specific for the remaining HBV-encoded antigens in multiple MHC backgrounds are needed to fully understand the relative ability of the response to each HBV antigen to cause liver cell injury in viral hepatitis. The current studies in the HBV transgenic mouse system also represent a model for the analysis of the immunobiology and pathogenesis of other viral diseases for which alternative experimental systems do not exist.

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10. Spleens were harvested from mice 1 week after the third immunization with 1×10^8 plaque-forming units (PFU) of vaccinia recombinant vKC1 at which time their antibody titer was 3.2×10^{-3}. The recombinant vaccinia virus vKC1 expresses the middle and major envelope polypeptides of HBV (subgroup adw) containing the preS(2) and HBs antigens (*14*). This construct is more immunogenic than vHBs4 with respect to the induction of HBsAg-specific CTLs (*16*). Nylon wool–adherent cells were incubated with MAb AT.83 (specific for Thy-1) plus rabbit complement (Cedarlane) to eliminate T cells [D. P. Dialynas *et al.*, *Immunol. Rev.* **74**, 645 (1983)]. Nylon wool nonadherent cells were further purified by incubation with MAb J11D (ATCC) plus rabbit complement to eliminate contaminating B cells. CTLs and helper T cells (Th) were further purified by the addition of MAbs RL172.4 [L3T4-specific; R. Ceredig, J. W. Lowenthal, M. Nabholz, H. R. MacDonald, *Nature* **314**, 98 (1985)] and 3.155 [Lyt 2-specific; D. P. Dialynas, above] plus complement, respectively.
Nontransgenic, syngeneic (B10D.2) mice were immunized twice with 1×10^8 PFU of vKC1. Primed spleen cells were cultured with irradiated (3000 rad) SV40-transformed transgenic hepatocytes (1×10^5 per well) and irradiated (3000 rad) syngeneic spleen cells (4×10^5 per well) in EHAA media (Gibco) that contained recombinant interleukin 4 (IL-4) [2 U/ml, a gift from H. Spiegelberg; L. Smith, *Nature* **326**, 798 (1987)] and IL-6 [32 U/ml, Boehringer Mannheim; M. Okada *et al.*, *J. Immunol.* **141**, 1543 (1988)] in 96-well flat-bottomed plates. Newborn hepatocytes from transgenic and nontransgenic pups were immortalized by transfection with SV40 DNA and were cultured [C. Woodworth, T. Secott, H. C. Isom, *Cancer Res.* **46**, 4018 (1986)]. The transgenic hepatocyte cell line secreted immunoreactive HBsAg into the culture supernatant. The transgenic hepatocytes were incubated with CTL line OH2 supernatant (10%) that contained gamma-interferon [O. Kanagawa, *J. Immunol. Methods* **110**, 169 (1988)] for 24 hours before incubation with primed spleen cells. Expression of MHC class I increased ten times, as observed by flow cytometric analysis with MAbs 34-5-8S and SF1-1.1.1 (ATCC), which are specific for H-2D^d and H-2K^d, respectively. Spleen cell cultures were restimulated weekly with irradiated SV40-transformed liver cells and feeder cells and with EL4 culture supernatant (5%) as a source of IL-2.
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HIV-1 Coat Protein Neurotoxicity Prevented by Calcium Channel Antagonists

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Coat protein gp120 from the human immunodeficiency virus type–1 (HIV-1) increased intracellular free calcium and injured rodent retinal ganglion cells and hippocampal neurons in culture. Highly purified recombinant gp120 envelope protein produced these effects in a dose-dependent fashion at picomolar concentrations. Immunoprecipitation with antibody to gp120, but not with control immunoglobulin-containing serum, depleted solutions of the viral envelope protein and also prevented both the rise in intracellular calcium and neuronal toxicity. The gp120-induced increase in intracellular calcium was abrogated by transiently lowering extracellular calcium or by adding the dihydropyridine calcium channel antagonist nimodipine (100 n*M*). Calcium channel antagonists also prevented gp120-induced neuronal injury. In addition, intracellular stores appeared to contribute substantially to the increase in calcium elicited by gp120. Since increases in intracellular calcium have been associated with neurotoxicity, it is possible that an injurious effect of gp120 on neurons might be related to this mechanism and that treatment with calcium channel antagonists may prove useful in mitigating HIV-1–related neuronal injury.

BOTH NATIVE AND RECOMBINANT VIral envelope protein gp120 of HIV-1 have been found, even in the absence of infectivity, to produce neuronal cell injury in the mammalian central nervous system, specifically in cultures of hippocampal (*1*) and retinal ganglion cell neurons (*2*). This finding may account at least in part for the neurological manifestations of dementia (*3*) and blindness (*4*) encountered in acquired immunodeficiency syndrome (AIDS). An increase in intracellular neuronal Ca^{2+} is associated with and apparently responsible for several forms of neurotoxicity, including that mediated by excitatory amino acids binding at the *N*-methyl-D-aspartate receptor (*5, 6*). Thus, a reasonable mechanism for gp120 neurotoxicity might be postulated to involve an increase in intracellular Ca^{2+}. Furthermore, since gp120 had been reported to increase intracellular Ca^{2+} in T lymphocytes (*7*), we decided to examine the effect of this viral envelope protein on Ca^{2+} in mammalian central neurons.

The concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was measured in postnatal rat retinal ganglion cells (*8*) by digital imaging microscopy with the Ca^{2+}-sensitive fluorescent dye fura 2 (*9*). Application of 200 p*M* of highly purified gp120 from a recombinant source (*10*) produced a striking increase in $[Ca^{2+}]_i$ (Fig. 1). Compared to control levels ($[Ca^{2+}]_i = 63 \pm 4$ n*M*, mean ± SEM, $n = 42$) obtained before the addition of coat protein, levels increased 33-fold

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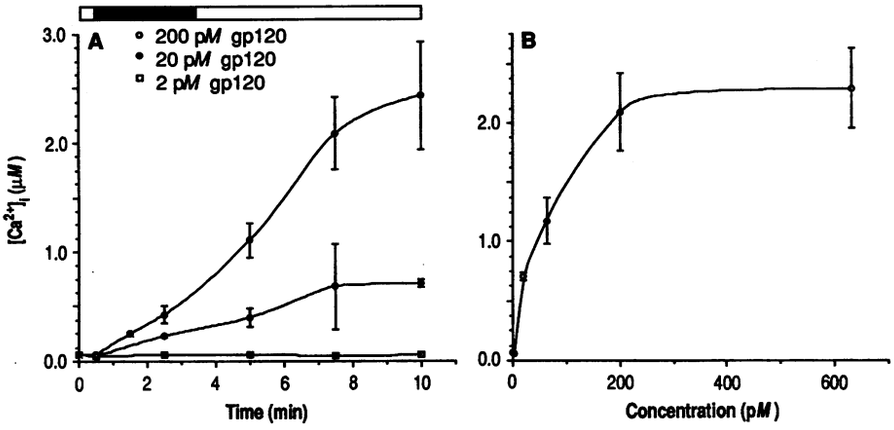

Fig. 1. HIV-1 envelope protein gp120 increases $[Ca^{2+}]_i$ in postnatal rat retinal ganglion cell neurons. Ganglion cells prepared by dissociation from the retina (*8*) were loaded with 10 μ*M* fura 2–AM (Molecular Probes) (*9*). Recombinant coat protein (rgp120-3B; 2 to 632 p*M*) was mixed in the bath solution and applied by pressure ejection from a microcapillary pipette (aperture 5 μm, 3 to 7 psi) located at a distance of ~10 to 20 μm from the neuronal cell body. The dose listed is that of gp120 in the pneumatic pipette. A somewhat smaller concentration would be expected to reach the neuronal surface. All experiments were performed at room temperature (21° to 24°C). (**A**) Kinetics of $[Ca^{2+}]_i$ in a retinal ganglion cell in response to various doses of gp120; the black bar at the top of the figure indicates a 3-min application of gp120. (**B**) Dose-response curve of gp120 concentration versus $[Ca^{2+}]_i$ (measured 7 min after addition of gp120, that is, 4 min after completion of a 3-min application). Values are mean ± SEM, $n = 24$.

within 7 min of gp120 application (2100 ± 330 n*M*, $n = 10$; range of values 934 to 3943 n*M*). Other preparations of gp120 purified from natural isolates (RF2 and 3B) produced similar results (*11*). All experiments shown here used the highly purified recombinant gp120. Similar effects were seen when gp120 was applied to hippocampal neurons (*12*). Overall, 200 p*M* gp120 produced an increase in $[Ca^{2+}]_i$ in 76% of the neurons tested ($n = 75$).

Several experiments indicated that gp120 was responsible for this rise in $[Ca^{2+}]_i$. Application of normal bathing medium did not produce a change in $[Ca^{2+}]_i$, although subsequent addition of gp120 to the same retinal ganglion cell neurons increased $[Ca^{2+}]_i$ to ~2 μ*M* ($n = 10$). Treatment of gp120 with trypsin followed by neutralization with soybean trypsin inhibitor (Sigma) resulted in a preparation that was no longer active in increasing $[Ca^{2+}]_i$. As the recombinant gp120 was produced from a construct with the herpes simplex virus glycoprotein D signal sequence (*10*), we applied glycoprotein D, made in exactly the same medium as recombinant gp120, to retinal ganglion cells ($n = 6$). Glycoprotein D exerted either no effect or resulted in a modest increase in $[Ca^{2+}]_i$ (<200 n*M*, $n = 6$), but never to the micromolar level typically observed after the addition of equimolar gp120.

Pressure ejection of 50 m*M* KCl on these neurons yielded an increase in $[Ca^{2+}]_i$ to the range of 600 n*M* (600 ± 99 n*M*, $n = 5$). Brief exposures to KCl for 30 s to 3 min produced these levels of Ca^{2+}, which peaked within 1.5 min of the beginning of the addition and recovered to levels of ~250 n*M* over the next few minutes (240 ± 21 n*M*, $n = 5$). In contrast, at least a 1-min addition of 200 p*M* gp120 was necessary to produce any rise in $[Ca^{2+}]_i$, and the effect was persistent and irreversible during the course of an experiment on a single cell (10 to 30 min of $[Ca^{2+}]_i$ monitoring, with measurements every 30 s). The time course of the change in $[Ca^{2+}]_i$ evoked by a 3-min application of various doses of gp120 is shown in Fig. 1A. The peak level was reached ~7 min after the beginning of the addition. Thus, there were both qualitative and quantitative differences in the observed increase in $[Ca^{2+}]_i$ in response to K^+ as opposed to gp120.

Extremely low doses of gp120, in the picomolar range, were effective in increasing $[Ca^{2+}]_i$ in a graded, dose-dependent fashion (Fig. 1B). As little as 20 p*M* gp120 produced increases in $[Ca^{2+}]_i$. Very high levels of free Ca^{2+} (≥2 μ*M*) were obtained with concentrations of gp120 at or above 200 p*M*.

The increases in $[Ca^{2+}]_i$ observed with gp120 could still have been caused by a contaminant in the purified preparation of the viral envelope protein, although this seems unlikely with the highly purified recombinant gp120 (*10*). As additional confirmation we performed immunoprecipitation experiments with goat antibody to gp120 (anti-gp120) coupled to protein A–coated Sepharose beads (*13*). One of three such experiments is shown in Fig. 2. Treatment with preimmune serum did not significantly alter the ability of gp120 to increase $[Ca^{2+}]_i$ (compare striped and grey bars: although the mean $[Ca^{2+}]_i$ was greater after application of gp120 treated with preimmune serum than after gp120 alone, this difference did not reach statistical significance). Both gp120 and preimmune serum–treated gp120 produced a significant increase in $[Ca^{2+}]_i$ compared to the control [($P < 0.01$, analysis of variance (ANOVA) followed by Scheffé multiple comparison of means; significance indicated by an asterisk]. In contrast, immunoprecipitation with postimmune serum containing anti-gp120 completely abrogated the gp120 effect (open bar).

We next studied whether alterations in extracellular or intracellular Ca^{2+} levels affect the action of gp120 on retinal ganglion cells. Free $[Ca^{2+}]_i$ (determined as in Fig. 1) did not vary when cells were incubated for 10 to 40 min in normal (2.5 m*M*) Ca^{2+} or in medium lacking added Ca^{2+}. Neurons bathed in a normal Ca^{2+} bath preceding

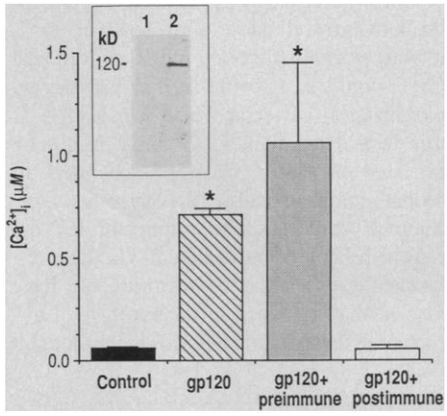

Fig. 2. Immunoprecipitation of gp120 with anti-gp120 prevents the rise in $[Ca^{2+}]_i$ in retinal ganglion cells engendered by the HIV-1 envelope glycoprotein. Neurons were loaded with fura 2 as described in Fig. 1. Values in each column represent the mean, with error bars indicating SEM, for $n = 42$, 4, 3, and 5 cells for columns 1 to 4, respectively. The solid bar displays the control $[Ca^{2+}]_i$ in the absence of the gp120 preparations represented in the other three bars. The Ca^{2+} levels shown were obtained 4 min after termination of a 3-min puffer application of the gp120 preparations. Immunoprecipitation was performed as described (*13*). (**Inset**) Immunoblot analysis demonstrating that immunoprecipitation with anti-gp120 depletes coat protein from the resulting preparation. Quantification of the amount of gp120 remaining after immunoprecipitation was performed by polyacrylamide electrophoresis and subsequent transfer to nitrocellulose for immunoblotting. Staining with the primary antibody (anti-gp120) was amplified with an avidin-biotin system to produce the necessary sensitivity for detection of small amounts of gp120. The result was a marked decrement in the gp120 concentration produced by immunoprecipitation (lane 1) compared to that observed after treatment with preimmune serum (lane 2, which contains a 120-kD band representing gp120).

gp120 application from a pipette that also contained normal Ca^{2+} responded with large increases in $[Ca^{2+}]_i$ (see above). When 200 p*M* gp120 was applied for 3 min from a puffer pipette containing no added Ca^{2+} or 5 m*M* EGTA to cells previously bathed in normal Ca^{2+} medium, there was no significant increase in $[Ca^{2+}]_i$ 4 min later ($[Ca^{2+}]_i = 0.11 \pm 0.04$ μ*M*). Thus, extracellular Ca^{2+} is necessary for gp120 to enhance $[Ca^{2+}]_i$. External Ca^{2+} could be important in an extracellular mechanism of action of gp120, such as binding, or might serve as a source for the increased $[Ca^{2+}]_i$. In an attempt to diminish intracellular Ca^{2+} stores, the cells were treated in a bath containing no added Ca^{2+} for 10 to 40 min (*14*). Subsequent application of 200 p*M* gp120 in normal Ca^{2+} to these cells produced a small but statistically insignificant increase in $[Ca^{2+}]_i$ (to 0.23 ± 0.07 μ*M*) (*15*). This result suggests that intracellular stores of Ca^{2+} contribute to the gp120-induced rise in $[Ca^{2+}]_i$.

Since the effect of gp120 was dependent on both extracellular and intracellular Ca^{2+}, it was possible that an influx of external Ca^{2+} would lead to the further increase by mobilization of intracellular stores (*16*). If this were true, then Ca^{2+} entry might be expected to occur via Ca^{2+} channels in retinal ganglion cells. Alternatively, Ca^{2+} channels could also be important for the replenishment of intracellular Ca^{2+} stores. Using the patch-clamp technique, we have shown that a major contributor to Ca^{2+} current in these central neurons is a prolonged

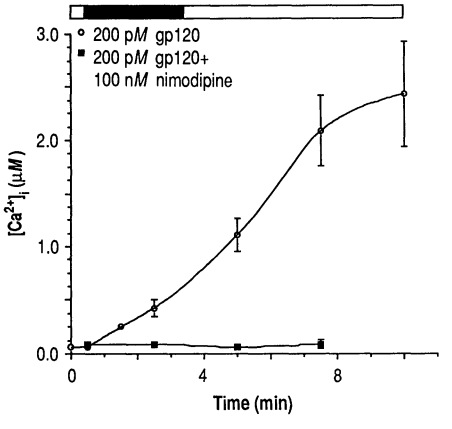

Fig. 3. The dihydropyridine Ca^{2+} channel antagonist, nimodipine, prevents a sustained increase in $[Ca^{2+}]_i$ after application of gp120. Retinal ganglion cells were loaded with fura 2 as in Fig. 1. The viral envelope protein gp120 (200 p*M*) was applied by puffer pipette to neurons previously bathed in normal medium or in medium containing 100 n*M* to 1 μ*M* nimodipine for several minutes (*24*). For neurons bathed in normal medium (○; $n = 10$), gp120 produced an increase in $[Ca^{2+}]_i$ as expected. In contrast, after treatment with nimodipine (■; $n = 11$), gp120 did not elicit a significant increase in $[Ca^{2+}]_i$.

component of the voltage-dependent Ca^{2+} current, which is sensitive to dihydropyridine antagonists such as nimodipine (*17*). In the present study when retinal ganglion cells were incubated in nimodipine (100 n*M*), gp120 no longer produced an increase in $[Ca^{2+}]_i$ (Fig. 3). Thus, entry of Ca^{2+} via dihydropyridine-sensitive channels appears to be important for the full manifestation of the gp120 effect or for its maintenance.

The exact mechanism whereby this viral envelope protein produces an increase in $[Ca^{2+}]_i$ in mammalian central neurons is not yet known. Whether gp120 is acting through a receptor, via a second messenger system, directly on Ca^{2+} channels, or even as a consequence of neuronal cell injury remains to be elucidated. In T lymphocytes, one report has shown that a natural isolate of gp120 increased inositol triphosphate (IP_3) and intracellular Ca^{2+} concentrations (*7*). Other investigators have been unable to confirm this finding with a recombinant gp120, similar to that used in our work (*18*). The gp120 coat protein binds to CD4, a lymphocyte surface protein that is a receptor for HIV-1 (*19*). However, the predominant form of CD4 in the mammalian brain is a

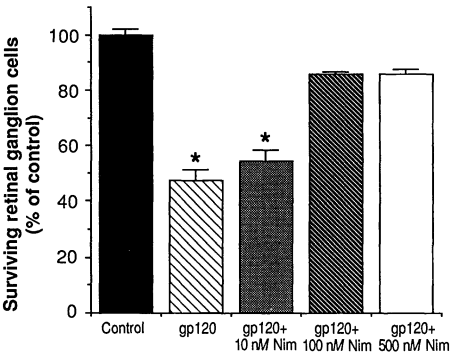

Fig. 4. Calcium channel antagonists prevent neuronal injury after exposure to gp120. Treated cultures received gp120 (20 p*M*) with or without nimodipine (10 to 500 n*M*) at the time of plating. Retinal ganglion cell survival was assayed 1 day later. The ability of retinal ganglion cells to take up and cleave fluorescein from fluorescein diacetate was used as an index of their viability and lack of injury as described previously (*6*). Incubation with gp120 resulted in significant neuronal injury compared to retinal ganglion cells in sibling control cultures. Treatment with 100 to 500 n*M* nimodipine prevented gp120-induced injury. Statistical testing with an ANOVA followed by Scheffé multiple comparison of means revealed the following rank order of results ($P < 0.01$ for each comparison): control = 500 n*M* nimodipine + gp120 = 100 n*M* nimodipine + gp120 > 10 n*M* nimodipine + gp120 = gp120. The asterisk indicates significant difference from control. Nifedipine (500 to 1000 n*M*) exerted a similar protective effect (*25*). Concentrations of the dihydropyridine Ca^{2+} channel antagonists ≤1 μ*M* had no effect on neuronal survival when administered by themselves, whereas doses >1 μ*M* were detrimental to survival and were therefore less useful (*25*).

truncated message that is apparently not expressed on neurons (*19, 20*). Along these lines, we found in retinal ganglion cells that antibody to rat CD4 did not prevent the increase in $[Ca^{2+}]_i$ evoked by gp120 (*21*). This implies that the action of gp120 causing an elevation of neuronal $[Ca^{2+}]_i$ may not involve CD4.

Whatever the mechanism for the marked increase in $[Ca^{2+}]_i$ produced by gp120 in central neurons, the fact that such a rise has been associated with neuronal cell death from various causes (*5, 6*) suggests that Ca^{2+} could be responsible, at least in part, for injury in the central nervous system of patients afflicted with AIDS. Figure 4 shows that Ca^{2+} channel antagonists, such as 100 n*M* nimodipine, not only abrogate the increase in $[Ca^{2+}]_i$, but also prevent neuronal injury mediated by gp120 in vitro (*22*). Although 100 n*M* plasma levels of nimodipine can be obtained with therapeutic doses of the drug, nimodipine is presently approved only for the treatment of neurological deficits associated with subarachnoid hemorrhage for a period of 21 days (*23*). The therapeutic potential of Ca^{2+} channel antagonists in the treatment of AIDS affecting the central nervous system is predicated on the premise that these findings in vitro reflect the sequence of events in vivo. This hypothesis is as yet unproven. Nevertheless, based on our data, we suggest that further research into this area is warranted.

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8. Retinal ganglion cells were cultured from 1- to 2-week-old Long Evans rats as described [D. Leifer *et al.*, *Science* **224**, 303 (1984); S. A. Lipton and D. L. Tauck, *J. Physiol.* **385**, 361 (1987)]. The ganglion cells were identified by the presence of retrogradely transported red fluorescent dyes (DiI or rhodamine-labeled microspheres) or by morphological and immunofluorescence criteria [the large or α-like ganglion cells stain with neurofilament antibodies; S. A. Lipton and D. L. Tauck, above; U. C. Dräger and A. Hofbauer, *Nature* **309**, 624 (1984)]. During Ca^{2+} measurements, unless otherwise stated the fluid bathing the neurons consisted of Hanks balanced salts: 137.6 m*M* NaCl, 1 m*M* $NaHCO_3$, 0.34 m*M* Na_2HPO_4, 0.44 m*M* KH_2PO_4, 5.36 m*M* KCl, 2.5 m*M* $CaCl_2$, 0.5 m*M* $MgSO_4$, 0.5 m*M* $MgCl_2$, 5 m*M* Hepes-NaOH, 22.2 m*M* glucose, and phenol red indicator (0.001% v/v); *p*H 7.2. Coat protein gp120 and other substances were usually applied to the neurons by pressure ejection after dilution in this

bath solution. High K^+ solutions were prepared by substituting KCl for NaCl.

9. Neuronal $[Ca^{2+}]_i$ was analyzed with fura 2–acetoxymethyl ester (AM) as described [G. Grynkiewicz *et al.*, *J. Biol. Chem.* **260**, 3440 (1985); D. A. Williams *et al.*, *Nature* **318**, 558 (1985); J. A. Connor *et al.*, *J. Neurosci.* 7, 1384 (1987); J. A. Connor *et al.*, *Science* **240**, 649 (1988); C. S. Cohan, J. A. Connor, S. B. Kater, *J. Neurosci.* 7, 3588 (1987); M. P. Mattson, M. Murrain, P. B. Guthrie, S. B. Kater, *ibid.* **9**, 3728 (1989)]. After adding Eagle's minimum essential medium containing 10 μ*M* fura 2–AM to retinal or hippocampal cell neurons, the cultures were incubated at 37°C in a 5% CO_2/95% air humidified chamber and then rinsed. The dye was loaded, trapped, and deesterified within 1 hour, as determined by stable fluorescence ratios and the effect of the Ca^{2+} ionophore ionomycin on measured $[Ca^{2+}]$. During Ca^{2+} imaging, the cells were incubated in a solution of Hepes-buffered saline with Hanks balanced salts (*8*). The $[Ca^{2+}]_i$ was calculated from ratio images that were obtained by measuring the fluorescence at 500 nm that was excited by 350 and 380 nm with a DAGE MTI 66 SIT camera mounted on a Zeiss Axiovert 35 microscope. Exposure time for each picture was 500 ms. Analysis was performed with a Quantex (Sunnyvale, CA) QX7-210 image-processing system. Since cells were exposed to ultraviolet light only during data collection (generally less than a total of 20 s per cell), bleaching of fura 2 was minimal.
10. Recombinant gp120 was produced by transfection of a Chinese hamster ovary (CHO) cell line with a plasmid containing the 3B envelope coding sequences from amino acids 61 to 531 [L. A. Lasky *et al.*, *Science* **233**, 209 (1986)]. The gene was truncated in this fashion to remove the native NH_2-terminal signal sequence and the COOH-terminal hydrophobic domains. This fragment was then ligated in-frame to the herpes simplex virus glycoprotein D signal sequence [P. W. Berman, T. Gregory, D. Crase, L. A. Lasky, *Science* **227**, 1490 (1985)] to allow the envelope protein to be constitutively secreted by the CHO cell line. Production in a mammalian cell ensured that the envelope protein was glycosylated. This envelope glycoprotein, rgp120-3B, was purified by immunoaffinity chromatography to greater than 99.9% purity.
11. E. B. Dreyer, P. K. Kaiser, S. A. Lipton, unpublished observations. Native gp120 was purified by immunoaffinity chromatography from two natural isolates, RF2 and 3B (*7, 13*) [S. W. Pyle *et al.*, *AIDS Res. Hum. Retrovir.* **3**, 387 (1988)]. The concentration of gp120 in the purified material was approximately 50% based on estimates from polyacrylamide gel electrophoresis (PAGE) and immunoblotting.
12. Hippocampal cortices of embryonic day 18 CD rats were dissociated with trypsin (0.027% w/v) and plated at a density of 600,000 cells per 35-mm culture dish, each dish containing five poly-L-lysine–coated glass cover slips. Growth medium [P. A. Rosenberg and E. Aizenman, *Neurosci. Lett.* **103**, 162 (1989)] was changed three times per week. In these experiments, Ca^{2+} measurements were made after 14 to 21 days in culture.
13. Immunoprecipitation of gp120 was performed as described (*7*) with some modifications. A 1:100 or 1:500 dilution of anti-gp120 or preimmune serum from the same goat, was bound to protein-A–coated Sepharose beads, washed, and incubated with a solution containing 7 n*M* gp120 for 18 hours at 4°C; this was followed by centrifugation. The supernatant of the material treated with preimmune serum had gp120 activity as evidenced by immunoblotting (Fig. 2, inset) and by producing an increase in $[Ca^{2+}]_i$ and cell death (after a dilution of 1:350 to ~20 p*M*); the material exposed to anti-gp120 had little or no activity.
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15. After a 10- to 40-min incubation in low Ca^{2+} solution, 50 m*M* K^+ applied in the presence of Ca^{2+} still produced a significant increase in $[Ca^{2+}]_i$, although not of the same magnitude as that observed when neurons were not incubated in nominally Ca^{2+}-free medium (*16*). The effect of K^+ under these conditions shows that the neurons are still capable of responding to external stimuli that lead to an increased influx of Ca^{2+}.
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21. Before Ca^{2+} imaging and the application of gp120, retinal neurons were incubated for 1 hour at 37°C with ascites containing W3/25 (~2.5 μg/ml) and MRC OX-35 (~2.5 μg/ml), two specific antibodies directed against the rat CD4 molecule. These concentrations of the antibodies are known to block effects mediated by high-affinity binding to the CD4 protein on the surface of rat T cells [W. A. Jeffries, J. R. Green, A. F. Williams, *J. Exp. Med.* **162**, 117 (1985)]. These antibodies do not compete with one another for binding to CD4, so they could be used simultaneously.
22. S. A. Lipton, P. K. Kaiser, J. T. Offermann, N. J. Sucher, E. B. Dreyer, in preparation. Both native and recombinant viral coat protein gp120 from HIV-1 were found to be toxic in a dose-dependent fashion at picomolar concentrations to rat retinal ganglion cell neurons in culture. Control experiments, similar to those performed in the Ca^{2+} imaging experiments, suggested that the lethal effects of the purified preparations of the envelope protein were caused by gp120 and not by a contaminant: (i) neurotoxicity could be abated by anti-gp120, but not by control preimmune sera; (ii) trypsin neutralized the deleterious effect of gp120 on retinal ganglion cells; (iii) at similar doses, glycoprotein D did not injure the neurons; and (iv) antibodies against CD4 (anti-CD4) did not prevent gp120-induced neuronal cell injury in our culture system. Although picomolar concentrations of gp120 appear to have potent effects, the actual level of gp120 shed by the virus in AIDS patients is unknown.
23. A. Scriabine, personal communication. In the presence of 5% rat serum in the culture medium used in our toxicity experiments (*6*), ≥96% of the nimodipine was protein bound, yielding a free concentration in the low nanomolar range.
24. Dihydropyridine antagonists have been shown to be more effective on depolarized neurons [B. P. Bean, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6388 (1984); M. C. Sanguinetti and R. S. Kass, *Circ. Res.* **55**, 336 (1984); A. M. Brown, D. L. Kunze, A. Yatani, *J. Physiol. (London)* **379**, 495 (1986)]. Retinal ganglion cells lying among small clusters of other neurons are known to experience frequent depolarizations because of synaptic inputs [S. A. Lipton, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9774 (1986)]. For this reason, the clustered neurons were examined for effects of nimodipine in these experiments. In the absence of nimodipine, the levels of intracellular Ca^{2+} of clustered retinal ganglion cells before and after application of gp120 were slightly greater than the corresponding levels in solitary cells; however this difference was not statistically significant, and in any case had no bearing on these results.
25. S. A. Lipton *et al.*, unpublished data.
26. We thank H.-S. Vincent Chen and L. Dawes for helpful discussions, S. Pyle and L. Arthur of the National Cancer Institute for providing purified isolates of native gp120-RF2 and gp120-3B, and L. Lasky, T. Gregory, P. Berman, and L. Riddle of Genentech for recombinant gp120-3B. We also thank W. Cruikshank and H. Kornfeld of Boston University Medical School for instructing us in their immunoprecipitation technique, T.-H. Lee for anti-gp120 and preimmune serum generated in his laboratory at Harvard Medical School, and A. F. Williams at the University of Oxford for antibodies to rat CD4. We are grateful to P. A. Rosenberg for the use of his hippocampal cultures and to E. Aizenman for reading an earlier version of the manuscript. Supported in part by grants from the NIH (EY 05477 to S.A.L. and NS 01395 to E.B.D.), by the Burroughs Wellcome Fund and National Society to Prevent Blindness (to E.B.D.), by Fight-for-Sight, Inc. of New York City (to P.K.K.), and by an Established Investigatorship of the American Heart Association (to S.A.L.).

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Defective Presentation of Endogenous Antigen by a Cell Line Expressing Class I Molecules

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Cytotoxic T lymphocytes (CTLs) recognize class I major histocompatibility complex (MHC) molecules associated with antigenic peptides derived from endogenously synthesized proteins. Binding to such peptides is a requirement for class I assembly in the endoplasmic reticulum (ER). A mutant human cell line, T2, assembles and transports to its surface some, but not all, class I MHC molecules. The class I molecules expressed on the surface of T2 do not present peptides derived from cytosolic antigens, although they can present exogenously added peptides to CTL. The transported class I molecules may interact weakly with an unknown retaining factor in the ER such that they can assemble despite the relative shortage of peptides.

THE PEPTIDES THAT ASSOCIATE WITH class I MHC molecules derive from viral or other proteins synthesized within the antigen-presenting cell (APC) (*1*), although the final subcellular location of the protein does not determine whether the peptides are presented in this way (*2*). Class I MHC–associated peptides can also be derived from proteins experimentally introduced into the APC cytoplasm (*3, 4*). The peptide–class I complex can be mimicked by the exogenous addition of short, synthetic peptides to APCs, probably by direct binding to MHC molecules at the surface (*5*).

Antigenic peptides probably associate with class I molecules in the ER (*6*). A mutant murine cell line, RMA-S, which fails

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