furthermore, a number of CNS-derived tumor cell lines and primary cells from the developing human nervous system can be infected in culture with HIV-1 (4, 5, 9, 10, 34). To clarify the importance of this surface molecule in the CNS pathology induced by HIV, we assessed the role of CD4 in these neural cell infections by a variety of methods.

MATERIALS AND METHODS

Cells. The CNS-derived cell line U373-MG was obtained from Dorothy Herling, Wistar Institute. It is derived from a glioblastoma and is positive for glial fibrillary acidic protein by Northern (RNA) blotting with glial fibrillary acidic protein molecular probe (unpublished results). The cells were maintained in Dulbecco modified Eagle medium containing 10% fetal calf serum. The medulloblastoma-derived cell line MED-217 (TE671) was obtained from John Trojanowski, Department of Pathology, University of Pennsylvania, and has been extensively characterized for its CNS tumor markers (15). It contains both glial and neuronal markers. This cell line was maintained in RPMI 1640 containing 10% fetal calf serum.

Human fetal dorsal root ganglia (DRG) cells were obtained and treated as described previously (34). HeLa T4 cells, which express the CD4 molecule, were obtained from Richard Axel, Columbia University. They were maintained in Dulbecco modified Eagle medium with 10% fetal calf serum and 500 μg of G418 per ml (Sigma Chemical Co., St. Louis, Mo.).

Virus stocks and assays. The experiments were performed with the IIIb strain of HIV-1 (obtained from R. Gallo), expanded in SUP-T1 cells as reported previously (16). The stock virus contained 10^4 to 10^5 50% tissue culture infection doses per ml when inoculated on SUP-T1 cells on microdilution plates and read 10 days after infection. The $p24^{gag}$ assay was performed as indicated by the manufacturer. The Coulter assay (Coulter Immunology, Hialeah, Fla.) was used to measure low concentrations of antigen (glial cells), and the Du Pont assay (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) was used when the expected concentration of $p24^{gag}$ was in the nanogram range (HeLa T4). The assay for particulate reverse transcriptase activity was performed as described elsewhere (16).

Immunofluorescence cytometry. The expression of cell surface antigens was determined by immunofluorescence flow cytometry with a FACstar cytometer (Beckton Dickinson and Co., Mountainview, Calif.) as described previously (16). The cells were trypsinized briefly (30 s) and removed from the monolayer primarily by mechanical force. They were then labeled in suspension with saturating concentrations of unconjugated monoclonal antibodies followed by $F(ab')_2$ fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Tago Laboratories, Burlingame, Calif.). Background levels of fluorescence were determined by using mouse monoclonal antibody P3X63, which is nonreactive in human cells. The two cell lines reacted with an

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antibody to HLA class I antigen (W6/32) that was used as a control. To ensure that trypsinization did not destroy the epitope defined by monoclonal antibodies OKT4A and OKT4, HeLa T4 cells, which also form monolayers, were treated in an identical fashion and assayed in parallel.

Antibodies. Antibodies OKT4, OKT4A, OKT8, and OKM1 were obtained from Ortho Diagnostics, Inc., Raritan, N.J. Monoclonal antibody Leu3a was obtained from Becton Dickinson. NF-M is a monoclonal antibody directed against the medium neurofilament protein and was obtained from Virginia Lee (Department of Pathology, University of Pennsylvania).

Analysis of RNA from U373 and MED 217 cells. Total cellular RNA was isolated from approximately 10^7 cells by using guanidinium thiocyanate (6). The polyadenylated population was selected with an oligo(dT) column as described elsewhere (3). Poly(A)$^+$ RNA (1 μg) was denatured for 5 min at 65°C in 1× MOPS (morpholinepropanesulfonic acid) buffer (40 mM MOPS [pH 6.3], 10 mM sodium acetate, 1 mM EDTA) with 50% formamide and 1.1 M formaldehyde and then subjected to electrophoresis in a 1% agarose gel containing 1.1 M formaldehyde in 1× MOPS. The RNA was transferred to a Nytran nylon membrane (Schleicher & Schuell, Inc., Keene, N.H.) in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) overnight, rinsed in 5× SSC, and then baked at 80°C for 1 h. The filter was incubated at 55°C in 50% formamide–5× SSPE (1× SSPE is 0.18 M NaCl, 1 mM EDTA, 10 mM $NaPO_4$)–5× Denhardt solution (1× is 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.02% Ficoll-400 [Pharmacia Fine Chemicals])–0.5% sodium dodecyl sulfate–100 μg of salmon sperm DNA per ml. The blots were probed overnight at 55°C with a 661-base-pair (bp) ^{32}P-labeled antisense CD4 RNA probe consisting of nucleotides −207 to +454 relative to the transcription start site (25) (pGEM-3Z CD4 subclone, obtained from R. Fisher, Biogen Research Corp., Boston, Mass.), with a final wash in 0.1× SSPE–0.5% sodium dodecyl sulfate at 60°C.

RNase protection. Total RNA (10 μg) was hybridized to a 284-bp ^{32}P-labeled antisense CD4 probe (in 80% formamide, 0.04 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid] [pH 6.7], 0.4 M NaCl, 1mM EDTA) for 16 h at 37°C, and the unhybridized RNA was digested with RNases A (5 μg/ml) and T1 (0.25 μg/ml) for 30 min at 30°C. The reaction was terminated by incubation with proteinase K (50 μg)-sodium dodecyl sulfate (1%) and by extraction with phenol-chloroform. After denaturation in 95% formamide with 10 mM EDTA, the products were subjected to electrophoresis through a 3.5% acrylamide gel containing 7 M urea and 1× TBE (50 mM Tris hydrochloride, 50 mM boric acid, 1 mM EDTA [pH 8]).

Blocking experiments. (i) Cell lines. U373-MG or MED 217 cells were incubated in 24-well plates with a 1:2 dilution of stock monoclonal antibody in 100 μl of serum-free medium (25 μg/ml) at 0°C. In experiments 1, 2, 4, and 5 the cells were washed, and HIV-1 (IIIb) was added to various dilutions. Incubation was done at 37°C for 1 to 24 h. The virus inoculum was removed, and the cells were washed with trypsin-EDTA and incubated at 37°C. In experiment 3 the cells were incubated with antibody for 30 min at 0°C, virus was added, and the cells were incubated at 0°C for an additional 2 h. The inoculum was then removed, and the cells were washed with trypsin-EDTA and incubated at 37°C. In all experiments the cells were trypsinized after 7 days, cocultivated with approximately 10^4 SUP-T1 cells, and watched for the development of a cytopathic effect (CPE), which was then recorded as reported above. The specificity of the CPE was confirmed by an antigen-capture assay for p24^{gag} (obtained from Du Pont).

Control cells used included MRC-5 and other glioma cell lines from which virus could not be rescued. A p24 assay of the postinoculation wash was performed on several occasions; results were negative for the presence of residual inoculum. The control cells were utilized in each experiment reported.

(ii) Fetal cells. Human fetal neural cells were isolated and maintained in vitro on collagen-coated glass cover slips for 20 to 24 h at 37°C prior to experimentation. The cells were then treated with the indicated antibody at a concentration of 5 μg/ml for 1 h at 37°C, washed, and infected with HIV-1 (reverse transcriptase activity, 3×10^6 cpm/ml) in the presence of 2.5 μg of antibody per ml for 20 to 24 h. The inoculum was removed, and medium containing the same concentration of antibody was added. Three days after infection, the cells were washed with phosphate-buffered saline, fixed in methanol-acetone (1:1) for 30 min, and stained for HIV-1 p24 and p17 as previously described (34). The nonneuronal cell population, representing approximately 75% of the plated cells, was identified morphologically, and the percentage of these cells expressing HIV-1 *gag* antigens was determined by counting an average of 10 fields (≈400 cells) for each experimental point.

Blocking with OKT4A and subsequent induction by phorbol esters. MED 217 cells were induced to replicate virus by the addition of phorbol myristate acetate (PMA) to the medium. Approximately 10^4 cells were plated onto 24-well plates, allowed to adhere overnight, and washed; 2.5 μg of antibody was then added in 100 μl of serum-free medium (or medium only was added in the control). The cells were kept on ice for 30 min and then infected with HIV-1 for 2 h at 36°C. The inoculum was removed, and the cells were washed twice with medium, trypsinized until removed from the monolayer, and again allowed to adhere. Supernatant samples were collected, and 50 h later the medium from the MED 217 cells was removed and PMA (50 ng/ml; Sigma) was added. Samples were collected at 24 and 48 h, and the PMA was removed. Further samples were collected after removal of PMA.

HeLa T4 cells were similarly blocked, but assay for p24^{gag} was performed in unstimulated cells, since the HeLa T4 cells produce virus.

RESULTS

HIV-1 infection of the glioblastoma cell line U373-MG and the medulloblastoma line MED 217 resulted in a latent, noncytopathic infection from which virus could be consistently recovered by cocultivation with a susceptible indicator line (SUP-T1) or with other CD4-positive cells (Table 1). Virus production could be induced by phorbol esters and by placing the cells in serum-free medium (see Fig. 3) (unpublished results). Although the recovery of virus from these cells was consistent, infectious center assays have determined that the proportion of cells capable of producing virus during cocultivation is low (1:500 to 1:1,000). This proportion may increase if PMA is used.

CD4 protein and mRNA. To assess the importance of CD4 in HIV-1 neuropathogenesis, we examined both human neural cell lines and primary human neural cell populations for the physical presence of CD4 as well as the potential functional role of CD4 in infection of these cell populations in vitro. Flow cytometry of the tumor cell lines (Fig. 1) and

TABLE 1. Characteristics of HIV-1 (IIIb) infection of neural cells[a]

Cell	Source	CPE	Cell p24	Supernatant p24	Supernatant virus	Rescue
U373 MG	Glioblastoma	−[b]	ND[c]	−	*[d]	+[e]
MED 217	Medulloblastoma	−	ND	−	*	+
Fetal	Fetal CNS	−	+	ND	−	−

[a] Cells were infected with HIV-1 (IIIb) and assayed for intracellular $p24^{gag}$ as described in Materials and Methods and in a previous report (34). Assay for $p24^{gag}$ in the supernatant was performed by using a commercial antigen-capture assay. Viral rescue was done by cocultivating infected neural cells with SUP-T1 cells, observing for CPE, and confirming the rescue by assay for $p24^{gag}$ and by passage of the filtered supernatant.

[b] −, Negative result.

[c] ND, Not done.

[d] *, Both U373-MG and MED 217 can be induced to produce cell-free virus by treatment with phorbol esters.

[e] +, Positive result.

of primary fetal glial cells (data not shown) with the monoclonal antibodies OKT4A (which is located close to the HIV-1-binding site) and OKT4 was performed by using methods previously described (16). Surface antigen was not detected with either of the CD4 antibodies in any of the populations. Control antibodies, including one to a common HLA class I antigen, were positive, indicating that the preparation for flow cytometry did not strip the cell membrane of surface protein. To further control for the possibility that these CD4 epitopes could be destroyed by trypsinization, we simultaneously analyzed a population of HeLa cells constitutively expressing the CD4 antigen (24) (Fig. 1B) and obtained a strong positive result.

We then analyzed these cells for the presence of mRNA hybridizing to a molecular probe of CD4, since others, using RNase protection experiments, have reported the presence of CD4 mRNA in glioblastoma cells (10). To perform these experiments we isolated total cellular RNA from approximately 10^7 cells by using guanidinium thiocyanate (6) and analyzed the poly(A)$^+$ fraction with a 661-bp antisense probe representing nucleotides −207 to +454 relative to the transcription start site (25). No signal was obtained with either cell line (Fig. 2B).

To further confirm the absence of CD4 mRNA in these cell lines, we subjected cellular RNA obtained from both cell lines to an RNase protection experiment. Total RNA was hybridized to a 284-bp ^{32}P-labeled antisense CD4 probe for 16 h at 37°C, and the unhybridized RNA was digested with RNases A and T1. After denaturation, the products were subjected to electrophoresis in a 3.5% acrylamide gel containing 7 M urea and 1× TBE. There was no detectable 284-bp protected CD4 RNA species in RNA isolated from U373-MG or MED 217 cells (Fig. 2A).

Blocking infection. Although we did not detect any CD4 protein by flow cytometric analysis, it is possible that low-level expression could go undetected by this technique. Therefore, to examine the neural cell lines for CD4 with even greater sensitivity, we performed blocking studies with monoclonal antibodies OKT4A and Leu3a, which are close to the HIV-1-binding site and which block infection of lymphoid cells (17, 19, 26, 32). To assess the infection of the CNS tumor lines, we infected cells with HIV-1 (IIIb) at various multiplicities and cocultivated them with SUP-T1 cells 1 week after infection, using the number of days required for the development of CPE in the SUP-T1 cells as an index of the amount of virus present in these latently

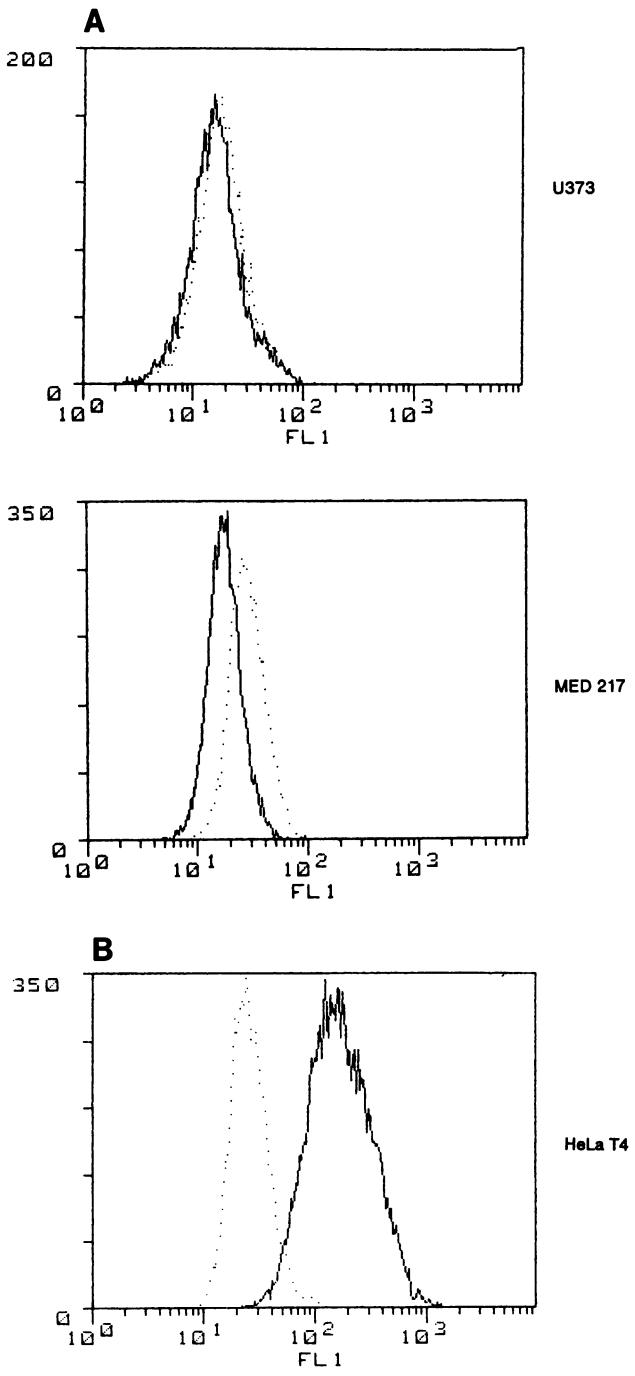

FIG. 1. Immunofluorescence cytometry of U373-MG and MED 217 cells. The expression of cell surface antigens was determined by immunofluorescence flow cytometry with a FACstar cytometer. The cells were trypsinized for 30 s and labeled in suspension with saturating concentrations of unconjugated monoclonal antibodies followed by $F(ab')_2$ fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Tago). Background fluorescence was determined with mouse monoclonal antibody P3X63, which is nonreactive in human cells. All the cells reacted with an antibody to HLA class I antigen (W6/32). (A) Reaction of antibody OKT4A (solid line) superimposed on results for a nonreactive antibody (mouse monoclonal antibody P3X63) (dotted line) in U373-MG and MED 217 cells. (B) Results for HeLa T4 cells, used as a positive control.

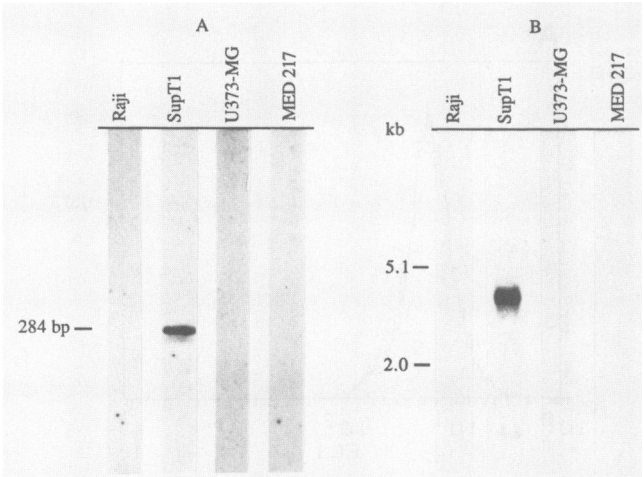

FIG. 2. Analysis of RNA isolated from U373-MG and MED 217 lines for CD4 mRNA. (A) Results of the RNase protection assay. Total RNA was hybridized with a 284-bp antisense RNA CD4 probe and then digested with RNases A and T1. The protected RNA was denatured and analyzed by electrophoresis in a 3.5% acrylamide gel containing 7 M urea and 1× TBE. (B) Results of the RNA blot hybridization analysis. Poly(A)$^+$ RNA (1 μg) was denatured and subjected to electrophoresis in a 1% agarose gel containing 1.1 M formaldehyde in 1× MOPS. The RNA was transferred to a nylon membrane, hybridized with a 661-bp antisense RNA CD4 probe as described in Materials and Methods, and given a final wash in 0.1× SSPE–0.5% sodium dodecyl sulfate at 60°C. kb, Kilobases.

infected cells (Table 2). Recovery of virus from cells that had been pretreated with antibodies OKT4A or Leu3a was the same as recovery from cells pretreated with OKT4, which is directed against a portion of the CD4 molecule removed from the HIV-1 gp120-binding site (17), and from cells pretreated with completely irrelevant antibodies or phosphate-buffered saline. To control for the possibility that input virus could have remained present up to the time of cocultivation, we trypsinized the cells once immediately after infection and once again prior to cocultivation. We also simultaneously inoculated a fibroblastic line (MRC-5) with HIV-1 and did not rescue virus from these cells upon cocultivation. The efficacy of the OKT4A antibody was assessed by using a similar blocking protocol for infection of HeLa T4 cells, which contain a functional CD4 molecule on the background of HeLa cells (24), and U937 cells, a monocytoid line of cells which express the CD4 molecule on their surfaces (2). Using the same concentrations of antibody, we found a definite (10-fold) inhibition of the production of p24 antigen in the supernatant of infected cells when assessed 1 week after infection, the point at which the neural cell lines were cocultivated (see below) (Fig. 3C).

TABLE 2. Effect of pretreatment of tumor cell lines with CD4-specific monoclonal antibodies[a]

Antibody	No. of days to rescue[b] in expt no.:				
	1	2	3	4	5
None	9	9	9	ND[c]	8
OKT4	9	5	11	11	8
OKT4A	9	12	8	10	8
Leu3a	ND	12	9	10	ND
NF-M	ND	ND	ND	10	8

[a] U373-MG cells (experiments 1 to 3) or MED 217 cells (experiments 4 and 5) were incubated in 24-well plates with monoclonal antibody (25 μg/ml) at 0°C. In experiments 1, 2, 4, and 5 the cells were washed and HIV-1 (IIIb) was added to various dilutions. After incubation at 37°C for 1 to 24 h, the virus inoculum was removed, and the cells were washed with trypsin-EDTA and incubated at 37°C. In experiment 3, the cells were incubated with antibody for 30 min at 0°C, virus was added, and the cells were incubated at 0°C for an additional 2 h. The inoculum was then removed, and the cells were washed with trypsin-EDTA and incubated at 37°C. In all experiments the cells were trypsinized after 7 days, cocultivated with approximately 10^4 SUP-T1 cells, and watched for the development of CPE, which was then recorded. The specificity of the CPE was confirmed by an antigen-capture assay for p24^{gag}.

[b] Of virus from latently infected cells by cocultivation with SUP-T1 cells.

[c] ND, Not done.

Induction of replication in MED 217 and U373-MG cells. To further confirm that the virus detected by cocultivation represents induction of the provirus and not residual trypsin-resistant inoculum, we infected both cell lines with or without blocking antibody and induced viral replication with PMA. The results of a representative experiment are shown in Fig. 3. Approximately 2 days (50 h) after infection, the MED 217 and U373-MG cells were treated with PMA (50 ng/ml), and samples of the supernatant were assayed for the presence of p24^{gag}. We pretreated the cells with OKT4 or OKT4A and found no difference in the amount of antigen production (Fig. 3A and B) between pretreated and control cells. The infected cells that had not been treated with PMA did not produce any detectable p24^{gag} in the supernatant fluid, as indicated. HeLa T4 cells were used to control for the amount of antibody used, and supernatants were collected immediately after infection (Fig. 3C). A significant reduction in p24 production was noted if infection was preceded by blocking antibody OKT4A but not by antibody OKT4, as expected (17).

Fetal cells. In parallel with studies involving glial cell lines, similar analyses were done with primary cultures derived from fetal nervous system tissue. Previous studies have demonstrated that a subpopulation of human fetal DRG glial cells is susceptible to infection with HIV-1 (34). The infected DRG glial cells, which constituted approximately 70% of the nonneuronal cells in culture, expressed intracellular antigens (p17^{gag} and p24^{gag}), as determined by indirect immunofluorescence, from 1 to 12 days after infection, with maximal fluorescence observed at 3 days (Table 1 and 3 [34]). Unlike infected tumor cell lines, infected human fetal DRG glial cells are not permissive for virus replication, even by cocultivation. We therefore determined the number of cells expressing these *gag*-related proteins after attempting to inhibit infection by treatment with OKT4A or Leu3a. There was only a very small reduction in the number of HIV-1 antigen-positive neural cells when infection was preceded by treatment with OKT4A or Leu3a but not with OKT4 or other irrelevant antibodies (Table 3). Therefore, the majority of the DRG glial cell population was not protected by treatment with OKT4A or Leu3a monoclonal antibodies, indicating that an OKT4A-independent mechanism is of great importance in establishing human fetal DRG glial cell infection. The small reduction after treatment with OKT4A was reproducible and may indicate that a small subpopulation of cells in the DRG is susceptible to blocking with antibody directed against the T4A epitope and therefore may use the T4A epitope on the CD4 molecule as a receptor.

DISCUSSION

These results demonstrate that HIV-1 infection of CNS-derived cell lines and primary fetal DRG glial cells involved an entry pathway distinct from that of lymphoid cells. The

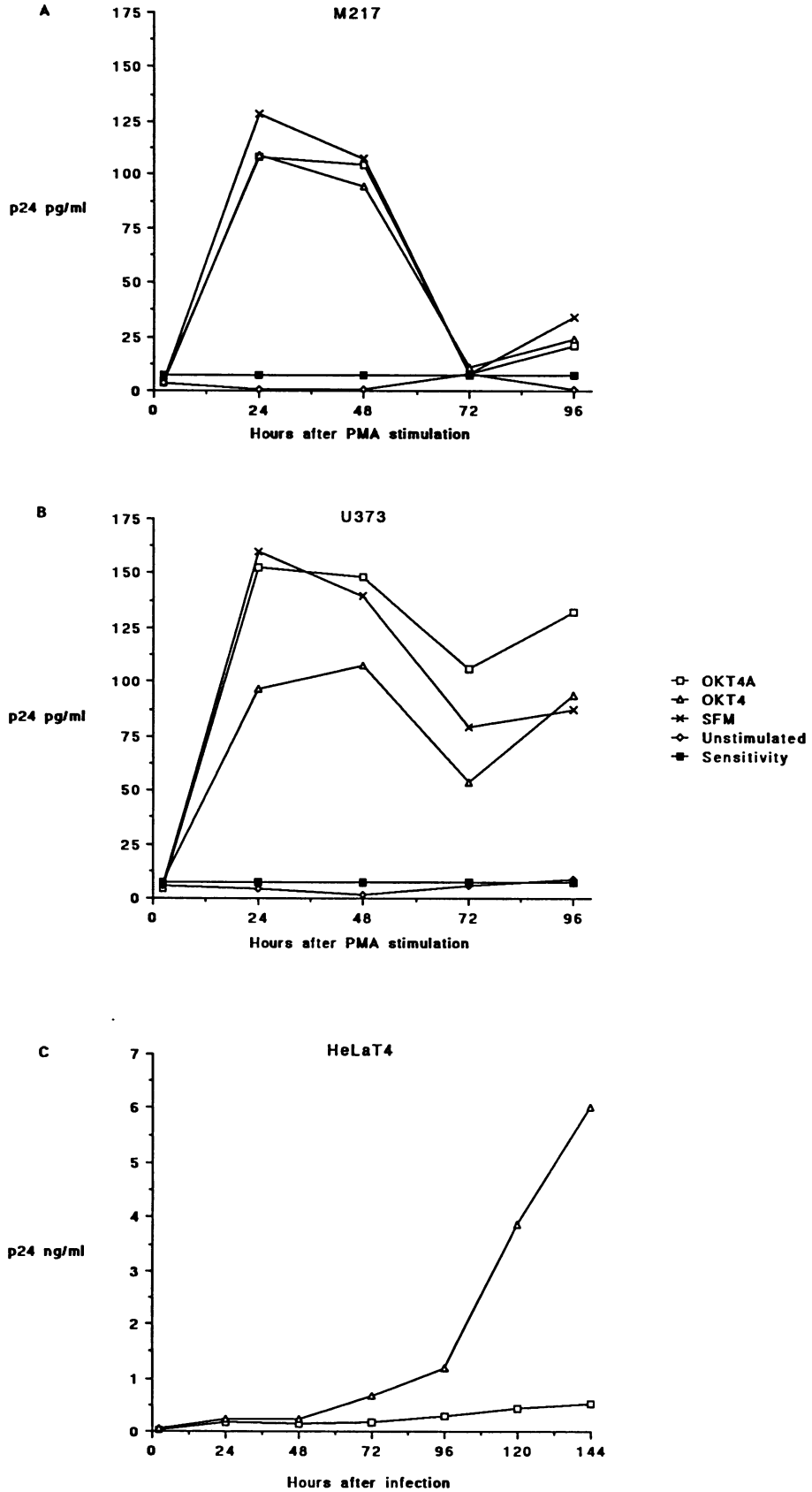

FIG. 3. Induction of p24 production from MED 217 cells (A) and U373-MG cells (B). U373-MG and MED 217 cells were infected with HIV-1 after treatment with monoclonal antibodies as described in Materials and Methods. Approximately 50 h after infection, the cells were stimulated with 50 ng of PMA per ml, and supernatants were collected at the indicated time points and assayed for $p24^{gag}$. The PMA was removed at 48 h, and further samples were collected at 72 and 96 h. A similar blocking experiment was conducted with HeLa T4 cells (C) but PMA was omitted. Note that the scales are different, since HeLa T4 cells produce more p24 under the usual infection conditions.

TABLE 3. Effect of pretreatment of human fetal neural cells with CD4-specific monoclonal antibodies[a]

Antibody	% HIV p17-p24-positive nonneuronal cells[b] in expt no.:		
	1	2	3
None	75	66	79
OKT8	74	60	88
OKM1	72	ND[c]	ND
OKT4	72	72	82
OKT4A	65	37	71
Leu3a	63	ND	ND

Human fetal neural cells were isolated and maintained in vitro on collagen-coated glass cover slips for 20 to 24 h at 37°C prior to experimentation. The cells were then treated with the indicated antibody, washed, and infected with HIV-1 in the presence of 2.5 μg of antibody per ml for 20 to 24 h. The inoculum was removed, and medium containing the same concentration of antibody was added. Three days after infection, the cells were washed with phosphate-buffered saline, fixed in methanol-acetone, and stained for HIV-1 p24 and p17 as previously described (34). The nonneuronal cell population, representing approximately 75% of the plated cells, was identified morphologically, and the percentage of these cells expressing HIV-1 *gag* antigens was determined by counting an average of 10 fields (≈400 cells) for each experimental point.

[b] At 3 days after infection.

[c] ND, Not done.

medulloblastoma and glioblastoma cells had no detectable CD4 on their surfaces, and we could not demonstrate any transcription of the CD4 gene using either RNA blot hybridization or nuclease protection assays. Therefore, it is unlikely that CD4 has a role in the infection of these cell lines. However, as one might expect, infection by HIV-1 was much more inefficient than in lymphoid cells and involved only a small proportion of the cultures. Thus, the role of CD4 as the primary receptor for HIV-1 is undisputed. However, neural cell infection was efficient enough to provide a reservoir of virus that would consistently infect susceptible cells placed in cocultivation and that could be induced to produce infectious virus by exogenous factors, including phorbol esters.

The data for the primary fetal glial cells also demonstrate that infection by HIV-1 proceeded via a pathway different from that of lymphoid cells, since once again there was no blocking of the HIV-1 infection in a majority of the DRG glial cells by monoclonal antibodies directed against the T4A or Leu3a epitopes. Immunofluorescence flow cytometry of the DRG population with anti-CD4 also did not show any detectable surface CD4 (C. Kunsch and B. Wigdahl, submitted for publication). These may be more difficult to interpret, given the heterogeneity of the cells obtained from primary culture.

Other investigators have established that colorectal and glial cells that do not express any CD4 on their surface, as detected by flow cytometry, can be infected with HIV, and that the number of cells infected in such cultures (up to 1:1,000) is in the same range as in our cell lines (1, 4). However, in contrast to our findings, the colorectal cells contain CD4 mRNA, leading to speculation that some CD4 protein below the level of detection of the flow cytometer is being expressed and used as the viral receptor. No such RNA was seen in our cell lines, and the blocking studies confirm the absence of a functional CD4 protein.

We can only speculate on the mechanism of infection of these neural cells, since our data cannot distinguish between two main possibilities: (i) that infection proceeds via attachment to a specific neural receptor and (ii) that entry into these cells is nonspecific and occurs through direct fusion at the plasma membrane or by endocytosis. With regard to the second possibility, the existence of a "fusion" receptor has been suggested, particularly for cells susceptible to infection with paramyxoviruses (31), and a similar HIV-1-specific molecule may exist as well. Fusion regions within HIV-1, possibly in the amino terminus of gp41 (14), could then mediate entry without the specific binding conferred by the gp120-CD4 interaction (22). However, such a fusion receptor would not be present in all cells, since in our study not all cell lines (not even all neural cell lines) were susceptible to HIV-1 infection.

These results suggest that therapeutic approaches intended to prevent HIV spread by inhibiting the interaction between gp120 and CD4 may have no effect on the infection of glial elements. This has already been shown for soluble forms of CD4 prepared by recombinant DNA methods (6a) and may also apply to antibodies derived by immunization against the specific regions of gp120 that interact with CD4. Furthermore, if an alternative entry pathway can be demonstrated in cells other than those derived from nervous tissue, it would expand the number of tissues that are potentially susceptible to HIV infection.

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