at least five times more surviving cones than heavily attacked trees. In 1983, a mast year, the total number of mature, undamaged cones averaged 20.7 ± 11.90 cones per tree for shrublike trees in comparison to 103.5 ± 30.94 cones per tree for upright trees ($t = 2.499$, d.f. = 38, $P < 0.01$). Although the cone crops in 1982 and 1984 were substantially lower, the same patterns held. Uninfested, upright trees produced 10.3 times more cones in 1982 and 8.2 times more cones in 1984 than infested shrublike trees ($t = 4.236$, d.f. = 38, $P < 0.01$; $t = 2.625$, d.f. = 38, $P < 0.01$, respectively). Since these measures of cone production span both low and mast cone production years, it appears that infested trees do not compensate for previous years of low cone production.

Although the most heavily infested trees lose their female cone-bearing function, they do not compensate for this loss by increasing production of male strobili. No statistically significant relation was detected between male strobili production and shoot mortality ($r^2 = 2.0$ percent, $n = 40$, $P >> 0.05$).

This acute and chronic herbivory by a native pest resulted in significant changes in plant architecture, reduced growth rates and cone crops, and caused a shift in sexual expression. Changes in these traits should have important evolutionary implications for the host (for example, evolution of resistance, allocation strategies for the use of plant defenses, or alteration of the mating system in which the cost of female function has increased relative to male function), the herbivore (for example, counter-adaptations against plant defenses), and the rest of the community (for example, the mutualistic relation between pinyon jay, the major seed disperser, and pinyon may be altered by the reduced cone crop). Since some plants suffer the negative effects of herbivory while others largely escape, the latter should enjoy greater reproductive success and realize a selective advantage.

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20. Because *D. albovitella* is sensitive to Cygon for only a brief time, insecticide applied 2 weeks later than optimum has little control effect. Five trees sprayed after the optimum for herbivore control showed no significant decline in herbivory the next year and no associated changes in bud and conelet production.
21. Analyses of soils (derived from volcanic ash and cinders) from the root zone show no significant differences in soil moisture or particle size between trees with different growth forms or infestation levels.
22. Although *D. albovitella* attacks and kills second-year cones, first-year conelets are not attacked. Thus censuses of conelets should accurately reflect cone initiation in the spring.
23. Author order was decided by a coin toss. We thank A. Mutuura for species confirmation and V. Artman, R. Balda, W. J. Boecklen, S. L. Cohn, D. Conklin, L. Floyd, L. von der Heydt, W. J. Mattson, A. Neas, M. Pederson, P. W. Price, K. Reinhard, A. M. Robinson, D. Takahashi, and M. R. Wagner for assistance in the field and commenting on the manuscript. Supported by USDA grant 84-CRCR-1-1443, NSF grant DEB-8005602, and Organized Research and the Bilby Research Center of Northern Arizona University. We thank the Forest Service for permission to conduct our studies in the Coconino National Forest.

8 November 1984; accepted 26 February 1985

Major Glycoprotein Antigens That Induce Antibodies in AIDS Patients Are Encoded by HTLV-III

Abstract. *Antibodies from the serum of patients with the acquired immune deficiency syndrome (AIDS) or with the AIDS-related complex and from the serum of seropositive healthy homosexuals, recognize two major glycoproteins in cells infected with human T-cell lymphotropic virus type III (HTLV III). These glycoproteins, gp160 and gp120, are encoded by the 2.5-kilobase open reading frame located in the 3′ end of the HTLV-III genome, as determined by amino terminus sequence analysis of the radiolabeled forms of these proteins. It is hypothesized that gp160 and gp120 represent the major species of virus-encoded envelope gene products for HTLV-III.*

Human T-cell lymphotropic viruses (HTLV) are a group of exogenous retroviruses that have been implicated in a variety of clinical syndromes (*1–5*). HTLV-III, which is the probable etiologic agent of the acquired immune deficiency syndrome (AIDS) (*4, 5*) has several characteristics in common with HTLV-I and -II. These characteristics include an apparent tropism for $OKT4^+$T cells (*4, 6*), a reverse transcriptase with Mg^{2+} preference (*4, 7*), an ability to *trans-*

Table 1. 5′ Nucleotide sequence and predicted amino acid sequence of the HTLV-III envelope gene region. The HTLV-III sequence is derived from Ratner *et al.* (*17*). The dark arrow indicates the site for cleavage of the leader sequence from the envelope glycoproteins resulting in the protein species gp160 and gp120. Asterisks indicate cysteine, leucine, and valine residues determined by radiolabel sequence analysis. The first nucleotide presented corresponds to nucleotide sequence 5802 of the HTLV genome.

```
ATG AGA GTG AAG GAG AAA TAT CAG CAC TTG TGG AGA TGG GGG TGG AGA TGG GGC ACC ATG
 M   R   V   K   E   K   Y   Q   H   L   W   R   W   G   W   R   W   G   T   M20

CTC CTT GGG ATG TTG ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC AGA GTC TAT TAT
 L   L   G   M   L   M   I   C   S   A ▼ T   E   K   L*  W   V*  T   V*  Y   Y40

GGG GTA CCT GTG  TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA GCA
 G   V*  P   V*   W   K   E   A   T   T   T   L*  F   C*  A   S   D   A   K   A60

TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC
 Y   D   T   E   V*  H   N   V*  W   A
```

activate retroviral transcription in infected cells (*8*), and an association with immunosuppression (*4, 9, 10*). That HTLV-I and HTLV-II are highly related is indicated by their primary nucleotide sequences (*11, 12*) and their serological cross-reactivities (*5, 13*). HTLV-III, which is presumably closely related to the lymphadenopathy-associated virus (*14*), contains limited regions of nucleic acid homology with the HTLV-I (*15–17*), partial serological cross-reactivity for the major *gag* and *env* gene products (*5, 10, 18*), and most recently has been shown to contain regions of homology with lentiviruses (*19*).

According to cell membrane immunofluorescence and immunoprecipitation studies with serum samples from infected individuals, the most immunogenic proteins of HTLV-I and HTLV-II are cell surface–expressed glycoproteins (*12, 18, 20*). These glycoproteins are derived from the *env* gene of HTLV, gp61–68 for type I and gp67 for type II (*12*), and are thought to be precursor envelope proteins that may subsequently be processed to an exterior glycoprotein (gp46–52) and smaller transmembrane protein (gp21 or 22) (*12*). The most immunogenic proteins recognized in HTLV-III–infected cells by the sera of patients with AIDS or AIDS-related complex (ARC), hemophiliacs, and exposed healthy homosexuals are also glycoproteins (*21*) of approximately 160 kD (gp160) and 120 kD (gp120). Using Western blot techniques, investigators at other laboratories have reported an additional protein (p41) which is predominant in virus preparations and is thought to be a glycoprotein (*5*). P41 is only weakly reactive in our cellular preparations when analyzed by radioimmunoprecipitation.

Recently, HTLV-III proviruses were cloned from an HTLV-III–infected H9 cell line (*16*). HTLV-III contains, in addition to *gag* and *pol*, a 2.5-kilobase open reading frame located in the 3′ end of the genome corresponding to the *env* and *lor* gene regions of HTLV I and HTLV-II (*17*). The nucleotide and amino acid sequence for the first 210 nucleotides of this region are given in Table 1. We report that the major glycoproteins gp160 and gp120, recognized by the sera of HTLV-infected individuals, are encoded at least in part by this 2.5-kb open reading frame of HTLV-III.

To determine the HTLV-III–specific coding region for gp160 and gp120, we analyzed the sequence of the NH_2-terminus by Edman degradation of the proteins labeled with [^{3}H]leucine and [^{35}S]cysteine. The proteins were prepared by immunoprecipitation and isolation from sodium dodecyl sulfate (SDS)–polyacrylamide gels. Gp160 and gp120 gave the same sequence of labeled amino acids for the first 40 degradation cycles (Fig. 1). Leucine peaks were observed at positions 4 and 22 and a cysteine peak was observed at position 24. The repeti-

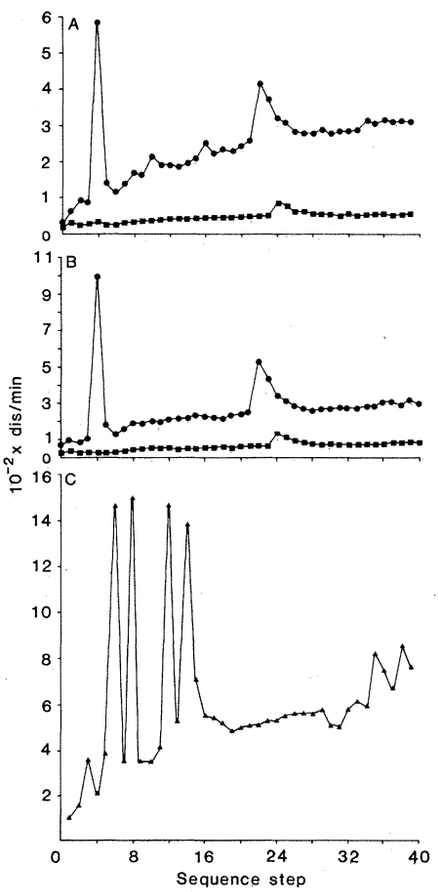

Fig. 1. Amino acid sequence analysis of the NH_2-terminus of the HTLV-III glycoproteins gp160 (A) and gp120 (B) labeled with [^{35}S]cysteine and [^{3}H]leucine and subjected to Edman degradation as described (*18, 24*). Radioactivity is shown for each degradation cycle: ●, [^{3}H]leucine; ■, [^{35}S]cysteine. Leucine peaks were observed at positions 4 and 22, and a cysteine peak was determined at position 24. Gp120 and gp160 were also labeled with [^{3}H]-valine as shown in (C) for gp160. Valine peaks were observed at 6, 8, 12, 14, 35, and 38 (▲). Proteins were purified from radioimmunoprecipitated HTLV-infected-cell, lysate by SDS-PAGE as described (*18*). Briefly, approximately 30×10^6 HTLV-III–infected cells (H9) were radiolabeled with 10 mCi of [^{3}H]leucine and 5 mCi of [^{35}S]cysteine or 10 mCi of [^{3}H]valine for 8 hours in appropriate media. Cell lysates were cleared with 200 μl of normal human serum prior to immunoprecipitation with 200 μl of reference HTLV-III–positive serum and the eluted proteins were subjected to electrophoresis on 10 percent SDS–polyacrylamide gels. The protein bands were then cut from the gel, electroeluted, dialyzed, and lyophilized before being sequenced.

tive yield for these sequences was gr
er than 93 percent. A number of mi
radioactive peaks were evident in
gp160 profile and are believed to
related to contaminating proteins. Gp
and gp120 were also labeled with [^{3}H]
line and again sequenced from the N
terminus. Valine peaks were eviden
both at positions 6, 8, 12, 14, 35, and
(Fig. 1C). Comparing this result with
deduced amino acid sequence based
the primary nucleotide sequence
HTLV-III, we conclude that gp160
gp120 are encoded by the 2.5-kb o
reading frame of HTLV-III. When
compares the leucine, cysteine, and
line positions in the first 40 amino ac
of gp120 and gp160 to the predic
sequence (Table 1), one finds a perf
match. The probability of finding suc
sequence in any given HTLV-spec
protein by chance alone is less t
$(1/19)^{1+2+6} \times (17/20)^{40-9}$ or 1.97×10
An initation codon is present 30 am
acids upstream from the NH_2-termin
based on the nucleotide sequence.
analogy with results previously obtai
with HTLV-I and HTLV-II (*10*),
because of their relative hydrophobic
these 30 residues probably represent
signal sequence which is removed dur
glycosylation.

To demonstrate a precursor-prod
relationship for these glycoproteins,
performed pulse-chase studies. HTI
III–infected cells were starved for 1 h
in cysteine-free medium and then m
bolically labeled for 5 minutes v
[^{35}S]cysteine (0.2 mCi/ml). The c
were then chased with excess cystein
complete medium, harvested at 0,
0.4, 1, 2, 4, and 8 hours, immunoprec
tated and subjected to SDS-PAG
Gp160 was initially the most promin
glycoprotein species observed, wher
gp120 became discernible only afte
hours, with a concurrent loss of gp1
Furthermore, we compared the gly
proteins seen in virus preparations v
those of cell lysates. When we immu
precipitated [^{35}S]cysteine-labeled p
teins from virus harvested at 12 ho
and concentrated from cell culture
pernatants, only gp120 was observed
contrast, both gp160 and gp120 w
seen in the cell lysates which sugge
that gp120 is a more mature envel
gene product.

The 2.5-kb open reading frame
HTLV-III is predicted to code fo
protein of approximately 90 kD. Tuni
mycin treatment of HTLV-III–infec
cells was performed to characterize
non-glycosylated forms of the HTLV
proteins. Tunicamycin is a glycosylat

inhibitor and has been used to identify the nascent envelope proteins of many other animal retroviruses, such as Rous sarcoma virus (*22*). A new protein species of approximately 88 kD was observed when the cell lysate was immunoprecipitated and subjected to SDS-PAGE (Fig. 2A). Although there is a difference of approximately 70 kD between gp160 and p88, this could conceivably be due to glycosylation alone since there are 31 potential glycosylation sites predicted from the gene sequence. The loss of both gp160 and gp120 and the appearence of only one new protein band suggests either that both the unglycosylated forms of gp160 and gp120 are identical or that the inhibition of glycosylation prevents further processing. We therefore isolated gp160 and gp120 by lentil lectin affinity chromatography and subjected them to endoglycosidase H digestion (Fig. 2B). The loss of radioactivity associated with gp160 and gp120 was accompanied by the appearance of two new proteins of approximately 88 kD (p88) and a smaller protein that migrated as a broad band.

Retrovirus biosynthesis typically involves the processing of an *env* gene precursor protein to a mature exterior glycoprotein and a transmembrane protein derived from the carboxyl terminus. Additionally, the glycosylation of the envelope proteins is usually modified from envelope precursors that contain high mannose residues and are sensitive to endoglycosidase H to an exterior glycoprotein containing complex sugar residues. Thus p88 may represent the native unglycosylated form of gp160 which is also seen in tunicamycin studies. The broad protein band between 70 and 80 kD may represent a partially glycosylated form of gp120. On the basis of the nucleotide sequence, one can predict from the cleavage site for the transmembrane protein an unglycosylated exterior glycoprotein of approximately 55 kD. We therefore isolated gp120 by preparative SDS-PAGE and treated the protein with endoglycosidase F, which digests both complex and high mannose sugar moieties. A broad protein band from about 58 to 68 kD was observed, lending further evidence that gp120 may be a cleavage product of gp160, although it is not fully digested as evidenced by the broad band and by the somewhat larger size than that predicted for the exterior glycoprotein.

In general, the *env* genes of animal retroviruses encode glycoproteins that are the most immunogenic proteins as detected by the sera of infected animals

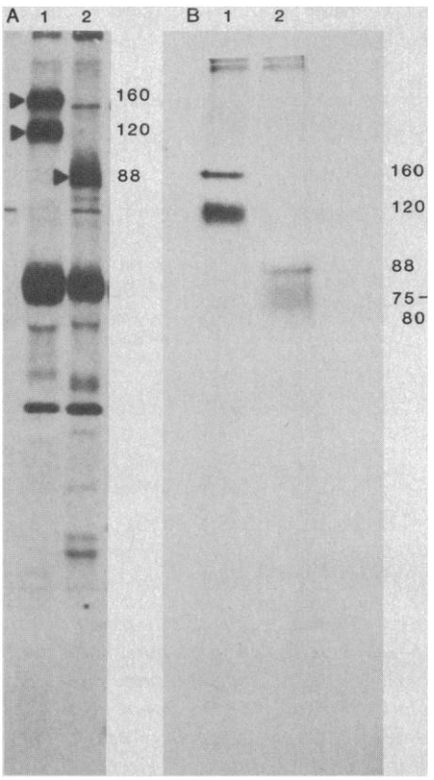

Fig. 2. Analysis of HTLV-III glycoproteins. (A) H9 cells infected with HTLV-III were treated with tunicamycin (*18*) and subjected to radioimmunoprecipitation and SDS-PAGE. Lane 1, proteins immunoprecipitated from untreated [^{35}S]cysteine-labeled infected H9 cells; lane 2, proteins from tunicamycin-treated cells. The infected H9 cells (4×10^6 cells) were incubated with tunicamycin (20 μg/ml) for 2 hours and then labeled with [^{35}S]cysteine for an additional 3 hours in the presence of tunicamycin. Cell lysates were prepared, cleared with 20 μl of negative human serum, and immunoprecipitated with 20 μl of known HTLV-III–positive serum. (B) HTLV-III glycoproteins were purified with lentil lectin and digested with endoglycosidase H (*18*). The [^{35}S]cysteine-labeled glycoproteins from HTLV-III–infected cells were immunoprecipitated and incubated (lane 1) in the absence of endoglycosidase H or (lane 2) in the presence of endoglycosidase H and run on 10 percent SDS–polyacrylamide gels. Procedures for endoglycosidase H digestion were described previously (*18*). Briefly, HTLV-III glycoproteins were first incubated with lentil lectin Sepharose 4B for 4 hours and then eluted with 0.2*M* methyl mannoside. The resulting proteins were then immunoprecipitated with HTLV-III reference serum, and the precipitates bound to protein A Sepharose were dissociated from antibody by boiling for 2 minutes in the presence of 0.1 percent SDS and 0.15*M* sodium citrate, *p*H 5.5. Equal portions were then incubated for 3 hours at 37°C in the presence or absence of 0.25 μg of endoglycosidase H. The reaction was terminated by the addition of five volumes of cold 95 percent ethanol, and the proteins were precipitated overnight at −20°C. The samples were then centrifuged at 12,000*g* for 15 minutes and the proteins were reconstituted with electrophoresis sample buffer, boiled for 3 minutes, and subjected to electrophoresis.

(*23*). In separate studies (*21*) we found that gp120 and gp160 were the HTLV-related species most probably precipitated by the antibodies found in patients with AIDS or AIDS-related complex, healthy homosexual men who had been exposed to HTLV-III, and asymptomatic hemophiliacs. The evidence presented clearly identifies two large glycoproteins that are encoded by the *env* gene of HTLV-III, and we suggest that gp160 represents the envelope gene precursor protein and gp120, a mature envelope glycoprotein. We have not yet identified the transmembrane protein of HTLV-III, although a 41-kD protein has been observed in virus preparations by Western blotting (*5*).

Further characterization of gp160 and gp120 is likely to be important for serodiagnosis of the HTLV-III carrier state as well as for prospective immunoprophylactic approaches to the prevention of AIDS. The possibility that these glycoproteins are directly involved in the pathologic consequences of HTLV-III infection on the T helper cell population must also be considered.

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Virus Envelope Protein of HTLV-III Represents Major Target Antigen for Antibodies in AIDS Patients

Abstract. *In this study, two glycoproteins (gp160 and gp120) that are encoded by human T-cell lymphoma virus type III (HTLV-III) were the antigens most consistently recognized by antibodies found in patients with the acquired immune deficiency syndrome (AIDS) and with the AIDS-related complex (ARC) and in healthy homosexual males. The techniques used to detect the glycoproteins were radioimmunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (RIP/SDS-PAGE). Although most antibody-positive samples from ARC patients and from healthy homosexual males also reacted with the virus core protein p24, less than half of the AIDS patients revealed a positive band with p24 under the same conditions. The ability to detect antibodies against a profile of both the major* env *gene encoded antigens and the* gag *gene encoded antigens suggests that the RIP/SDS-PAGE may be a valuable confirmatory assay for establishing the presence or absence of antibodies to HTLV-III in human serum samples.*

One of the first suggestions that a retrovirus might have a role in the etiology of the acquired immune deficiency syndrome (AIDS) was the finding that a minority of patients with AIDS and AIDS-related complex (ARC) had antibodies that reacted with antigens found in cells infected with human T-cell leukemia virus type I (HTLV-I) (*1*). The low titers of these antibodies and the observation that only about 25 percent of the patients had antibodies that would precipitate the HTLV-I encoded gp61 or p24 proteins suggested that cross-reacting antibodies to a related agent could be responsible for the initial observation (*1*, *2*). Subsequently, retroviruses that were cytopathic for T-helper lymphocytes were isolated from numerous AIDS and ARC patients. Although these viruses were usually designated HTLV-III (*3*), some were designated lymphadenopathy-associated virus (LAV) (*4*, *5*) or AIDS-related virus (ARV) (*6*). HTLV-III, LAV, and ARV probably represent the same class of agent (*7*, *8*), but on the basis of their antigenic cross-reactivity and several short stretches of amino acid homology they appear to be only distantly related to HTLV-I (*2*, *7*, *9*). However, both HTLV-I and HTLV-III infect T-helper lymphocytes and have a Mg^{2+}-dependent reverse transcriptase (*3*).

The major envelope (*env*) gene products of HTLV-III have been identified as glycoproteins of 160 kD (gp160) and 120 kD (gp120 (*10*). These two glycoproteins have the same amino acid sequence at the amino terminus (*10*). We now report that gp120 and gp160 are the HTLV-III proteins detected most readily in radioimmunoprecipitation assays of serum samples from patients with AIDS or ARC. In AIDS patients, the *env* gene encoded proteins are detected about twice as readily as p24, which is the *gag* gene encoded protein of HTLV-III that represents the major antigen found in virus particles.

Serum samples were initially screened for antibodies to HTLV-III by indirect cell membrane immunofluorescence (MIF) using the H9/HTLV-III cell line as described (*11*) (Table 1). Whereas 48 of 50 (96 percent) of the AIDS patients and 43 of 50 (86 percent) of the ARC patients were positive, 34 of 73 (47 percent) of the healthy homosexual males but none of 27 healthy laboratory workers were positive.

All of the samples from the same 190 individuals were also tested by radioimmunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (RIP/SDS-PAGE) with [^{35}S]cysteine-labeled H9/HTLV-III and uninfected H9 cells (*11*). As shown in Table 1, samples that were positive by MIF were also positive by RIP/SDS-PAGE, and one sample from an AIDS patient and three samples from ARC patients that were negative by MIF were also positive by RIP/SDS-PAGE.

Figure 1 shows the RIP/SDS-PAGE reactions for 15 representative samples of human serum that were positive by MIF (lanes 2 to 16) and one sample that was negative (lane 1). Whereas all the HTLV-III membrane antigen antibody-positive samples regularly reacted with the *env* gene encoded gp120 and gp160 proteins, only 85 of 131 (62 percent) also detected the p24 protein of HTLV-III. Although

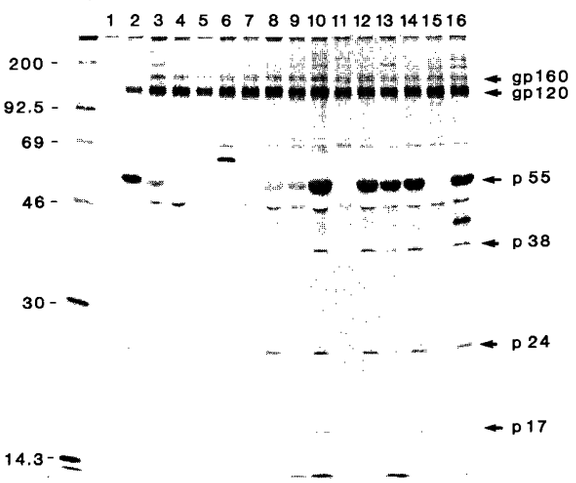

Fig. 1. Reactivity of serum samples for antibody to HTLV-III proteins as determined by RIP/PAGE (*10*, *11*). Cell lysates were prepared from [^{35}S]cysteine-labeled HTLV-III–infected H9 cells and immunoprecipitated with sera from the following individuals: a laboratory worker whose serum was negative for antibody to HTLV-III membrane antigens (lane 1); seven ARC patients (lanes 2, 6, 7, 10, 11, 12, and 16); three AIDS patients (lanes 3, 5, and 15); and five healthy homosexuals who were positive for antibody to HTLV-III membrane antigens (lanes 4, 8, 9, 13, 14).