Functional Interaction and Partial Homology Between Human Immunodeficiency Virus and Neuroleukin

MARK R. LEE, DAVID D. HO, MARK E. GURNEY*

Dementia is common in patients with AIDS, but the mechanism by which the human immunodeficiency virus type 1 (HIV-1) causes the neurological impairment is unknown. In this study the possibility that an antigen of HIV-1 suppresses neuronal responses to neurotrophic factors was examined. Both HIV-1 and a related retrovirus, simian immunodeficiency virus (SIV), inhibited the growth of sensory neurons from chick dorsal root ganglia in medium containing neuroleukin (NLK) but not in medium containing nerve growth factor. An unrelated type D retrovirus, simian acquired immunodeficiency syndrome virus, did not affect the growth of neurons in the presence of either neurotrophic factor. The inhibition by HIV-1 of neuron growth in the presence of NLK was found to be due to the gp120 envelope glycoprotein. Regions of sequence homology between gp120 and NLK may account for this inhibitory property of gp120 and functional interactions between gp120 and NLK may be important in the pathogenesis of the AIDS dementia complex.

DEMENTIA IN PATIENTS WITH THE acquired immune deficiency syndrome (AIDS) is characterized by poor memory, inability to concentrate, apathy, and psychomotor retardation (*1*). This AIDS dementia complex is associated with direct infection of the brain by the human immune deficiency virus type 1 (HIV-1) (*2*). Within the brain, the monocyte-macrophage appears to be the target of HIV infection, suggesting that HIV may cause AIDS dementia indirectly (*3*). For example, HIV-1 infection may activate inappropriate secretion of monokines, such as interleukin-1 or tumor necrosis factor, which may impair neuronal function or compromise the integrity of the blood-brain barrier (*3*). A second alternative is that release of HIV-1 antigens from infected monocyte-macrophages may interfere directly with neuronal function. This would be much like the mechanism of immunosuppression by oncogenic retroviruses. Infection by feline leukemia virus causes immunosuppression by the direct suppressive effects of the retroviral envelope glycoprotein on immune cell function (*4*). In support of an antigen hypothesis of AIDS dementia is the correlation between the amount of HIV-1 core antigen detected in the cerebrospinal fluid and the severity of dementia in AIDS patients (*5*). The homology of a region of the amino acid sequence of neuroleukin to a highly conserved domain of the HIV-1 external envelope glycoprotein (gp120) led us to examine the hypothesis that an HIV-1 antigen directly suppresses neuronal responses to neurotrophic factors (*6*).

During development, neurotrophic factors influence neuronal growth and survival (*7*). The role of these factors in the adult is less well defined. They may regulate plasticity (*8*), maintain neuronal metabolism, or influence neurotransmitter synthesis (*9*), but they have less influence on neuronal survival unless adult neurons are axotomized or otherwise damaged (*10*). We examined the interaction of HIV-1 with two different neurotrophic factors, nerve growth factor (NGF) (*11*) and neuroleukin (NLK). HIV-1 has no sequence homology to NGF.

Sensory neurons were cultured from embryonic chick dorsal root ganglia (DRG). Early in development, virtually all DRG neurons require NGF for growth and survival in tissue culture (*12*). As development proceeds, most neurons lose responsiveness to NGF because of loss of NGF receptors (*13*). Some of these become responsive to NLK (*6, 12*). Late in development, DRG neurons that retain responsiveness to NGF express characteristics of differentiated pain neurons (*14*). At embryonic day 10, about 40% of the DRG neurons respond solely to NGF in tissue culture, 40% respond solely to NLK, and about 10% respond to both factors. By embryonic day 16, dually responsive sensory neurons are absent, as are neurons that can be grown with NGF, while 40% of the DRG neurons retain responsiveness to NLK (*6, 12*). The dual-responsive neurons may represent a transitional phenotype indicating a switch from NGF to NLK responsiveness. Thus, by preparing cultures from embryonic day 10 DRG, we could examine the effects of HIV-1 antigens on neurons responsive solely to NLK or NGF or to both factors.

In preparing the cultures, we removed most of the non-neuronal cells by "preplating." The neurons had processes and expressed the 200-kD and 160-kD intermediate neurofilament proteins. The remaining polygonal, neurofilament-negative, ganglionic non-neuronal cells accounted for 10% of the cells cultured. NLK primarily influences the survival and growth of cultured neurons; previous studies have shown that NLK is not a mitogen, nor does NLK influence the survival of non-neuronal cells (*6*).

In the absence of NLK or NGF, only 3% to 5% of the neurons developed processes and survived 48 hours in vitro (Fig. 1a). The remaining 90% of the cells plated failed to elaborate processes and died. In contrast,

Table 1. Disrupted HIV-1 or SIV inhibits chick sensory neuron growth with NLK but not with NGF. Viral concentrates (diluted 1:1000) and NLK (1 n*M*) or NGF (3 n*M*) were added to the culture medium. The results are expressed as number of neurons per culture well. The culture wells were seeded with 2050 ± 120 cells scored 3 hours after plating. Process-bearing cells were scored microscopically after fixation. Values tabulated are mean ± SD for four replicate culture wells. The culture plates were coded and scored blind. The experiment was repeated multiple times with similar results. The HTLV-III_B isolate of HIV-1 was grown on H9 cells; SIV was grown on HUT-78 cells; and SAIDS-D was grown on Raji B-lymphoblastoid cells. Productive infections were obtained as assayed by reverse transcriptase (RT) activity and by cytopathology (*27*). The RT activities (per milliliter of culture supernatant) observed were 1.6×10^6 cpm for the HIV-1 infection, 1.9×10^6 cpm for the SIV infection, and 2.2×10^6 cpm for the SAIDS-D infection. The 40-ml samples of culture supernatants were cleared of cells and debris by centrifugation at 9000*g* for 20 minutes. The virions were then subjected to ultracentrifugation at 100,000*g* for 60 minutes. Each pellet was resuspended in 0.4 ml of TX-100 buffer containing 0.1% Triton X-100, 5 m*M* dithiothreitol, 100 m*M* KCl, and 50 m*M* tris-HCl (*p*H 7.4) to disrupt the virions and destroy infectivity.

Neurotrophic factor	Control	Virus added		
		HIV-1	SIV	SAIDS-D
NLK	1010 ± 56	320 ± 72*	360 ± 53*	890 ± 130¶
NGF	1050 ± 140	1070 ± 170	1110 ± 140	1020 ± 110
NLK + NGF	1640 ± 140	1100 ± 110‡	1130 ± 170†	1610 ± 170

*$P < 0.005$; †$P < 0.01$; ‡$P < 0.03$; paired one-tailed *t* test with Bonferroni correction against number of sensory neurons scored in the respective control. ¶No significant difference, although in this experiment neuron survival was slightly decreased.

M. R. Lee, Department of Neurology, University of Chicago, Chicago, IL 60637.
D. H. Ho, Division of Infectious Diseases, Department of Medicine, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA 90048.
M. E. Gurney, Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

*To whom correspondence should be sent.

when grown in the presence of either NLK or NGF, up to 45% of the cells rapidly developed processes within 12 hours (Fig. 1, b and c). Each factor maintained the survival of the differentiated neurons for the duration of the culture period.

The survival of 50% of the maximum number of sensory neurons in the 48-hour culture assay was elicited by 19 ng of NLK per milliliter ($3.4 \times 10^{-10} M$). To obtain maximum survival of sensory neurons, we routinely used 1 n*M* NLK and 3 n*M* NGF.

We tested detergent-disrupted, noninfectious preparations of HIV-1, simian immunodeficiency virus (SIV), and simian acquired immunodeficiency virus type D (SAIDS-D) for suppression of sensory neuron growth in NLK or NGF. HIV-1 and SIV are related, type C cytopathic retroviruses (*15*), whereas SAIDS-D is an unrelated type D retrovirus (*16*). SIV causes a disease in macaques that resembles human AIDS, including primary retroviral encephalopathy (*15*). Rhesus monkeys infected with SAIDS-D also develop immunodeficiency, but the disease differs from human AIDS in that it leads to a depletion of both T and B cells and the ratio of helper to suppressor T cells does not reverse (*16*). Brain infection by SAIDS-D with consequent neurological dysfunction has not been reported.

Concentrates of HIV-1 and SIV suppressed the growth of sensory neurons in NLK at virus dilutions of 1:1000. Further dilution diminished the inhibition of neuron growth in NLK. The TX-100 disruption buffer did not interfere with the assay at dilutions greater than 1:1000. Neither HIV-1 nor SIV affected sensory neuron survival in NGF. SAIDS-D did not inhibit neuron growth in either NGF or NLK at the virus dilutions tested. In repeated experiments HIV-1 and SIV consistently suppressed the growth of sensory neurons in NLK without affecting the growth of sensory neurons in NGF (Table 1). In cultures grown with both NLK and NGF and treated with either HIV-1 or SIV, the number of neurons obtained was equivalent to the number obtained in cultures grown solely with NGF. Thus, HIV and SIV inhibited the growth of sensory neurons in the cultures that were responsive solely to NLK, but did not inhibit the growth of dually responsive neurons, which presumably were maintained by the NGF.

The suppression of sensory neuron growth in NLK was demonstrated with many different preparations of HIV-1, including sucrose banded HIV harvested from the H9/HTLV-III$_B$ producer cell line (Litton Bionetics); density gradient purified HIV-1(LAV) grown on phytohemagglutinin-stimulated human peripheral blood mononuclear cells; and pelleted HIV-1 (HTLV-III$_B$) grown on the T-lymphocytic cell lines H9, Molt-4, and Molt-3B; and HIV-1(HLTV-III$_B$) grown on the monocytic cell lines U937, A3.5, and A4.5. Both detergent-disrupted HIV-1(LAV) preparations and heat-inactivated HIV-1(LAV) (56°C for 3 hours) suppressed sensory neuron growth in NLK. No inhibition of NGF bioactivity was observed with any of the HIV-1 preparations.

The effects of disrupted HIV-1 on the growth of sensory neurons in NLK may be interpreted in at least two ways. HIV-1 may be selectively toxic to NLK-dependent sensory neurons and this toxicity may be independent of the biological activity of NLK. Alternatively, HIV-1 antigens may inhibit the biological activity of NLK by binding to a surface component of the sensory neuron; such binding could render the neuron refractory to NLK action. To distinguish between the two hypotheses, we compared the effects of incubating the neurons with HIV-1 before exposing them to NLK, exposing them to HIV-1 for the entire culture period (0 to 48 hours), and exposing them to HIV-1 after growth had been established (24 to 48 hours). In a control experiment we added a monoclonal antibody to NLK (designated B130.6) to cultures grown under similar conditions (*6, 17*).

Dissociated DRG cells were incubated for 3 hours on tissue culture plastic in culture medium to remove adherent non-neuronal cells and were treated concurrently with either disrupted HIV-1 or B130.6. The cells

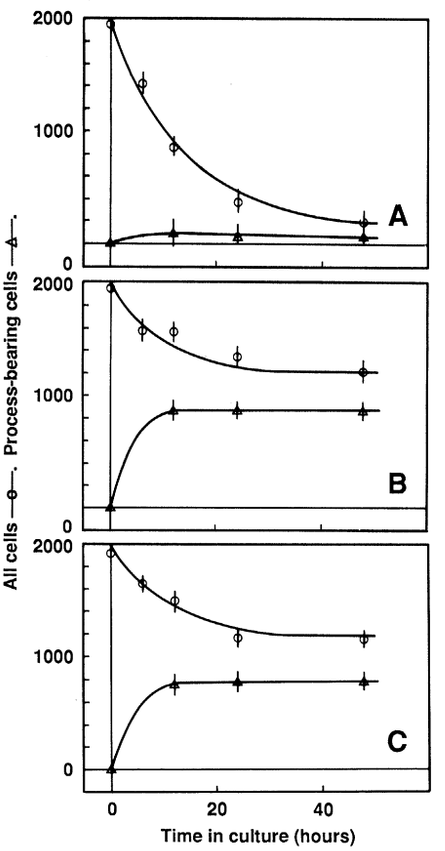

Fig. 1. The influence of 1 n*M* NLK and 3 n*M* NGF on the survival of cultured chick sensory neurons. Total cells (○) and cells with neurites (△) were counted in four replicate wells at the indicated times. Error bars represent mean ± SD. In the absence of NLK or NGF, most cells die within 24 hours in culture (**A**). In the presence of NLK (**B**) or NGF (**C**), up to 45% of the neurons show processes within 12 hours of culture and they survive throughout the culture period. Chick embryonic day 10 ganglia were dissected under sterile conditions, digested with 0.1% trypsin for 20 minutes at 37°C, washed in culture medium, and then triturated into a single-cell suspension. Cell suspensions were plated in 10-cm plastic tissue culture dishes for 3 hours at 37°C; nonadherent neurons were harvested and seeded in 16-mm culture wells coated with poly-ornithine (100 μg/ml in 150 m*M* borate buffer) and then laminin (Collaborative Research, 10 μg/ml). Cells attached to the culture substrate and process-bearing cells were counted at the indicated times in a swath of ten microscope fields per culture well (4% of the culture surface). The NLK and NGF were added to the culture medium, which consisted of Dulbecco's modified Eagle's medium containing glucose at 6 mg/ml, 10% heat-inactivated horse serum, penicillin at 100 U/ml, and streptomycin at 100 μg/ml. Recombinant mouse NLK was produced by transient expression in monkey COS cells as described previously (*6*).

Table 2. Effect of timed addition of HIV-1 or monoclonal antibody to NLK on the response of sensory neurons to 1 n*M* NLK. Sensory neurons were incubated with HIV-1 or with the B130.6 monoclonal antibody to NLK (*17*) for 3 hours (−3 hours to 0 hour) and then washed twice with medium prior to seeding in the culture well. Alternatively, HIV-1 or B130.6 was included in the culture medium during the entire 48-hour culture period (0 to 48 hours) or was added for only the second 24 hours of culture (24 to 48 hours). The wells were seeded with 1700 ± 180 cells scored at 3 hours after plating. Process-bearing cells were scored microscopically after fixation. Values tabulated are neurons per well (mean ± SD) for six replicate culture wells.

Period of treatment	Addition to culture		
	NLK	HIV-1	B130.6
−3 to 0 hour	760 ± 160	290 ± 76*	720 ± 120
0 to 48 hours	860 ± 150	220 ± 35*	192 ± 70*
24 to 48 hours	810 ± 31	680 ± 73	660 ± 70†

*$P < 0.002$; †$P < 0.05$; paired, one-tailed t test with Bonferroni correction against number of sensory neurons scored in the respective control. The experiment was repeated once and a similar result was obtained.

were not exposed to NLK during this period. At the end of the incubation period nonadherent neurons were collected, washed twice with culture medium, and then plated in pORN-laminin coated culture wells with NLK at 1 n*M*. As expected, prior incubation with B130.6 did not inhibit growth in NLK because the monoclonal antibody does not react with a surface component of the sensory neurons. In contrast, prior incubation with HIV-1 made the neurons refractile to growth in NLK (Table 2). Toxicity due to preincubation with HIV-1 was not evident as the yield of nonadherent neurons was normal and their subsequent plating efficiency in the culture wells was not affected.

No toxic effects of HIV-1 on sensory neurons were observed once sensory neuron growth in NLK had been established (Table 2). Both HIV-1 and B130.6 had to be added when the cultures were initiated in order to suppress growth of the neurons in NLK.

To show that the inhibitory activity in HIV-1 preparations was associated with retroviral antigens rather than contaminating serum or cellular proteins, we performed immunoprecipitation studies. Samples of HIV-1 antibody positive sera from three AIDS patients were tested for their reactivity with HIV-1 antigens by Western blotting (Fig. 2). Serum BA85-E41 had the greatest reactivity with gp120. Other studies have shown that, by radioimmunoprecipitation, antibody to gp120 can be detected in virtually all HIV-1 antibody-positive human sera, although the titer of the antibody varies (*18*). Sera from all three AIDS patients reacted with viral antigens of 66 kD and 55 kD, which presumably are products of the viral *pol* gene. Serum BA85-E41 and serum BA85-E43 reacted strongly with the gp41 transmembrane envelope protein and the *gag* antigens. BA85-E41 immunoprecipitated the inhibitory factor from the disrupted HIV-1 preparation, whereas sera from the other two AIDS patients did not (Table 3).

The result of this experiment suggested that gp120 in the HIV-1 preparations inhibited the bioactivity of NLK. To test the hypothesis directly, we tested two monospecific antisera produced (one in goat, D-2935, and one in rabbit, D-13381) (*19*), against immunoaffinity-purified gp120 for their ability to immunoprecipitate the inhibitory factor from disrupted HIV-1 preparations. Normal rabbit and goat sera were used as controls. Both antisera to gp120 removed the inhibitory factor from disrupted preparations of HIV-1, whereas the normal rabbit and goat sera were ineffective (Table 3). Thus gp120 is the factor in disrupted HIV-1 preparations that inhibits the biological activity of NLK in the sensory neuron bioassay. Removal of other HIV-1 antigens from the disrupted virus preparations by immunoprecipitation with the BA85-E43 and BA85-E45 HIV-1 antibody-positive sera did not remove the inhibitory factor.

To show directly that gp120 inhibits the biological activity of NLK, we tested the effects of purified gp120 and a bacterially expressed fragment of gp120 (designated PE3) in the culture assay. The gp120 was purified from HIV-1–infected H9 cells by immunoaffinity chromatography and was homogeneous by SDS-PAGE (*19*). PE3 (gp120 43-279) is a 28-kD protein expressed from the Kpn I–Bg II fragment of the *env* gene that contains the amino terminal half of gp120 (*20*). The carboxyl terminus of PE3 contains the gp120 sequence with homology to NLK. P121 (gp41 56-138) is an internal fragment of gp41 that contains an immunodominant epitope reactive with the majority of HIV-1 antibody-positive human sera (*21*). P3′ORF is ex-

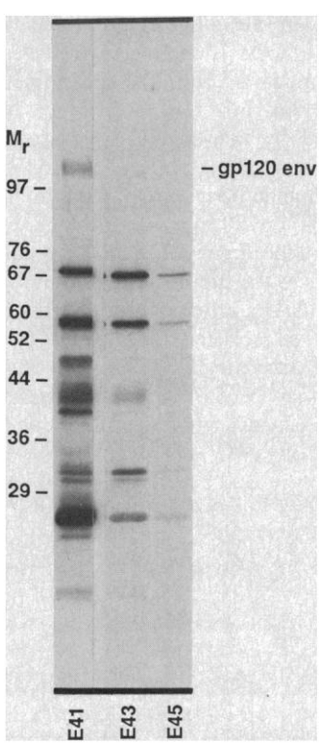

Fig. 2. Western blots of an HIV-1 preparation probed with HIV-1 antibody-positive sera from three AIDS patients (BA85-E41, E43, and E45). Molecular weight standards are indicated. The protocol for Western blotting was described previously (*6*).

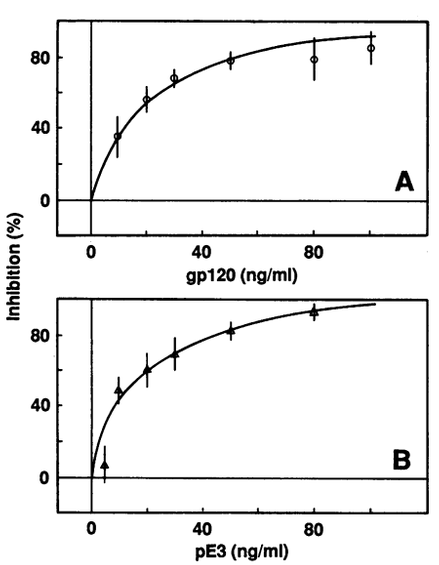

Fig. 3. Inhibition of 1 n*M* NLK bioactivity by gp120 and PE3. The gp120 and PE3 were added at the indicated concentrations. Experiments were done in four replicate wells and the data show mean ± SD. Zero percent inhibition corresponds to 1110 ± 75 cells per culture well grown with NLK; 100% inhibition corresponds to the number of neurons in control wells without NLK (380 ± 37 cells per well). Similar results were obtained in three separate experiments.

Table 3. Immunoprecipitation of the inhibitory factor from the disrupted HIV-1 preparation by HIV-1 antibody-1 positive human sera and by antisera to purified gp120. The wells were seeded with 1800 ± 270 cells scored 3 hours after plating. Values tabulated are neurons per well (mean ± SD) for four replicate culture wells. The experiment was repeated once and a similar result was obtained. Antibody to HIV-1 was detected in the human sera (CDC BA85 E-41, E-43, E-45) by ELISA and by Western blotting (Fig. 2). The rabbit (D-13381) and goat (D-2935) antisera to gp120 were prepared by immunization with highly purified gp120 (*19*). Normal rabbit and goat sera were used as controls. The protein A–Sepharose beads were incubated with 20 μl of serum for 30 minutes at room temperature, washed extensively, and then incubated overnight at 4°C with a 1:10 dilution of the HIV-1 concentrate in phosphate-buffered saline with 10% horse serum. The beads were then centrifuged and the supernatant was added at a final dilution of 1:1000 to the culture medium. The culture medium contained 1 n*M* NLK.

Serum used for immunoprecipitation	Neurons per well
Human HIV-1 antibody-positive sera	
CDC BA85-EA41	810 ± 110
CDC BA85-EA43	350 ± 89*
CDC BA85-EA45	360 ± 60*
Rabbit antiserum to purified gp120	
Antiserum to gp120	880 ± 130
Normal rabbit serum	340 ± 80†
Goat antiserum to purified gp120	
Antiserum gp120	940 ± 100
Normal goat serum	420 ± 66‡
Controls	
NLK (1 n*M*)	860 ± 89
NLK + HIV (1:1,000)	370 ± 89†
NLK + TX-100 buffer (1:1000)	890 ± 95

*$P < 0.002$; †$P < 0.01$; ‡$P < 0.02$; paired, one-tailed *t* test with Bonferroni correction against number of sensory neurons scored in the respective control. The immunoprecipitations were coded and analyzed blind.

pressed from the 3′*orf* gene. All three bacterially expressed proteins contained 30 to 40 vector-encoded amino acids and were purified by immunoaffinity chromatography.

Only gp120 and PE3 inhibited the growth of sensory neurons in NLK (Table 4). Addition of P121 or P3′ORF at up to 1 μg/ml was not inhibitory. Dose-response curves for the inhibition of NLK bioactivity by various concentrations of gp120 or PE3 were obtained with NLK at 1 n*M* (Fig. 3). Fifty percent inhibition of NLK bioactivity was obtained with gp120 at 20 ± 0.9 (SD) ng/ml (three experiments) and with PE3 at 18 ± 1.9 ng/ml (three experiments). The corresponding molar concentrations are $1.7 \times 10^{-10} M$ for gp120 and $6.6 \times 10^{-10} M$ for PE3. Thus the two proteins are potent inhibitors of NLK. At equimolar concentrations in the bioassay (1 n*M*), gp120 completely inhibits the bioactivity of NLK.

In a search of the protein or nucleic acid sequences of the National Biomedical Research Foundation (NBRF) and GenBank, we found that the only sequence with significant homology to NLK was a region of the gp120 envelope glycoprotein of HIV (Fig. 4).

The Lipman and Pearson algorithm FASTP (ktup: 1) (*22*) identified internal sequences within gp120 and NLK that align at 14 of 45 positions (31% homology). FASTP additionally scores replacements of chemically similar amino acids (glycine → valine) as well as replacements of amino acids that occur frequently in evolution (methionine → leucine or isoleucine). When such amino acid replacements are scored, the alignment increases to 73%. The best alignment is obtained without gaps. Similarities in secondary structure also are predicted for the two sequences. The Chou-Fasman algorithm (PepPlot implemented by the University of Wisconsin Genetics Computer Group) predicts prominent β-pleated sheets for the amino terminal regions of both sequences followed by α-helical domains toward their carboxyl termini.

Random alignments between NLK and sequences in the NBRF data bank generate a mean score of 28.8 ± 6.8 (SD), and conversion of the score for alignment with gp120 to a *z*-value [(76-28.8)/6.8] generates a score of 6.9. Lipman and Pearson set a $z \geq 6$ as probably significant and a $z \geq 10$ as highly significant (*22*). No other sequences in the NBRF data bank yielded *z* scores above 6. The carboxyl terminus of PE3 contains the region of sequence homology with NLK (Fig. 4).

The homology with NLK is conserved in all isolates of HIV-1 for which sequence information is available (Fig. 4) (*23*). Within gp120, a strong pattern of alternating variable and constant regions of sequence is observed. The first variable domain corresponds to the signal peptide for secretion of the primary translation product of the *env* messenger RNA. NLK is partly homologous to the carboxyl half of the second conserved domain of gp120, which is ≥80% homologous among different HIV-1 isolates (*23*). In comparison, sequence conservation is only 21 to 30% within the three variable domains of gp120. Small, in-frame insertions and deletions occur within the variable domains, as well as amino acid replacements due to point mutations (*23*). Thus, selective pressure presumably maintains the amino acid sequence within the two conserved domains of gp120, although their functional importance is not understood. Sequence variation within the second and third variable domains may not be constrained by functional requirements or may permit antigenic variation of gp120 during the course of a natural infection (*23*). Since tropism for CD4 cells is seen with all isolates of HIV-1 (*23, 24*), positive selection presumably maintains the sequences within gp120 necessary for infection and cell fusion. Both processes are mediated by binding of gp120 to the CD4 antigen (*25*).

Our experiments show that gp120 specifically inhibits the biological response of cultured neurons to NLK but not to NGF. Extension of these results to the etiology of AIDS dementia will depend on elucidating the role of NLK and other neurotrophic factors in the adult brain. Infection and killing of $CD4^+$ lymphocytes by HIV-1 may not entirely account for the immunodeficiency seen in AIDS (*26*). Since NLK is secreted by activated T lymphocytes and induces immunoglobulin secretion by cultured human peripheral blood mononuclear cells (*6*), gp120 may also interfere with the lymphokine function of NLK. If this contributes to the suppression of immune function in AIDS, it may account for the conservation of sequence homology to NLK among different HIV-1 isolates. If so, the brain may be an "innocent bystander" in AIDS, a chance target of HIV-1 due to viral

Table 4. Inhibition of growth of NLK-dependent sensory neurons by purified gp120 and a recombinant fragment of gp120. Immunoaffinity-purified gp120 (*19*) was used at 50 ng/ml; PE3 (gp120 43-279) (*20*) was used at 50 ng/ml; P121 (gp41 56-138) (*21*) and P3′ORF were used at 100 ng/ml. NLK was used at 1 n*M* and NGF at 3 n*M*. The culture wells were seeded with 2050 ± 120 (SD) cells scored 3 hours after plating. Values tabulated are neurons per well (mean ± SD) for four replicate culture wells. The data were collected as part of the experiment presented in Table 1. The experiment was repeated four times with similar results.

Neurotrophic factor	Control	gp120	PE3	P121	P3′ORF
NLK	1010 ± 56	290 ± 61*	310 ± 84*	960 ± 120	910 ± 75
NGF	1050 ± 140	980 ± 120	1060 ± 21	1070 ± 140	920 ± 68

*$P < 0.005$; paired one-tailed *t* test with Bonferroni correction against number of sensory neurons scored in the NLK control. The culture plates were coded and scored blind.

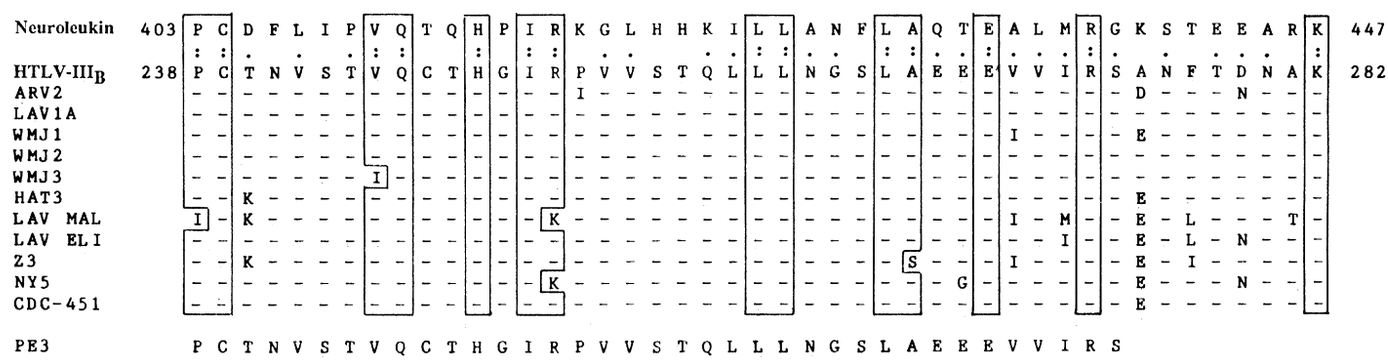

Fig. 4. Sequence homology between NLK and a conserved domain within the gp120 protein of HIV-1. Sequences of 12 different isolates are listed (*23*). Amino acid identities are indicated by double dots; conserved replacements are indicated by a single dot. The carboxyl terminal sequence of PE3 is indicated.

inhibition of a factor that is used by both the brain and the immune system.

REFERENCES AND NOTES

1. W. D. Snider *et al.*, *Ann. Neurol.* **14**, 403 (1983); R. M. Levy, D. E. Bredesen, M. L. Rosenblum, *J. Neurosurg.* **62**, 475 (1985); B. A. Navia, B. D. Jordan, R. W. Price, *Ann. Neurol.* **19**, 517 (1986); B. A. Navia, E-S. Cho, C. K. Petito, R. W. Price, *ibid.*, p. 525.
2. G. M. Shaw *et al.*, *Science* **227**, 177 (1985); J. A. Levy, J. Shimabukuro, H. Hollander, J. Mills, L. Kaminsky, *Lancet* **1985-II**, 586 (1985); D. D. Ho *et al.*, *New Engl. J. Med.* **313**, 1493 (1985).
3. S. Koenig *et al.*, *Science* **233**, 1089 (1986); C. A. Wiley, R. D. Schrier, J. A. Nelson, P. W. Lampert, M. B. A. Oldstone, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7089 (1986); M. H. Stoler, T. A. Eskin, S. Benn, R. C. Angerer, L. M. Angerer, *J. Am. Med. Assoc.* **256**, 2360 (1986); S. Gartner *et al.*, *Science* **233**, 215 (1986); D. D. Ho, T. R. Rota, M. S. Hirsch, *J. Clin. Invest.* **77**, 1712 (1986); D. H. Gabuzda *et al.*, *Ann. Neurol.* **20**, 289 (1986).
4. L. E. Mathes *et al.*, *Nature (London)* **274**, 687 (1978); L. E. Mathes *et al.*, *Cancer Res.* **39**, 950 (1979); E. A. Copelan *et al.*, *J. Immunol.* **131**, 2017 (1983); B. T. Rouse and D. W. Horohov, *Rev. Infectious Dis.* **8**, 850 (1986).
5. J. Goudsmit *et al.*, *Lancet* **1986-II**, 177 (1986); L. G. Epstein *et al.*, *Ann. Neurol.* **21**, 397 (1987); R. Yarchoan *et al.*, *Lancet* **1987-I**, 132 (1987).
6. M. E. Gurney, S. A. Heinrich, M. R. Lee, H.-S. Yin, *Science* **234**, 566 (1986); M. E. Gurney *et al.*, *ibid.*, p. 574.
7. R. Levi-Montalcini and B. Booker, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 373 (1960); *ibid.*, p. 384; H. Thoenen and D. Edgar, *Science* **229**, 238 (1985).
8. M. G. Menesini Chen, J. S. Chen, R. Levi-Montalcini, *Arch. Ital. Biol.* **116**, 53 (1978); M. C. Brown, R. L. Holland, W. G. Hopkins, *Annu. Rev. Neurosci.* **4**, 17 (1981); M. E. Gurney, *Nature (London)* **307**, 546 (1984).
9. D. Purves and A. Nja, *Nature (London)* **260**, 535 (1976); J. P. Schwartz, J. Pearson, E. M. Johnson, Jr., *Brain Res.* **244**, 378 (1984); K. M. Rich, H. K. Yip, P. A. Osborne, R. E. Schmidt, E. M. Johnson, Jr., *J. Comp. Neurol.* **230**, 110 (1984).
10. I. A. Hendry and J. Campbell, *J. Neurocytol.* **5**, 351 (1976); H. K. Yip, K. M. Rich, P. A. Lampe, E. M. Johnson, Jr., *J. Neurosci.* **4**, 2986 (1984); H. Gnahn, F. Hefti, R. Heumann, M. E. Schwab, H. Thoenen, *Dev. Brain Res.* **9**, 45 (1983); F. Hefti, *J. Neurosci.* **6**, 2155 (1986); L. R. Williams *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9231 (1986); L. F. Kromer, *Science* **235**, 214 (1987).
11. S. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 302 (1960); R. H. Angeletti and R. A. Bradshaw, *ibid.* **68**, 2417 (1971).
12. Y-A. Barde, D. Edgar, H. Thoenen, *ibid.* 77, 1199 (1980); M. R. Lee and M. E. Gurney, in preparation.
13. K. Herrup and E. M. Shooter, *J. Cell Biol.* **67**, 118 (1975); H. Rohrer and Y-A. Barde, *Dev. Biol.* **89**, 309 (1982); C. A. Raivich, A. Zimmerman, A. Sutter, *EMBO J.* **4**, 233 (1985).
14. P. I. Baccaglini and P. G. Hogan, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 594 (1983).
15. M. D. Daniel *et al.*, *Science* **228**, 1201 (1985); P. J. Kanki, J. Alroy, M. Essex, *ibid.* **230**, 951 (1985); H. Kornfeld, N. Riedel, G. A. Viglianti, V. Hirsch, J. I. Mullins, *Nature (London)* **326**, 610 (1987).
16. M. D. Daniel *et al.*, *Science* **223**, 602 (1984); M. Gravell *et al.*, *ibid.*, p. 74; P. A. Marx *et al.*, *ibid.*, p. 1083; K. G. Osborn *et al.*, *Am. J. Pathol.* **114**, 94 (1984).
17. M. E. Gurney, B. R. Apatoff, S. P. Heinrich, *J. Cell Biol.* **102**, 2264 (1986).
18. F. Barin *et al.*, *Science* **228**, 1094 (1985).
19. W. G. Robey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7023 (1986).
20. S. D. Putney *et al.*, *Science* **234**, 1392 (1986).
21. N. T. Chang *et al.*, *ibid.* **228**, 93 (1985).
22. D. J. Lipman and W. R. Pearson, *ibid.* **227**, 1435 (1985).
23. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985); M. A. Muesing *et al.*, *ibid.*, p. 450; S. Wain-Hobson, P. Sonigo, O. Danos, S. Cole, M. Alizon, *Cell* **40**, 9 (1985); R. Sanchez-Pescador *et al.*, *Science* **227**, 484 (1985); B. R. Starcich *et al.*, *Cell* **45**, 637 (1986); B. H. Hahn *et al.*, *Science* **232**, 1548 (1986); M. Alizon, S. Wain-Hobson, L. Montagnier, P. Sonigo, *Cell* **46**, 63 (1986); R. L. Willey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5038 (1986).
24. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500; F. Barré-Sinoussi *et al.*, *ibid.* **220**, 868 (1983).
25. J. S. McDougal *et al.*, *ibid.* **231**, 382 (1986); J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985); J. Sodroski, W. C. Goh, C. Rosen, K. Campbell, W. A. Haseltine, *Nature (London)* **322**, 470 (1986); J. D. Lifson *et al.*, *ibid.* **323**, 725 (1986).
26. D. Klatzmann and L. Montagnier, *Nature (London)* **319**, 10 (1986); A. S. Fauci, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9278 (1986).
27. D. D. Ho *et al.*, *Science* **226**, 451 (1984).

28. Supported by the Searle Scholars Program, the McKnight Foundation, the Muscular Dystrophy Association, grants from the University of California–wide Task Force on AIDS, and NIH grants 5P01 NS-21443 and K08-AI00685. We thank L. O. Arthur, Program Resources, Inc. and the NCI-Frederick Cancer Research Facility for immunoaffinity-purified gp120 and antisera to gp120; J. Ghrayeb, Centocor, Inc., for providing PE3, P121, and P3′ORF proteins; M. Daniel, New England Regional Primate Research Center, for providing SIV (macaque isolate); J. S. McDougal, Center for Disease Control, for providing HIV-1(LAV); and T. Perney, University of Chicago, for providing NGF.

23 April 1987; accepted 16 July 1987

Molecular Diversity of the Human T-Gamma Constant Region Genes

PIER GIUSEPPE PELICCI, MILAYNA SUBAR, ARTHUR WEISS, RICCARDO DALLA-FAVERA, DAN R. LITTMAN

The human T cell antigen-receptor γ chain, which is expressed on the surface of a subpopulation of CD3$^+$ T lymphocytes, exhibits size polymorphism and varies in its ability to form disulfide bonds with a second polypeptide. Analysis of both genomic and complementary DNA clones encoding the human γ polypeptide shows differences in lengths of the coding portions of the two constant region genes, Cγ1 and Cγ2. A single second-exon segment is always present in the Cγ1 gene. Cγ2 alleles containing either duplicated or triplicated second-exon segments are present in the normal human population and are expressed as messenger RNAs. Furthermore, a cysteine residue, encoded by the second exon of Cγ1 and probably involved in interchain disulfide bridging, is absent in all Cγ2 second-exon segments. These differences between Cγ1 and the two alleles of Cγ2 may explain the variability in molecular weight and disulfide bonding of γ molecules expressed in different cells.

MOST T LYMPHOCYTES EXPRESS AN antigen-receptor complex consisting of polymorphic α and β T cell receptor (TCR) subunits in association with several nonpolymorphic chains designated as CD3 (*1–3*). A subset of T cells that lack the surface αβ heterodimer express another receptor, known as γ, in association with the CD3 complex (*4–7*). The TCR γ molecules consist of variable (V), joining (J), and constant (C) regions, and, like immunoglobulins and the TCR α and β chains, are generated by somatic rearrangement of gene segments (*8–12*). Human cell lines representative of the subset containing TCR γ express γ chains of various sizes in association with another protein of 40 to 45 kD, designated δ (*4, 13–16*). In some cell lines the components of the heterodimers are noncovalently associated, and in other lines they are linked by disulfide bonds. In contrast, the murine γ chain has been found in all cases to be disulfide-linked to a second chain of 45 kD (*7*).

The human TCR γ constant region is composed of two tandemly organized and highly homologous genes, Cγ1 and Cγ2 (*9, 12*). Analysis of patterns of TCR γ gene rearrangements in T cells has shown that both the Cγ1 and Cγ2 genes take part in recombination events (*17*). We now show that these constant regions are structurally distinct and there is an allelic polymorphism for the number of exons in the Cγ2 gene. Taken together, these findings may explain the presence or absence of disulfide linkage as well as the size polymorphism of the human TCR γ chains.

Our experimental approach involved the isolation and characterization of several TCR γ complementary DNAs (cDNAs) and genomic clones of the TCR γ constant region locus. Three distinct cDNA libraries obtained from human T cells were screened with a mouse Cγ cDNA probe. Two cDNA libraries were prepared from the Fro 2.2 and PEER human T cell lines that display rearrangements of the Cγ2 gene (*17, 18*). The

P. G. Pelicci, M. Subar, R. Dalla-Favera, Department of Pathology and the Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016.
A. Weiss, Department of Medicine and Howard Hughes Medical Institute, University of California, San Francisco, School of Medicine, San Francisco, CA 94143.
D. R. Littman, Department of Microbiology and Immunology, University of California, San Francisco, School of Medicine, San Francisco, CA 94143.