(Fig. 1). Other membrane components, such as minor glycoproteins and macroglycolipids, probably also are present, and in principle could contribute to the inhibition. However, because band 3 is the major component and liposomes are effective at low concentrations, a reasonable working hypothesis is that band 3 is the active component in these liposomes. The precise mechanism of inhibition by band 3 and glycophorin remains to be established. Explanations based on toxicity to parasites or alteration of erythrocyte membrane properties are unlikely, since controls with band 3 from a different species or with liposomes alone were much less effective. A plausible interpretation is that liposomes containing band 3 bind to surface sites on the parasites and thereby block their attachment to band 3 on the erythrocyte membrane.

Participation of band 3 in invasion is not surprising, since this cell-surface protein is present in 1 million copies per cell. Band 3 is the principal component of intramembrane particles visualized by freeze-fracture electron microscopy (*8*). Attachment of band 3 to merozoite surface components could explain the rearrangement of intramembrane particles into a ring surrounding a particle-free region that occurs at the junction between merozoite and erythrocyte membrane (*12*). Band 3 is attached on the cytoplasmic surface of the membrane to the spectrin-actin membrane cytoskeleton by linkage to ankyrin (*13*). Thus removal of band 3 from the particle-free zone (the site of merozoite entry) would also clear this region of the spectrin meshwork and allow penetration of the parasite.

Malarial parasites infect many vertebrate species, including reptiles, birds, and mammals. It is likely that different species share some fundamental features of the process of invasion. Band 3 has closely related homologues in these species and may represent a common receptor for all malarial strains. It is pertinent to note that band 3 has been implicated in invasion of rhesus monkey erythrocytes by *P. knowlesi* (*7*).

It will be important to identify the putative band 3 receptor of *P. falciparum* merozoites. Such a protein would be the logical target for vaccines against malaria.

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Trans-Acting Transcriptional Regulation of Human T-Cell Leukemia Virus Type III Long Terminal Repeat

Abstract. *Human T-cell leukemia virus type III (HTLV-III) was recently identified as the probable etiologic agent of the acquired immune deficiency syndrome (AIDS). Here it is shown that, in human T-cell lines infected with HTLV-III, gene expression directed by the long terminal repeat sequence of this virus is stimulated by more than two orders of magnitude compared to matched uninfected cells. The rate of transcription of the HTLV-III long terminal repeat is more than 1000 times that of the SV40 early promoter in one infected cell line. Thus, HTLV-III, like HTLV-I, HTLV-II, and the bovine leukemia virus, is characterized by* trans*-activation of transcription in infected cells. The efficiency of* trans*-activation in the case of HTLV-III may account, at least in part, for the virulent nature of HTLV-III infection.*

The human T-cell leukemia viruses (HTLV) are retroviruses associated with disorders of the OKT4$^+$ (helper) subset of T lymphocytes. HTLV type I (HTLV-I) is the probable etiologic agent of adult T-cell leukemia (*1*). HTLV type II (HTLV-II) is a rare isolate originally derived from a patient with a T-cell variant of hairy cell leukemia (*2*). HTLV-III is the probable etiologic agent of the acquired immune deficiency syndrome (AIDS), a disease characterized by depletion of the OKT4$^+$ cell population (*3*). In cells infected with HTLV-I or HTLV-II, the rate of transcription of heterologous genes directed by the viral long terminal repeat (LTR) sequences is greatly augmented (*4*). This phenomenon, called *trans*-acting transcriptional regulation, is also shared by bovine leukemia virus (BLV), a virus that appears to be structurally and functionally related to HTLV-I and -II (*5*). The genomes of HTLV-I, HTLV-II, and BLV have a long open reading frame (LOR) located between the *env* gene and the 3′ LTR

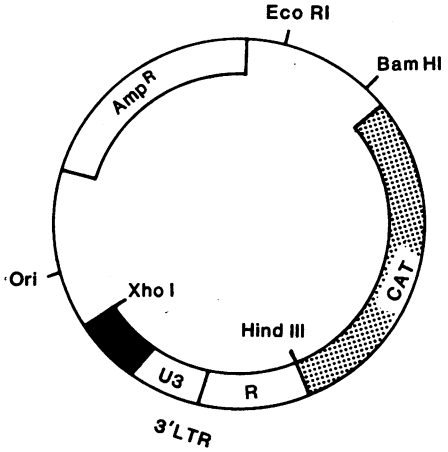

Fig. 1. Construction of pU3R-III plasmid. The diagram depicts the pU3R-III plasmid (Amp^R, ampicillin resistance gene; ori, bacterial origin of replication). The pU3R-III plasmid was constructed by isolating from the HTLV-III LTR cDNA clone C15 an Xho I–Hind III fragment containing the entire U3 and approximately 75 nucleotides of the R region (*11*). This fragment was inserted into the Xho-I–Hind III vector fragment of pSVIXCAT as previously described (*4*). Approximately 180 nucleotides of viral sequence 5′ to the LTR are included in the inserted fragment (solid black box). All recombinant DNA techniques were performed according to the enzyme manufacturer's specifications. Plasmids were purified by centrifugation in $CsCl_2$ gradients prior to transfection.

Table 1. Relative CAT activity in transfected cells. The percentage conversion of chloramphenicol to its acetylated forms in cells transfected with HTLV-CAT recombinants was normalized against the percentage conversion in similar cells transfected with pSV2CAT. The values represent the percentage acetylation per unit time relative to that directed by pSV_2CAT. The pU3R-I, pU3-II, and pBLV-CAT plasmids contain the U3 sequences of the LTR's of HTLV-I, HTLV-II and BLV, respectively, located 5′ to the CAT gene as described (*4, 5*). The experiments were repeated a minimum of two times each with less than 20 percent variation in the values reported. ND, not determined.

Cell line	Description	pU3R-III	pU3R-I	pU3-II	pBLV-CAT	pSV_2CAT
	Cells not infected with HTLV-III					
HOS	Human osteosarcoma cells	2.0	0.9			1.0
HOS/PL	HTLV-I–infected HOS cell line	1.6	75			1.0
FLK	Fetal lamb kidney cells	1.9	1.6			1.0
FLK-BLV	BLV-infected FLK cells	2.5	0.9			1.0
CCC S+L−	Feline epithelial cells	1.2	0.7			1.0
Raji	Human B-lymphocyte line	2.5	2.0			1.0
MT2	HTLV-I–infected human T lymphocyte line	1.4	130			1.0
C81-66-45	HTLV-I–immortalized nonproducer T-lymphocyte line	2.8	76			1.0
C3-44	HTLV-II producer T-lymphocyte line	1.7	105			1.0
	Uninfected and HTLV-III–infected cell lines					
H9	Human T lymphocytes	3.6	2.5	<0.1	<0.1	1.0
H9/HTLV-III	Human T lymphocytes infected with HTLV-III	1160	3.2	<0.1	<0.1	1.0
L8460D Jurkat	Human T lymphocytes	1.8	ND	ND	ND	1.0
L8460D Jurkat HTLV-III	Human T lymphocytes infected with HTLV-III	500	ND	ND	ND	1.0

that is not related by primary sequence to vertebrate genomic DNA (*6*). Proteins encoded by the LOR region of HTLV-I and II were recently identified (*7*). We have proposed that the protein product of the LOR region mediates *trans*-acting transcriptional regulation as well as some of the biological effects of virus infection (*4*). HTLV-III is distantly related to HTLV-I and -II by primary nucleotide sequence (*3, 8*) and by antigenic cross reactivity between the 24,000 dalton (p24) proteins and envelope proteins (*9*). In addition, there is a sequence distantly related to the LOR genes of HTLV-I and II near the 3′ terminus of the HTLV-III genome (*8*). These observations prompted us to examine the ability of the HTLV-III LTR to function in uninfected cell lines and cell lines infected with members of the HTLV-BLV family.

The transcriptional control elements of retroviruses lie within the U3 region of the LTR (*10*). To examine the ability of the HTLV-III LTR to act as a promoter, we inserted the entire U3 and about 75 nucleotides of the R region from an HTLV-III complementary DNA (cDNA) clone (*11*) 5′ to a chloramphenicol acetyltransferase (CAT) gene (Fig. 1) (*12*). This plasmid (pU3R-III) was introduced into eukaryotic cells via transfection and the level of CAT activity, which is correlated with steady-state CAT messenger RNA levels (*4, 12, 13*), was measured 48 hours after transfection. The CAT activity was normalized to that observed following transfection of pSV_2CAT, a plasmid that contains the SV40 early region promoter 5′ to the CAT gene (*12*), to control for differences in the ability of different cell types to take up and express foreign DNA.

The results show that the HTLV-III LTR functions as an efficient promoter in a variety of animal and human lymphoid and nonlymphoid cell lines (Table 1). Apparently, the HTLV-III LTR, like the HTLV-I LTR (*4*), is not dependent on virus-associated *trans*-acting factors for efficient promoter activity, and is not functionally restricted to lymphoid cells. There was no significant stimulation of CAT activity directed by pU3R-III in cells infected with HTLV-I, HTLV-II, or BLV. We conclude that the *trans*-acting transcriptional factors associated with HTLV-I, HTLV-II, or BLV (*4, 5*) do not stimulate gene expression directed by the HTLV-III LTR.

We then examined whether the HTLV-III LTR might be transcriptionally activated by factors present in HTLV-III-infected cells. The pU3R-III plasmid was transfected into uninfected human T-cell lines and matched cell lines infected in vitro with HTLV-III (*3*) (Table 1 and Fig. 2). The HTLV-III LTR was about three to four times as efficient as the SV40 early promoter at directing CAT activity in the uninfected H9 lymphocytes. In contrast, there was a dramatic increase in the CAT activity directly by the HTLV-III LTR in the infected H9 cells. The level of CAT activity of pU3R-III in HTLV-III–infected H9 cells was more than 1100 times that of pSV_2CAT. This relative increase in the

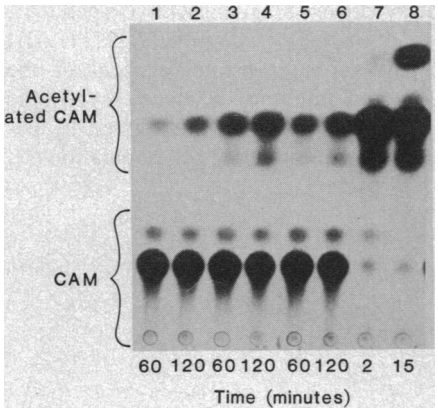

Fig. 2. Transient expression of the CAT gene in uninfected and HTLV-III–infected cells. Uninfected and HTLV-III–infected H9 cells (*3*) were transfected by a modification of the DEAE-dextran technique (*16*). Approximately 5×10^6 cells were washed once in serum-free medium and resuspended in 3 ml of serum-free medium containing 250 μg of DEAE-dextran per milliliter and 50 m*M* tris-chloride, *p*H 7.3 to which 5 to 10 μg of plasmid DNA had been added. After incubation at 37°C for 1 hour, cells were centrifuged, rinsed in serum-free medium, and resuspended in medium supplemented with fetal bovine serum. Cells were harvested 48 hours after transfection and cellular extracts were prepared by freeze-thawing three times. After removal of cell debris by centrifugation, extracts (100- to 200-μg protein content) were analyzed for CAT activity as described (*12*), with 3 m*M* acetyl coenzyme A being used in the reaction mix. Percentage conversion of chloramphenicol to the acetylated forms was determined by ascending thin-layer chromotography and liquid scintillation counting of spots cut from the plate. The figure depicts a typical CAT assay. Lanes 1 to 4, uninfected H9 cells; lanes 5 to 8, H9 cells infected with HTLV-III. The plasmids used for transfection and the duration of the CAT enzymatic assay were as follows: pSV_2CAT, 60 minutes (lane 1) and 120 minutes (lane 2); pU3R-III, 60 minutes (lane 3) and 120 minutes (lane 4); pSV_2CAT, 60 minutes (lane 5) and 120 minutes (lane 6); and pU3R-III (lanes 7 and 8). Note that the time course of the CAT enzymatic assay for lanes 7 and 8 was only 2 minutes and 15 minutes, respectively.

ability of the pU3R-III plasmid to direct CAT gene expression was also seen in another lymphocyte line, Jurkat L8460D, infected in vitro with HTLV-III (*14*) (Table 1). As the majority of transiently expressing DNA is extrachromosomal, factors present in HTLV-III–infected H9 cells that modulate gene expression by the HTLV-III LTR must act in *trans*. We conclude that factors associated with HTLV-III viral infection *trans*-activate transcription directed by the HTLV-III LTR.

To examine whether the *trans*-acting factors present in HTLV-III–infected cells could stimulate CAT gene expression directed by other HTLV-BLV LTR's, we introduced plasmids that contain the U3 region of HTLV-I (pU3R-I), HTLV-II (pU3-II), and BLV (pBLV-CAT) (*4, 5*) into HTLV-III–infected and uninfected H9 cells. In all cases there was no significant difference in the level of CAT activity in infected and uninfected cells (Table 1). We conclude that the viral *trans*-acting factors in HTLV-III–infected H9 cells do not transcriptionally activate the LTR's of HTLV-I, HTLV-II, or BLV.

The magnitude of the transcriptional activation of the HTLV-III LTR in the infected H9 and Jurkat cell lines is noteworthy. Relative to the activity of the SV40 promoter, the level of CAT activity directed by the HTLV-III LTR in the infected cells was about five to ten times the maximum level of CAT activity directed by the HTLV-I or HTLV-II LTR's and about 50 to 100 times that observed for the BLV LTR in the appropriate infected cell type (*4, 5*). Efficient *trans*-activation of the viral LTR may be related to the high levels of virus production observed in some HTLV-III–infected cell lines (*3*). High levels of virus production may be responsible, at least in part, for the ability of HTLV-III to establish a chronic viremia, which is unusual for the other HTLV's or BLV (*1, 15*).

Transcriptional *trans*-activation of the LTR in infected cells places HTLV-III in the retroviral family that includes HTLV-I, HTLV-II, and BLV, in accord with demonstrated similarities among the genomes and proteins of these viruses (*3, 8, 9*). The ability of these viruses to alter the transcriptional environment of the host cell suggests that they might exert their phenotypic effects via transcriptional regulation of specific host cellular genes. The specific activation of LTR sequences of the infecting virus suggests that sequences recognized by HTLV-III–associated *trans*-acting factors differ from those recognized by factors present in HTLV-I– or HTLV-II–infected cells. The cellular genes regulated by such factors might also differ, leading to cell death consequent to infection by HTLV-III and to cell proliferation following infection by HTLV-I or HTLV-II.

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Sequence Homology and Morphologic Similarity of HTLV-III and Visna Virus, a Pathogenic Lentivirus

Abstract. *A study was conducted of the genetic relation between human T-cell lymphotropic retroviruses and visna virus. The human T-cell lymphotropic viruses include those associated with T-cell malignancies (HTLV-I and HTLV-II) as well as the etiologic agent of the acquired immune deficiency syndrome (HTLV-III). Visna virus, a slowly replicating and pathogenic but nononcogenic retrovirus of sheep, is a member of the subfamily Lentivirinae. Results obtained by molecular hybridization and heteroduplex analysis indicated that a greater extent of nucleotide sequence homology exists between HTLV-III and visna virus than between HTLV-III and any of the other viruses. The homology observed under conditions of low stringency spanned the entire genome, but was strongest in the* gag/pol *region. The morphogenesis and fine structure of HTLV-III and visna virus also demonstrated striking similarities. The data provide strong evidence for a close taxonomic and thus evolutionary relation between HTLV-III and the Lentivirinae subfamily.*

The family Retroviridae consists of animal viruses that contain an RNA-dependent DNA polymerase (or reverse transcriptase). These viruses replicate by way of DNA intermediates and often integrate into the host genome and can, in some instances, be transmitted through the germ line. Their gross morphogenesis is similar in that an encapsidated viral RNA genome is released