For the synthesis of the corresponding 2′-amino derivatives, the amino group was protected by trifluoroacetylation (*25*). The oligoribonucleotides were prepared by automated oligoribonucleotide synthesis on an Applied Biosystems 380B DNA Synthesizer on a 1-μmol scale with the monomeric ribonucleotide phosphoramidites supplied by Milli-Gen/Biosearch. The oligomers were worked up according to the specifications of the supplier with the following modification: after removal of the protecting groups, the oligoribonucleotides were worked up according to Scaringe and co-workers (*26*). The dried pellets were taken up in 50 μl of water and subjected to PAGE. Bands were visualized by ultraviolet shadowing or, in the case of [5′-^{32}P]oligoribonucleotides (*27*), autoradiography, and were cut out. The RNA was isolated by freezing and then crushing the gel piece and eluting at 37°C overnight in sodium acetate (3 M, pH 6.0). The eluate can be concentrated either by SepPak cartridge reverse-phase chromatography or by ethanol precipitation. Concentrations were determined with an extinction coefficient per nucleotide of 6600 M^{-1} cm^{-1} (*9*). Aqueous solutions of the oligoribonucleotides were stored at −20°C.

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Deleted HTLV-I Provirus in Blood and Cutaneous Lesions of Patients with Mycosis Fungoides

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Mycosis fungoides, a rare form of cutaneous T cell leukemia/lymphoma, is suspected of having a viral etiology on the basis of certain similarities to adult T cell leukemia, which is associated with human T cell leukemia/lymphoma virus type I (HTLV-I) infection. Cell lines were established from peripheral blood mononuclear cells (PBMC) of an HTLV-I–seronegative patient with mycosis fungoides. DNA hybridization analysis revealed the presence of HTLV-I–related sequences with unusual restriction endonuclease sites. Sequence analysis of subcloned fragments demonstrated the presence of a monoclonally integrated provirus with a 5.5-kilobase deletion involving large regions of *gag* and *env* and all of *pol*. Additional evidence for the presence of deleted proviruses was found by polymerase chain reaction (PCR) amplification of DNA from cutaneous lesions of five other HTLV-I–seronegative patients. The findings suggest that HTLV-I infection may be involved in the etiology of at least certain cases of mycosis fungoides.

THE CUTANEOUS T CELL LYMPHOMAS (CTCLs) are a group of rare disorders that include mycosis fungoides, Sézary syndrome, Woringer-Kolopp disease, and adult T cell leukemia/lymphoma (ATL) (*1*). ATL is an aggressive disorder with characteristic early visceral spread and poor prognosis and has been shown to be associated with infection by HTLV-I (*2, 3*). Phenotypically, the proliferating leukemic cells are predominantly $CD4^+$ and Tac^+. The HTLV-I provirus is monoclonally integrated into the abnormal cell population, and the large majority of patients are seropositive for HTLV-I (*4*). In contrast, mycosis fungoides and its variant Sézary syndrome are relatively indolent disorders. Although cutaneous involvement may be extensive, the disease progresses slowly, and visceral involvement may not occur for many years (*5*). In contrast to ATL, the leukemic cells of these disorders are $CD4^+$ and Tac^-.

The etiology remains unknown, and studies to implicate HTLV-I or a related retrovirus have yielded ambiguous results. Serological studies by enzyme-linked immunosorbent assay (ELISA) on more than 200 American patients with CTCL showed that fewer than 1% were HTLV-I–seropositive (*6*), whereas several studies of European patients yielded seropositivity rates of up to 12% (*7*). More recently, Manzari and co-workers (*8*) reported the isolation of a HTLV-I–related retrovirus, HTLV-V, from a cell line derived from an Italian patient with mycosis fungoides. Southern hybridization analysis showed that the provirus, although similar to HTLV-I, was unique, and it was proposed that HTLV-V may be involved in the etiology of some CTCLs in Italy.

To study a possible viral etiology in this disease, we grew lymphocytes from an American patient with mycosis fungoides in leukemic phase in RPMI medium containing 20% fetal bovine serum and 10% interleukin-2 (v/v) after an initial 72-hour stimulation with phytohemagglutinin (PHA). The patient was seronegative for HTLV-I in both ELISA and Western blot assays after being repeatedly tested over a 2-year period (*9*). After 10 days, cells were cocultivated with three different samples of cord blood lymphocytes stimulated with PHA. The lines (MF-B1, MF-B3, and MF-B6) proliferated spontaneously and grew in large clumps. Although no syncytia formation was evident, many of the cells were polynucleated. Comparative phenotypic analysis showed them to be predominantly B cells, which is in contrast to findings in the peripheral blood where the majority of cells were $CD4^+$ Tac^-. The B cells expressed surface heavy (immunoglobulin M, immunoglobulin D) and light (kappa) chains, and the phenotypic markers have remained constant in more than a year of culture. The cells have been shown to be Epstein-Barr virus (EBV)–infected by Southern hybridization and clonal on the basis of immunoglobulin heavy and light chain gene rearrangements (*10*). The proliferation and establishment of B cell lines from this patient, who had a $CD4^+$ leukemia, was not considered unusual because B cell lines with concomitant EBV and HTLV-I infections are often established from patients with ATL (*11*).

Although structures morphologically similar to C-type particles were infrequently observed by electron microscopy, no budding particles or evidence of virus assembly or formation at the cell membranes was ever observed (*12*). No significant levels of either Mg^{2+}- or Mn^{2+}-dependent reverse transcriptase (RT) activity were detected in concentrated supernatant fluids (*13*), and as such there was no evidence that the cell lines were productively infected with a retrovirus.

A probe containing the entire HTLV-I provirus hybridized specifically and under high stringency washing conditions [0.1× saline sodium citrate (SSC) containing 0.1%

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SDS at 60°C] to DNA from cultured cells (Fig. 1A). However, the restriction enzyme profiles were quite different from those obtained from DNA from HTLV-I–infected cells (Fig. 1B). No common signals were observed after Eco RI, Bam HI, Pst I, or Hind III digestion. A single 5.7-kb fragment was observed after Eco RI digestion. This was unexpected because the HTLV-I provirus does not contain internal Eco RI sites (*14*), and signals greater than 9 kb, the expected provirus size, are always observed (Fig 1B). Similarly, HTLV-I contains at least three internal Pst I sites and would be expected to produce three signals at 2.6, 1.8, and 1.6 kb, which represent internal fragments (Fig. 1B). In the MF-B6 cell line, signals at 3.4 and 2.0 kb were observed (Fig. 1A).

For further analysis of the 5.7-kb Eco RI fragment, chromosomal DNA was digested to completion with Eco RI and a library of DNA in the molecular size range of 5.4 to 6.0 kb was prepared in λ ZAP-II bacteriophage (*15*). The library was screened with the HTLV-I provirus probe and two subclones (pEP3, 0.8 kb; pPP, 2.0 kb) of the cloned 5.7-kb fragment (Fig. 2A) defined by internal Pst I sites were prepared in plasmid pHSG-399. All attempts to subclone the remaining 2.8-kb fragment (EP1) in a number of vectors were unsuccessful. The subclones pEP3 and pPP were used in Southern hybridization analysis of DNA from HTLV-I–infected cells. Both contained sequences related to the HTLV-I long terminal repeats (LTRs), but pEP3 also possibly contained *gag*-related sequences and the PP2 contained sequences related to the p*X* region (Fig. 2B), suggesting that the 2.8-kb sequence represented by the combined pEP3 and pPP subclones contained a deleted or truncated HTLV-I–related provirus.

This conclusion was confirmed by DNA sequence analysis (*16*). The pEP3 subclone (768 bp) contained sequences from the 5′ LTR and *gag* genes having greater than 95% similarity to HTLV-I (*17*). Single base changes in the 5′ LTR created the unexpected restriction site for Eco RI. The start signal for the expected open reading frame for the *gag* polyprotein (coding for core proteins p19, p24, and p15) was present. However, there was clearly a large deletion because the expected *gag* sequence ends abruptly and the 3′ of pEP3 contains the terminal sequences of *env* and the 5′ of the p*X* (Fig. 3).

The similarity of the sequences flanking the deletion suggests that homologous recombination is part of this process. The pPP2 subclone contained the remaining sequence of p*X* and the beginning of the 3′ LTR. The p*X* sequence has a number of single base pair changes that have eliminated the expected Pst I restriction sites reported for this region (*16, 17*). Thus, the HTLV-I–related sequence in the MF-B6 cell line is a deleted form of the HTLV-I provirus having a sequence organization 5′LTR-*gag*-*env*-p*X*-LTR3′, and it appears to be monoclonally integrated. The presence of two flanking LTR sequences strongly supports the view that the initial infection was by an exogenous virus, almost certainly HTLV-I or a very closely related agent. The possibility that this represents an endogenous provirus sequence cannot be excluded, but, given this evidence, it seems much less likely.

The same deletion was also observed in uncultured peripheral blood lymphocytes of the patient (Fig. 2C). Unfortunately, because of insufficient blood sampling we were unable to fractionate the lymphocytes to (i)

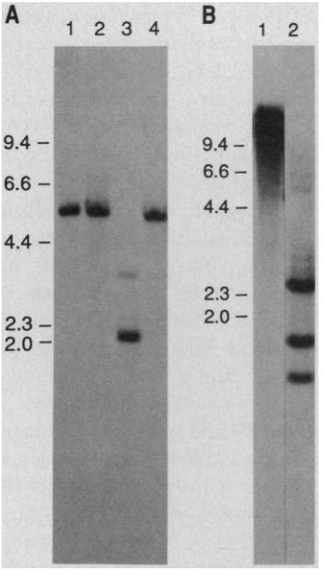
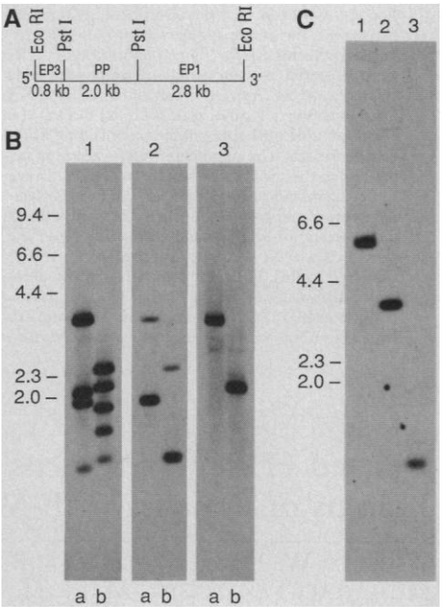


Fig. 1. Southern hybridization of DNA from MF-B6– and HTLV-I– (NS-78) infected cells with a probe (λ 23-3) that contained the entire HTLV-I provirus. (**A**) MF-B6 DNA (10 μg) digested with Bam HI (lane 1), Eco RI (lane 2), Pst I (lane 3), and Hind III (lane 4). (**B**) HTLV-I (NS-78) DNA (10 μg) digested with Eco RI (lane 1) and Pst I (lane 2). Hybridization conditions were similar to those described (*21*). DNA was isolated by phenol-chloroform extraction, digested with the indicated restriction endonucleases (U.S. Biochemical Corporation), separated by electrophoresis on 0.8% agarose gels, and transferred to nitrocellulose (Schleicher & Schuell) membranes. Membranes were prehybridized in 3× SSC containing 50% formamide, 10% Dextran, 1% SDS, and salmon sperm DNA (0.2 mg/ml) for 4 hours at 42°C and then hybridized in the same solution containing a [α-^{32}P]dCTP HTLV-I provirus (λ 23-3) probe that was random primer labeled. After hybridization, membranes were washed twice with 2× SSC containing 0.1% SDS for 15 min, and then with 0.1× SSC containing 0.1% SDS at room temperature for 20 min and at 60°C for 45 min. Membranes were exposed to X-Omat AR film with intensifying screens at −70°C for 24 hours.

Fig. 2. (**A**) Restriction enzyme map of the 5.7-kb cloned fragment from MF-B6 DNA digested with Eco RI. Two subclones (pEP3, 0.8 kb, and pPP, 2.0 kb) were prepared in plasmid pHSG-399 (*15*) and used as probes in Southern hybridization analysis of DNA from HTLV-I– (NS-78) infected cells. (**B**) Southern hybridization analysis of HTLV-I– (NS-78) infected cells with (panel 1) HTLV-I (λ 23-3) provirus probe, (panel 2) pEP3, and (panel 3) pPP. DNAs were digested with (a) Sst I and Bam HI and (b) Sst I and Pst I. (**C**) Analysis of DNA from uncultured PBMCs of the patient. PBMCs were isolated on Ficoll-Hypaque gradients and collected by centrifugation. We digested 10 μg of DNA with (lane 1) Eco RI, (lane 2) Pst I, and (lane 3) Eco RI and Pst I and analyzed as above. Exposure time was 5 days, in contrast to the 1-day exposure used in the study described in Fig. 1 and in (B).

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                                        640          640
CCAGATCCCGTCCCGTCCCGCGCCACCGCCGCCGTCACCCCC   ACCCCCATTACTCTCTTATAAACCCTGAGTCATCCCTGTA
::::::::::::::::::::::::::::::::::::::::: :::X ::::::::::::::::::::: :::::::::::::::::
CCAGATCCCGTCCCGTCCCGCGCCACCGCCGCCGTCATCCCC   ACCCCCATTACTCTCTTATAAAACCTGAGTCATCCCTGTA
          gag                          1120          6610          env
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Fig. 3. Nucleotide sequence of the region of the provirus mf (top) containing the 5.5-kb deletion compared to the published sequence of HTLV-I (bottom). At sequence position 639 of pEP3 there is a deletion of the entire *pol* and parts of the *gag* and *env* regions. The potential sites of recombination are underlined. Sequence analysis was carried out as described (*22*), and the complete sequence of the deleted provirus is described elsewhere (*16*).

identify the populations that contained the deleted provirus and (ii) determine whether the leukemic cells contained the deleted provirus. It also remains unclear whether the deleted provirus is defective in replication. However, repeated attempts to detect mRNA synthesis by Northern hybridization analysis were unsuccessful. Similarly, immunoprecipitation studies with [^{35}S]methionine–labeled cells with HTLV-I–specific sera and the patient's sera were unable to detect unique proteins of the expected size (*10*).

The sequence analysis also shows that the deletion would eliminate the donor sites for the mRNAs of the p40^{tax} and p27^{rex} proteins encoded by the p*X* gene (*18*). The combined action of these gene products is considered responsible for the characteristic expression of Tac on the CD4$^+$ leukemic cells in ATL (*19*). In mycosis fungoides there is a proliferation of CD4 lymphocytes that are Tac-negative. Changes or deletions similar to those described here could be responsible for the absence of Tac on the leukemic cells in this disorder.

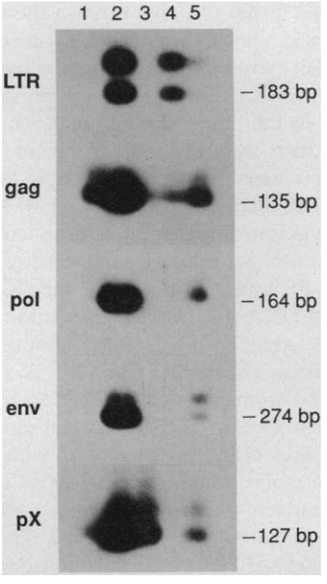

Fig. 4. PCR amplification of DNA from cutaneous lesions of mycosis fungoides. Five patients from Göteborg, Sweden, who were HTLV-I–seronegative and who had histopathologically confirmed disease were included. Punch biopsies of affected skin were immediately frozen in liquid N_2 and ground to a powder with a mortar and pestle. Samples were suspended in TEG buffer (0.01 M tris-HCl, 0.001 M EDTA, 20% glucose, pH 7.6) containing proteinase K (100 μg/ml), incubated at 62°C for 6 hours, then digested again at 37°C for 24 hours. DNA was extracted by standard phenol-chloroform extraction and precipitated in ethanol. PCR amplification was carried out as described (*21, 23*) with 0.5 μg of DNA per reaction. Amplified products were separated by electrophoresis on 1.5% agarose gels, transferred to nitocellulose filters, and probed with end-labeled internal oligonucleotide probes.

To more definitively establish a role for HTLV-I in mycosis fungoides, we used PCR amplification to analyze DNA extracted directly from cutaneous lesions of five additional HTLV-I–seronegative patients. These individuals were from Sweden, an area considered to be nonendemic for HTLV-I infection. All five DNA samples contained HTLV-I–related sequences, but the patterns of amplification varied (Fig. 4). In four patients, *gag*-related sequences were detected. Four of the DNAs also contained p*X* sequences, three of which were *gag*-positive. Two samples contained *pol* and *env* (patients 2 and 5), and two contained LTR sequences (patients 2 and 4). In only one patient could DNA be amplified from all gene regions (patient 2).

The different patterns of amplification suggest that there may be variable deletions of regions of the provirus in the cutaneous lesions. As control, DNA from the skin of two normal individuals was also analyzed. No HTLV-I sequences were detected, and this evidence would support the view that the positive signals were not due to endogenous sequences (*10*). PCR analysis of DNA from PBMCs of the five patients demonstrated that one (patient 2), the only patient in leukemic phase, had HTLV-I provirus sequences, and the amplification pattern was identical to that of the skin (*10*). Southern hybridization analysis of two of the patient DNA samples was negative, most likely because of low proviral copy number (*10*).

Although leukemic cells from patients with ATL usually contain an intact monoclonally integrated provirus, defective forms, although rare, have also been described (*20*). In ATL there is active replication of the intact provirus, albeit at a low level, and an antibody response to all of the virus proteins occurs. In contrast, our findings would suggest that in mycosis fungoides, after virus infection there may be one or more deletions, presumably random, of various regions of the integrated provirus. Thus, it could be anticipated that although integration and presumably alteration of normal cell function occurs, the deletion may subsequently limit or prevent active replication. Thus, no antibody response or perhaps only a limited antibody response occurs. The results of our studies extend the spectrum of disease associated with HTLV-I infection and raise the possibility that there may be alternative mechanisms involved in HTLV-I–associated leukemogenesis. The findings also highlight the limitations of using only serological studies to establish virus-disease associations.

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9. We carried out ELISA and Western blot (WB) assays using commercial kits (Abbott) according to the manufacturer's instructions. Positive control sera were from patients with ATL and asymptomatic HTLV-I–infected carriers. No reactivity was noted with the patient's serum on ELISA, and no immunoreactivity to any of the virus proteins was observed on WB. Positive control sera were consistently reactive on ELISA and demonstrated reactivity to p19, p24, and gp46 on WB.
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12. For electron microscopy, cells were collected by centrifugation, washed in phosphate-buffered saline (PBS), and fixed in 2.5% glutaraldehyde in PBS. Thin sections were stained with uranyl acetate and examined in a Zeiss electron microscope.
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15. Chromosomal DNA was digested to completion with Eco RI and separated by electrophoresis on 0.8% agarose gels. DNA migrating in the 5.4- to 6.0-kb region was electroeluted, purified by phenol-chloroform extraction, and ligated into λ ZAP-II bacteriophage (Stratogene) digested with Eco RI according to the manufacturer's instructions. Plaques were screened with a HTLV-I provirus (λ 23-3) probe. The HTLV-I–related cloned insert was digested with Pst I and two subclones (pEP3, 0.8 kb, and pPP2, 2.0 kb) prepared in the plasmid pHSG-399 (Japanese Cancer Research Bank, Tokyo, Japan). All attempts to subclone the remaining fragment (EP1) in a number of plasmid and phage vectors were unsuccessful. The subclones were used in Southern hybridization analysis and further subcloned into bacteriophage M13 for single-strand DNA sequence analysis.
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22. We carried out DNA sequencing of single- and

double-stranded templates using the dideoxy chain termination method (Sequenase, U.S. Biochemical Corporation). For single-strand sequencing a sequential series of overlapping clones were prepared by selective digestion with T4 DNA polymerase followed by religation into M13 (Cyclone 1 Biosystem, IBI).

23. Primers used in PCR studies were as follows: LTR (bp 561–582) 5′TGACCCTGCTTGCTCAACTCTA3′, (bp 602–634) 5′TCTCTCCTAGGAGTGCTATAG3′; *gag* (bp 1402–1423) 5′CCATCACCAGCAGCTAGATAGC3′, (bp 1513–1535) 5′GCTGGTATTCTCGCCTTAATCC3′; *pol* (bp 4757–4778) 5′CCCTACAATCCAACCAGCTCAG3′, (bp 4919–4942) 5′GTGGTGAAGCTGCCATCGGGTTTT3′; *env* (bp 5405–5424) 5′TACCATGCCACCTATTCCCT3′, (bp 5659–5678) 5′GCTCGACTAGAAGGGAGAAG3′; p*X* (bp 7574–7595) 5′CCAATCACTCATACAACCCCCA3′, (bp 7719–7700) 5′CTGGAAAAGACAGGGTTGGGAG3′. Corresponding internal oligonucleotide probes for the LTR, *gag*, *pol*, *env*, and p*X* amplified products were 5′GGGTGGAACTTTCGATCTGTAACGGCGCAGAA3′, 5′TAATACCTCGGGTTTCGCCTCTGATATAAG3′, 5′GTACTTTACTGACAAACCCGACCTAC3′, 5′TCCCTCATTGGACTAAGAAGCCAAACCG3′, and 5′GTGCTGCCCAAGGGTGGGTTCCATGTATCC3′, respectively. Oligonucleotides were obtained from Research Genetics, Huntsville, AL. Amplification of DNA was performed in a total volume of 50 μl in a reaction mixture containing 225 μM each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTp), and deoxythymidine triphosphate (dTTP), 125 pmol each of primer, 50 mM KCl, 2.5 mM $MgCl_2$, and 10 mM tris-HCl (pH 8.3), and 2 units of *Taq* polymerase (Cetus). Solutions were covered with mineral oil to prevent condensation. Thirty cycles of denaturation for 60 s at 94°C, primer annealing for 90 s at 54°C, and chain elongation for 120 s at 72°C were carried out in a DNA thermal cycler (Perkin-Elmer Cetus). Aliquots (10 μl) of amplified products were separated by electrophoresis on 1.5% agarose gels and transferred to nitrocellulose membranes (Schleicher & Shuell) that had been presoaked in 6× SSPE. [1× SSPE contains 0.15 M NaCl, 0.01 M NaH_2PO_4, and 0.001 M EDTA (pH 7.4)]. Membranes were dried in a vacuum oven for 1 hour at 80°C and prehybridized in 4× SSPE, 5× Denhardt's solution, 25% formamide, 0.5% SDS for 1 hour at 42°C. Hybridization was continued overnight at 42°C in the same solution containing the corresponding oligonucleotide probe end-labeled with [γ-^{32}P]ATP. After hybridization, membranes were washed twice at room temperature with 2× SSPE containing 0.1% SDS for 5 min, twice with 0.2× SSPE containing 0.1% SDS for 15 min, and finally once with 0.2× SSPE containing 0.1% SDS at 56°C for 15 min. Membranes were exposed to XAR-2 Kodak film with intensifying screens at −70°C overnight.

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Inhibition of Entry of HIV-1 in Neural Cell Lines by Antibodies Against Galactosyl Ceramide

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Although the CD4 molecule is the principal cellular receptor for the human immunodeficiency virus (HIV), several CD4-negative cell lines are susceptible to infection with one or more HIV strains. These findings indicate that there are alternate modes of viral entry, perhaps involving one or more receptor molecules. Antibodies against galactosyl ceramide (galactocerebroside, or GalC) inhibited viral internalization and infection in two CD4-negative cell lines derived from the nervous system: U373-MG and SK-N-MC. Furthermore, recombinant HIV surface glycoprotein gp120 bound to GalC but not to other glycolipids. These results suggest a role for GalC or a highly related molecule in HIV entry into neural cells.

THE CD4 MOLECULE IS THE PRINCIpal cellular receptor for the type 1 HIV (HIV-1) (*1*). However, many cell lines of nervous system, liver, and fibroblast origin do not express CD4 but can be infected, albeit less efficiently, with one or more strains of HIV-1 (*2–4*). These findings suggest that, in some cells, HIV entry is mediated by one or more alternate receptor molecules (*3*). To identify the HIV-1 receptor in cells of nervous system origin, we began screening antibodies against cell surface components for their ability to inhibit viral internalization and infection in two infectible cell lines, U373-MG and SK-N-MC (*3, 4*).

The SK-N-MC and U373-MG lines were used for these experiments because they represent two ends of the spectrum of CD4-negative cells susceptible to HIV infection. The U373-MG line was derived from a glioma (*3*); it can be infected with several HIV-1 strains, but infection can only be detected by cocultivation with CD4-positive cells or by polymerase chain reaction (PCR) analysis for viral DNA (*5*). In contrast, the SK-N-MC line, derived from a peripheral neuroblastoma, is much more permissive for viral replication (*4*). Both $p24^{gag}$ antigen and infectious virus can be detected in the supernatant of infected SK-N-MC cells, although the amount is much lower than in CD4-positive lymphoblastoid cells or in a HeLa line (HeLaT4) that constitutively expresses CD4 (*4, 6*).

We tested the inhibition of viral uptake in the U373-MG cell line with rabbit antisera raised against neural cell adhesion molecule, whole glioma membranes, gangliosides, GalC, and irrelevant antigens. Three antisera, 4BGC2, 8586, and 674, raised against GalC, a glycolipid common to oligodendrocytes and Schwann cells (*7–10*), inhibited viral entry, whereas other rabbit antisera did not (Fig. 1). None of these antisera affected the uptake of virus into HeLaT4 cells (Fig. 1), and we did not detect significant viral uptake in two noninfectible lines, HTB-138 and HeLa (Fig. 1). Using both enzyme-linked immunosorbent assay (ELISA) and high-performance thin-layer chromatography (HPTLC), we determined that the three antibodies against GalC (anti-GalC) reacted equally well with GalC and with galactosyl sulfatide, a nervous system glycolipid that differs from GalC by a single sulfatide group (*11*). To confirm the specificity of inhibition, we incubated one of the anti-GalC with GalC liposomes, decreasing its titer in ELISA and concomitantly diminishing its inhibition of viral uptake (*6*).

We then examined the effect of the anti-GalC on infection of U373-MG and SK-N-MC cells. Because infection of U373-MG results in low or undetectable production of virus unless the cells are stimulated (*3*), we used a PCR assay to determine the presence of viral DNA 3 days after inoculation with HIV-1 (strain III_B) (Fig. 2A). Treatment of the cells with immunoglobulin G (IgG) prepared from antiserum 4BGC2 (*12*) resulted in absence of the PCR *gag* signal in U373-MG cells, but treatment with IgG from a rabbit immunized against an irrelevant antigen did not. However, 4BGC2 IgG had no effect on the signal obtained from infected HeLaT4 cells. Similar results were obtained with antibody 8586.

Because HIV-1 infection of the SK-N-MC line is less restrictive than in the U373-MG cells, a different assay was used to measure inhibition by the antibodies against GalC (Fig. 2B). SK-N-MC cells were infected and maintained in the presence of the test IgG, and their effect on viral $p24^{gag}$ production was determined (*13*). In the infected cells, there was a burst of p24 production 2 days after infection that rapidly decreased to a low, chronic level. The anti-GalC resulted in a marked decrease in the early peak of $p24^{gag}$, then a rapid return to undetectable levels. Even at the highest concentration (6 mg/ml before infection, then 0.1 mg/ml

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