Cleveland, OH). The Intelligenetics Suite programs (Intelligenetics, Mountain View, CA) were used for data analysis. DNA blots: 10 to 15 μg of genomic DNA from either sperm [B. Hogan, F. Constantini, E. Lacy, in *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986), p. 107] or liver was digested with the indicated restriction enzyme, fractionated by electrophoresis through 0.9% agarose gels, blotted, and hybridized (*29*).

31. The restriction map of the λ4 clone was deduced from agarose gel band patterns of digests of the clone with individual restriction enzymes or pairs of enzymes. Plasmid subclones of the 5.7-kb and 3.7-kb Eco RI fragments and the 3.6-kb Bam HI–Hind III fragment were also analyzed. Mapping gels were blotted and hybridized to the entire F115 cDNA probe. Mouse repetitive DNA sequence elements were detected [M. Steinmetz *et al.*, *Cell* **24**, 125 (1981)] in a ~1.7-kb region between the Bgl II and Xba I sites as indicated by probing restriction digests of λ-phage clone DNA (1 μg per lane) with ^{32}P-labeled whole genomic DNA. A mouse CD23 cDNA [S. O. Gollnick *et al.* in (*29*)] containing repetitive elements in its 3′-untranslated region was used as a control.
32. Databases screened were the EMBL nucleic acid sequence bank release 20, Genbank release 61, Swiss Prot protein sequence database release 12, and Protein Identification Resource release 21. No other herpes virus sequences in the databases had any sequence with such a marked relationship to CSIF, although a weakly homologous sequence was found in the varicella zoster virus genome [A. J. Davison and J. E. Scott, *J. Gen. Virol.* **67**, 1759 (1986); nucleotides 31965 to 32133; 24% identity over 59 amino acids]. The search did not reveal any proteins with significant homology to the ORF in the 3′-untranslated sequence of F115, although we noted an isolated seven amino acid identity between this ORF (nucleotides 1144 to 1164; Fig. 3A) and amino acids 62 to 68 of a mouse T cell receptor Vβ sequence (D. L. McElligott, S. B. Sorger, L. A. Matis, S. M. Hedrick, *J. Immunol.* **140**, 4123 (1988).
33. The authors thank E. Mocarski, C. Martens, A. O'Garra, R. Kastelein, H. Rugo, and P. O'Hanley for helpful discussions, F. Vega for oligonucleotides, L. Thompson-Snipes, A. Zlotnik, and V. Dhar for providing cells for RNA preparation, Y.-H. Chien for providing the mouse genomic DNA library, and D. Nomura for assistance in homology searches. P.V. was supported by a DNAX Research Institute postdoctoral fellowship. DNAX Research Institute is supported by Schering-Plough Corporation.

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Induction of CD4$^+$ Human Cytolytic T Cells Specific for HIV-Infected Cells by a gp160 Subunit Vaccine

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Cytolytic T lymphocyte (CTL) responses were evaluated in humans immunized with recombinant human immunodeficiency virus type 1 (HIV) envelope glycoprotein gp160. Some vaccinees had gp160-specific CTLs that were shown by cloning to be CD4$^+$. Although induced by exogenous antigen, most gp160-specific CTL clones also recognized gp160 synthesized endogenously in target cells. These clones lysed autologous CD4$^+$ T lymphoblasts infected with HIV. Of particular interest were certain vaccine-induced clones that lysed HIV-infected cells, recognized gp160 from diverse HIV isolates, and did not participate in "innocent bystander" killing of noninfected CD4$^+$ T cells that had bound gp120.

THE HOST RESPONSE TO VIRAL INfection depends on the lysis of infected host cells by virus-specific CTLs (*1*). Several candidate HIV vaccines are soluble recombinant forms of the HIV envelope glycoproteins gp160 and gp120 (*2*). Soluble protein antigens do not normally elicit a CD8$^+$ CTL response because the processing pathways for exogenous protein antigens do not allow association of these antigens with class I major histocompatibility complex (MHC) molecules (*3, 4*). Soluble protein vaccines, therefore, probably will not elicit CD8$^+$, class I–restricted CTLs like infection with HIV does (*5, 6*). Instead, exogenous protein antigens associate with class II MHC and are recognized by CD4$^+$ T cells (*7*). Because some virus-specific CD4$^+$ T cell clones have cytolytic activity (*8*), soluble subunit vaccines may also elicit specific CTLs. However, the usefulness of such CD4$^+$ CTLs in limiting the spread of viral infection will depend on whether the processing of endogenously synthesized viral protein in infected cells permits association of viral antigen with class II MHC and subsequent recognition by CD4$^+$ CTLs induced with soluble exogenous forms of the same viral protein. In other viral systems, the processing of endogenously synthesized viral proteins for recognition by CD4$^+$ T cells has been documented in some (*9*), but not other (*3, 10*), cases.

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We analyzed the CTL response in human volunteers immunized with an HIV subunit vaccine that consisted of a purified recombinant form of the envelope glycoprotein precursor, gp160 (*11*). Healthy HIV-seronegative volunteers received intramuscular injections of 40 or 80 μg of recombinant gp160 (MicroGeneSys, Inc., West Haven, Connecticut) with alum at 0, 1, 6, and 18 months (*12*). Other volunteers received alum alone or hepatitis B vaccine according to the same immunization schedule. CTL responses were evaluated immediately before and 2 weeks after the 18-month boost. Peripheral blood mononuclear cells (PBMC) from vaccinees and controls were stimulated in vitro with gp160 and then tested 7 days later for cytolytic activity against autologous Epstein-Barr virus–transformed B lymphoblastoid cell lines (B-LCL) pulsed with gp160 (Fig. 1). Cultures from normal, nonimmune, HIV-seronegative donors (n = 10) and from control vaccinees immunized with alum (n = 2) or with a hepatitis B vaccine (n = 3) lacked antigen-specific CTL activity. The culture from one of eight gp160 vaccinees was positive for gp160-specific cytolytic activity immediately before the 18-month boost, even though this volunteer received only 40 μg of antigen and it had been over 1 year since the last boost. When retested 2 weeks after the boost, three of eight volunteers had gp160-specific CTL activity, including the volunteer who was positive before the boost. Thus repeated immunization with low doses of soluble HIV envelope protein induced a CTL response in a significant fraction of those immunized, and in some cases activity persisted for over 1 year.

To characterize the cells responsible for the gp160-specific cytolytic activity, positive cultures were restimulated with antigen and then cloned by limiting dilution; all positive cultures were successfully cloned (Table 1). All of the clones had the CD4$^+$CD8$^-$ phenotype. The isolation of CD4$^+$ clones was not due to selection against CD8$^+$ cells during the cloning procedure because positive bulk cultures consisted almost exclusively of CD4$^+$ lymphoblasts (*14*). The cytolytic activity of vaccine-induced gp160-specific T cell clones was class II MHC-restricted.

In infected cells, the envelope protein precursor gp160 is cleaved by a cellular protease into an NH_2-terminal fragment, gp120, that contains the CD4 binding site and a COOH-terminal fragment, gp41, that contains the hydrophobic fusion and transmembrane anchor domains (*15*). These subunits remain noncovalently associated on the surface of infected cells and on the envelope of HIV virions. HIV isolates have sequence variability in both subunits, but it

is predominantly in gp120 (*16*). To determine whether CTL clones induced with gp160 recognized determinants in gp120 or gp41, clones were tested for lysis of B-LCL targets pulsed with purified recombinant gp160 or gp120 (*11*) or with a fusion protein containing a portion of gp41 (*17*). Six of 11 vaccine-induced CTL clones recognized epitopes on the gp120 subunit (Table 1). The remaining clones responded to gp160 but not gp120. One of these clones, C.28, recognized a determinant contained within residues 546 to 645 of gp41.

Although vaccine-induced $CD4^+$ CTL clones lysed target cells that had taken up and processed exogenous envelope protein, the potential in vivo efficacy of these CTLs depends on whether they lyse infected cells that are synthesizing the envelope protein endogenously. Therefore, vaccine-induced CTL clones were tested for lytic activity against target cells infected with recombinant vaccinia virus vectors carrying the HIV *env* gene (*18*) (Fig. 2A). The CTL clone B.8 lysed autologous B-LCL targets pulsed with soluble gp120 and targets infected with a vaccinia vector carrying the HIV *env* gene (vac-*env*). Targets cells infected with a control vaccinia vector lacking the HIV *env* gene (vac) were not lysed. Thus the HIV envelope protein can be processed for recognition by vaccine-induced $CD4^+$ CTLs when it is synthesized within the target cell, a result consistent with previous mechanistic studies (*19*). Not all vaccine-induced clones, however, recognized endogenously synthesized gp160 (Table 1). Because many vaccine-induced $CD4^+$ CTL clones lysed target cells that were synthesizing gp160 endogenously, we tested their lysis of HIV-infected T cells. Using the mitogen phytohemagglutinin (PHA), we generated $CD4^+$ T cell clones of irrelevant specificity from two different vaccine recipients. These clones (B.15 and C.30) were infected with HIV and then used as targets in assays with autologous gp160-specific CTL clones. Under the conditions of infection used (*20*), target cells were uniformly positive for expression of HIV gag proteins by indirect immunofluorescence analysis on permeabilized cells with monoclonal antibodies to p24 (Fig. 2B). The target T cell clones, B.15 and C.30, after infection with HIV, were lysed by autologous vaccine-induced CTL clones (B.8 and C.28, respectively) in an MHC-restricted fashion (Fig. 2C). Uninfected targets were not lysed. To evaluate the possibility that some of the observed lysis of HIV-infected cells was due to the uptake and processing of exogenous gp120 released by infected cells, noninfected target clones were pulsed with heat-inactivated, noninfectious supernatants from HIV-in-

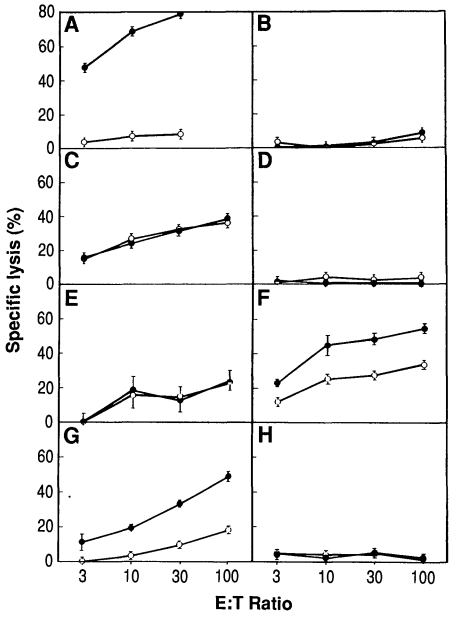

Fig. 1. Detection of gp160-specific CTLs in cultures from gp160 vaccine recipients. Ficoll-Hypaque purified PBMC from vaccinees and controls were stimulated in vitro with gp160 as described (*13*). After 7 days, responding cells were assayed for cytolytic activity against autologous B-LCL pulsed with a baculovirus control supernatant (○) or with gp160 (●) at 60 μg/ml for 16 hours at 37°C. CTL activity was measured in a standard ^{51}Cr release assay (*24*). Source of effector cell populations: (**A**) The $CD4^+$ gp120-specific CTL clone Een217 that recognizes amino acids 410 to 429 of gp160 (PV22 isolate) in association with the human class II molecule DR4 (*13*) was used as a positive control. (**B** and **C**) Representative day 7 cultures from two different nonimmune, HIV-seronegative volunteers. (**D**) Representative day 7 culture from a hepatitis B vaccinee. (**E**) Representative negative culture from a gp160 vaccinee. Cultures negative for gp160-specific CTL activity on day 7 remained negative following repeated in vitro antigen stimulation, demonstrating that day 7 cultures provided an accurate assessment of the magnitude of the CTL response. (**F**) Positive day 7 culture from a gp160 vaccinee tested immediately before the 18-month boost. (**G**) Restimulated day 14 culture from the same vaccinee. Cultures positive on day 7 invariably remained positive following further in vitro antigen stimulation. (**H**) Freshly isolated PBMC from a gp160 vaccinee whose day 7 culture was positive. The detection of lytic activity required in vitro antigen stimulation because even in gp160-vaccinees with a positive response, freshly isolated PBMC had no specific CTL activity. In contrast, HIV-specific CTL activity can often be detected without an in vitro restimulation step in freshly isolated PBMC from HIV-seropositive individuals (*5, 6*).

Table 1. Properties of gp160-specific CTL clones. CTL clones were derived by limiting dilution cloning from all vaccine recipients with positive bulk culture assays (*23*). Clones from two of these donors (B and C) were highly stable upon in vitro culture, and their properties are summarized below along with the properties of the described (*13*) gp120-specific clone Een217 that was isolated from a nonimmune, HIV-seronegative adult (donor A). The phenotype of the clones was determined by indirect immunofluorescence as previously described (*13*). MHC restriction was determined in blocking studies with monoclonal antibodies to class I (W632) and class II (MHM33). All clones were $CD4^+CD8^-$ and class II-restricted. Cytolytic activity of gp160-specific T cell clones was measured in standard ^{51}Cr release assays (*24*) at effector to target (E:T) ratios of 10:1. Similar results were obtained at E:T ratios of 30:1. Targets cells were autologous B-LCL or autologous $CD4^+$ T cell clones of irrelevant specificity. Targets were pulsed with the indicated recombinant proteins (*11, 17*) or infected with the indicated vaccinia virus vectors carrying the HIV *env* gene (vac-*env*) (*18*) or with HIV (*20*). Indicated in parentheses are the HIV isolates used for infection of targets or as a source of the *env* gene for production of protein or recombinant vaccinia vectors. Results are expressed in terms of percent antigen-specific lysis of target cells which is the percent specific lysis of antigen-bearing targets cells minus the percent specific lysis of appropriate control target cells. For target cells pulsed with recombinant envelope proteins, control targets were cells pulsed with media alone. For target cells infected with vaccinia vectors carrying the HIV *env* gene, control targets were cells infected with the control vaccinia vector vSC8 (vac) that lacks the HIV *env* gene. For HIV-infected target cells, control targets were mock-infected $CD4^+$ T cell clones; +++, antigen-specific lysis of >50%; ++, antigen-specific lysis of 25 to 50%; +, antigen-specific lysis of 15 to 25%; −, antigen-specific lysis of <10%.

Clone	Donor	Antigen-specific cytolysis							
		B-LCL targets						$CD4^+$ T cells	
		gp160 (BRU)	gp120 (BRU)	546–645 (BRU)	gp120 (SF2)	vac-*env* (BH8)	vac-*env* (MN)	gp120 (BRU)	HIV (IIIb)
Een217	A	+++	+++	−	+	+++	NT*	++	+
B.5	B	+++	+++	−	−	−	−	−	NT
B.8	B	+++	+++	−	−	+++	−	++	+++
B.10	B	++	+	−	−	−	−	−	NT
B.12	B	+++	+++	−	−	−	−	−	NT
B.14	B	+++	+++	−	−	+	NT	++	NT
B.16	B	+++	++	−	−	−	−	−	NT
C.22	C	+++	−	−	−	++	NT	−	NT
C.23	C	+++	−	−	−	++	NT	−	NT
C.28	C	+++	−	+++	−	+++	++	−	++
C.29	C	+++	−	−	−	+	NT	−	NT
C.31	C	++	−	−	−	+	NT	−	NT

*NT, not tested.

fected targets and then tested for susceptibility to lysis by autologous CTL clones (Fig. 2C). The gp120-specific clone B.8 did lyse noninfected autologous $CD4^+$ lymphoblasts pulsed with supernatants from infected cultures, but less well than HIV-infected cells. The gp41-specific clone C.28, as expected, did not lyse supernatant-pulsed cells. These data demonstrate that certain vaccine-induced CTL clones can recognize endogenously synthesized gp160 and directly lyse HIV-infected cells.

Assessment of the potential efficacy of vaccine induced CTLs is further complicated by the noncovalent nature of the association between gp120 and gp41 that allows gp120 to spontaneously dissociate; after release from gp41, gp120 can bind to CD4. Noninfected, activated $CD4^+$ T cells can take up and process exogenous gp120 for association with class II MHC gene products and can then be lysed by gp120-specific $CD4^+$ CTLs (*13*). To determine whether vaccine-induced CTLs could also mediate the destruction of noninfected $CD4^+$ T cells in the presence of gp120, activated autologous $CD4^+$ T cell clones of irrelevant specificity were pulsed with gp120, labeled with ^{51}Cr, and then used as targets in cytolytic assays with vaccine-induced CTL clones (Table 1). Two of 11 vaccine-induced CTL clones lysed noninfected autologous $CD4^+$ T cells pulsed with gp120. As expected, this reaction was observed for gp120-specific clones, but not for gp41-specific clones.

The most useful vaccine-induced CTLs would be able to recognize gp160 from divergent HIV strains. Cross-reactivity on purified gp120 from the divergent SF2 strain was observed for clone Een217, which recognizes a partially conserved gp120 epitope (*13, 21*). Other vaccine-induced clones with specificity for determinants in gp120 showed little reactivity toward SF2 gp120, indicating that some human T cell epitopes in gp120 are localized to the variable regions of the protein (Table 1). For the gp41-specific clone C.28, cross-reactivity was tested using a recombinant vaccinia vector carrying the *env* gene of the divergent MN isolate. This clone was cross-reactive on cells expressing MN envelope protein (Fig. 3), a result consistent with the lower degree of variability in the gp41 portion of the *env* gene. Thus some vaccine-induced CTL clones recognize highly conserved regions of the envelope protein.

Analysis of the immune response induced by the soluble gp160 vaccine revealed several unexpected results. First, even though all of the T cell clones isolated from vaccine recipients had the $CD4^+$ phenotype, most of them were cytolytic. Second, our data demonstrate that when the HIV envelope protein is synthesized in infected cells, it is processed for recognition by $CD4^+$ T cells. Thus, envelope protein subunit vaccines can elicit in humans CTLs active against HIV-infected cells. The mechanism by which endogenously synthesized envelope protein is processed for recognition by $CD4^+$ CTLs is unclear, but may involve the expression of the protein on the cell surface followed by internalization and processing in an endocytic compartment (*19*). Detailed studies of the specificity of vaccine-induced CTL clones revealed the existence of several different patterns of responsiveness (Table 1). Most importantly, these studies led to the identification of a subset of vaccine-induced CTL clones (such as C.28) with a combination of properties likely to be particularly advantageous in vaccine development: the ability to lyse HIV-infected cells, cross-reactivity on diverse HIV-1 isolates, and failure to mediate the gp120-dependent destruction of noninfected $CD4^+$ T cells. We propose that subunit vaccines that contain the extracellular portion of gp41, a region that is known to be immunogenic (*22*), may preferentially induce CTL clones of this type. In summary, although the induction of virus-specific CTL responses is traditionally associated with attenuated live viral vaccines, our results demonstrate that envelope protein subunit vaccines, which have greater inherent simplicity and safety, may also induce CTLs active against HIV-infected cells.

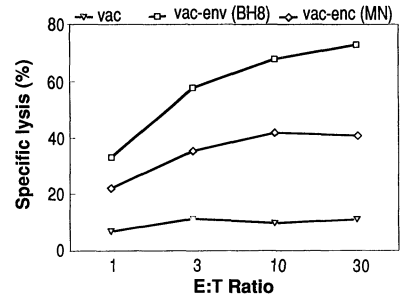

Fig. 3. Lysis of target cells expressing the HIV *env* gene from a divergent HIV strain. The vaccine-induced CTL clone C.28 was tested for cytolytic activity against autologous B-LCL pulsed with media alone, or infected with vac-*env* vectors carrying HIV *env* gene from the BH8 isolate or from the divergent MN isolate, or with the control vaccinia vector (vac). The BH8 *env* sequence is similar to that of the BRU isolate used in the preparation of the gp160 vaccine.

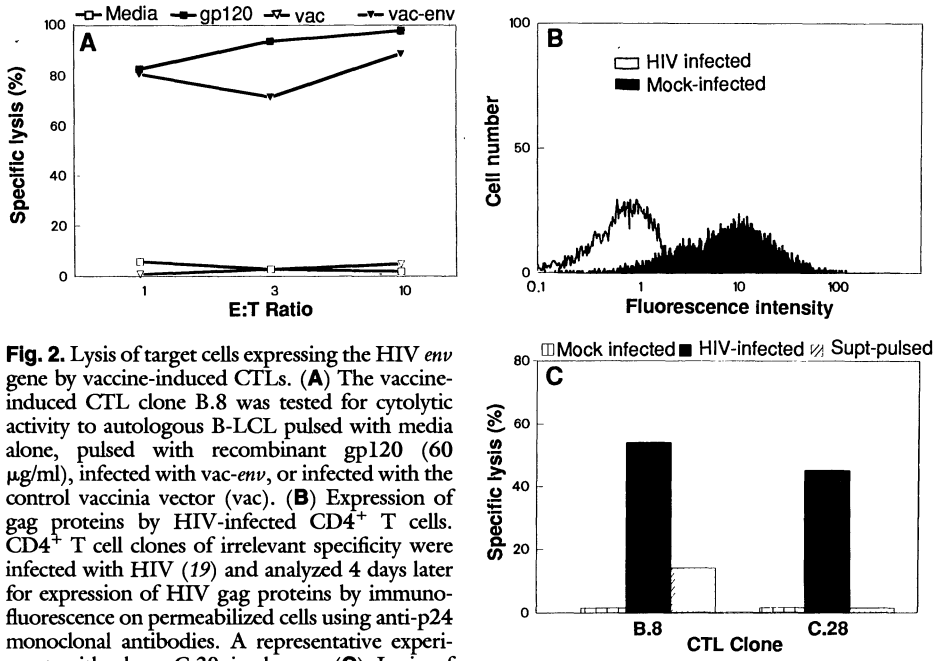

Fig. 2. Lysis of target cells expressing the HIV *env* gene by vaccine-induced CTLs. (**A**) The vaccine-induced CTL clone B.8 was tested for cytolytic activity to autologous B-LCL pulsed with media alone, pulsed with recombinant gp120 (60 μg/ml), infected with vac-*env*, or infected with the control vaccinia vector (vac). (**B**) Expression of gag proteins by HIV-infected $CD4^+$ T cells. $CD4^+$ T cell clones of irrelevant specificity were infected with HIV (*19*) and analyzed 4 days later for expression of HIV gag proteins by immunofluorescence on permeabilized cells using anti-p24 monoclonal antibodies. A representative experiment with clone C.30 is shown. (**C**) Lysis of HIV-infected $CD4^+$ T cells by vaccine-induced CTL clones. Targets were mock-infected T cell clones, clones infected with HIV, or clones pulsed with heat-inactivated (56°C, 30 min) supernatants from HIV-infected cells. Lysis by autologous vaccine-induced CTL clones was measured 4 days after infection in a standard 8-hour ^{51}Cr release assay. The E:T ratio was 1:1. Similar results were obtained at higher E:T ratios.

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11. Recombinant gp160 was produced in *Spodoptera frugiperda* cells infected with a recombinant baculovirus expression vector in which the HIV *env* gene (BRU isolate) was placed under transcriptional control of the *Autographa californica* Nuclear Polyhedrosis virus polyhedrin promotor. Recombinant gp120 was produced in a similar fashion (R. Dolin *et al.*, in preparation). Recombinant gp120 from the SF2 isolate was produced in transfected Chinese hamster ovary cells (N. L. Haigwood *et al.*, in preparation) and was obtained from N. Haigwood and K. Steimer through the AIDS Reference and Research Reagent Program, NIAID, NIH. Recombinant gp160 and 120 were used to pulse adherent stimulator and B-LCL target cells at 60 μg/ml. Recombinant gp120 was used to pulse $CD4^+$ T cells at 10 μg/ml.
12. Volunteers were immunized as with gp160 in a phase I vaccine trial organized by the NIAID AIDS Vaccine Clinical Trials Network. The design of the trial, including provisions for informed consent, will be described elsewhere (R. Dolin *et al.*, in preparation).
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17. A fragment of gp41 consisting of residues 546 to 645 of the envelope protein of the BRU isolate was expressed as a fusion protein in *Escherichia coli* using a glutathione S-transferase fusion protein expression vector (Glutagene, Medos Co., Victoria, Australia). Affinity-purified fusion protein was used to pulse B-LCL at a concentration of 60 μg/ml.
18. The use of vaccinia virus vectors for the expression of the HIV-1 *env* gene has been described [S. Chakrabarti, M. Robert-Guroff, F. Wong-Staal, R. C. Gallo, B. Moss, *Nature* **320**, 535 (1986)]. We used the vectors vPE7 and vPE16 which contain the *env* gene of IIIb-derived BH8 clone of HIV. Control infections were carried out with the vaccinia vector vSC8 (vac), which lacks the HIV *env* gene. Target cells were infected with recombinant vaccinia viruses at a multiplicity of infection of 10 for 12 to 16 hours at 37°C prior to labeling with ^{51}Cr and use in the cytolytic assay. Strain specificity was investigated using a vaccinia vector carrying the *env* gene from the MN strain (*6*).
19. Using a vaccinia vector carrying a truncated form of the HIV *env* gene with a stop codon at the end of the gp120 coding sequence, we showed that infected B-LCL that produced and secreted a large amount of gp120 did not process the protein for recognition by $CD4^+$, gp120-specific CTLs. Rather, attachment to a membrane anchor sequence was critical for delivery of gp120 to the compartment where this processing occurs [M. Polydefkis *et al.*, *J. Exp. Med.* **171**, 875 (1990)].
20. Three days after activation with PHA, targets were mock-infected or infected with HIV. Infections were done by resuspending the target clones in cell-free supernatants of supT1 cells infected 7 days previously with HIV-1 (IIIb isolate). After 4 hours at 37°C, cells were washed and then cultured in interleukin-2 (IL-2)–containing media. Infected cells were used as targets in CTL assays 4 days after exposure to HIV. At this time, infected cells showed no evidence of cytopathic effects and were uniformly positive for gag expression.
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23. Day 7 PBMC cultures positive for gp160-specific CTL activity were restimulated with irradiated (5000 R), gp160-pulsed autologous monocytes and cultured for an additional week in the presence of IL-2. Responding cells were then cloned by limiting dilution following stimulation with irradiated gp160-pulsed autologous monocytes or with PHA and irradiated allogeneic PBMC. Clones were screened for gp160-specific cytolytic activity as described above. Procedures for the characterization and long-term culture of human T cell clones have been previously described (*13*).
24. Cytolytic activity was measured as described (*13*) except that the assay period was 8 hours. All determinations were performed in quadruplicate. Data are expressed in the form of the mean + SEM of the % specific lysis determined as described [R. F. Siliciano, A. D. Keegen, R. Z. Dintzis, H. M. Dintzis, H. S. Shin, *J. Immunol.* **135**, 906 (1985)]. For all CTL assays, the SEM of the percent specific lysis was generally <5%.
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Light Pulses That Shift Rhythms Induce Gene Expression in the Suprachiasmatic Nucleus

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Lighting cycles synchronize (entrain) mammalian circadian rhythms by altering activity of cells in the suprachiasmatic nucleus (SCN) of the hypothalamus, a circadian pacemaker. Exposure of hamsters and rats to light pulses at those phases of the circadian rhythm during which light can shift the rhythm caused increased immunoreactivity for the product of the immediate-early gene c-*fos* in cells in the region of the SCN that receives retinal fibers. Light pulses also increased messenger RNA for the Fos protein and for the immediate-early protein NGFI-A in the rat SCN. Similar increases in mRNA for NGFI-A were seen in the SCN of hamsters. Thus cells in this portion of the SCN undergo alterations in gene expression in response to retinal illumination, but only at times in the circadian cycle when light is capable of influencing entrainment.

DAILY (CIRCADIAN) RHYTHMS ARE a pervasive feature of mammalian physiology and behavior (*1*). They are controlled by a system that includes a central pacemaker (or clock), which generates an endogenous near 24-hour periodicity, and an entrainment mechanism, which responds to environmental lighting cycles by adjusting the period of this rhythm to precisely 24 hours and synchronizing its phase to local time. The entrainment mechanism operates by producing daily phase shifts in the pacemaker, primarily in response to light exposure between dusk and dawn; the circadian system is relatively insensitive to light during the day (*2*).

In mammals, the dominant pacemaker for many daily rhythms is the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus immediately dorsal to the optic chiasm (*3*) (Fig. 1A). Two visual projections reach the SCN, one originating in the retina (*4, 5*) and one originating in a retinorecipient area of the lateral geniculate nuclei [the intergeniculate leaflet (IGL) and adjacent

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