DTIC FILE COPY

AD-A203 587

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited (Approved for public release)
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Yale University School of Medicine	6b. OFFICE SYMBOL (If applicable)
7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State, and ZIP Code) New Haven, CT 06510	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research and Development Command	8b. OFFICE SYMBOL (If applicable)
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-87-C-7065	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrickm Frederick MD 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
623105	3M2 623105H29	AA	044

DTIC ELECTE 13 JAN 1989 S E D

11. TITLE (Include Security Classification)

Sylvatic HTLV Related Viruses in South America

12. PERSONAL AUTHOR(S)

Francis L. Black, Lisa Regan, Sidney E.B.Santos, Roy A. Capper

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 12/15/86 TO 12/14/87	Mar 11, 1988	29

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	AIDS, HIV1, HIV2, HTLV-I, SIV, South America, Amerindian, New World Monkey, Platyrrhine Primate, Retrovirus, RA I
06	03		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Ninety one of 2441 serum specimens (3.7%) from persons in 18 of 25 Amerindian tribes distributed across most of the length and breadth of South America reacted positively in HIV1 ELISA tests. None of 148 specimens from non-Indians living near one tribe was positive. Twenty-seven mostly different individuals, of 1084 tested (2.5%) reacted positively in an HTLV-I ELISA test. Twelve sera that were positive in one or both of the ELISA tests reacted in Western Blot tests with the proteins of HIV1, but only one of these gave the pattern of bands usually associated with HIV1 infection. Similar partial reactions were obtained in Western Blot tests with SIV_{MAC} proteins. The positive sera included, more frequently than by chance, Marital couples, sibs and mother-child pairs.

Eighteen of 387 specimens (4.6%) from Platyrhine primates captured in various parts of the Amazon Basin were positive by a Protein A ELISA test for HIV1. The positive specimens included the genera Alouatta, Aotus, Ateles, Cebus and Snguinus. Fourteen of 30 ELISA

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs Virginia M. Miller	22b. TELEPHONE (Include Area Code) 301/663-7325 — 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

89 1 12 032

Blck 19, con't.

positive sera were also positive for one or more proteins by HIV2 Western Blot. One a Cebus, positive for P18, 24, 34, 110 and 140, fulfilled the criteria for specificity.

Some of the positive results may be non-specific and due to reactions with falciparum malaria antibody. However, the pattern of distribution does not follow that of malaria and Western Blot reactions and suggests the existence of one or more viruses related to, but different from, that of AIDS in the Amazon area. Regardless of the cause, false positive serological reactions, complete with Western Blot confirmation, are to be encountered in South America. The distribution of these reactions is very wide, both geographically and in terms of host species. They react with HIV1, HIV2 and HTLV-I, butare stronger with HIV2 than HIV1 and stronger with HIV1 than HTLV-I. (KT)

ADDENDUM

At the Workshop on "Epidemiology of Retroviral Infections", 30 Nov, 1988, it was suggested that P. falciparum antibodies correlate with HIV1 false positive reactions in the Phillipines and that the same phenomenon might explain the data described in this report. Malaria antibody titers were available on many of the same sera and have since been analyzed in this regard.

Antibody to Plasmodium falciparum, P. vivax and P. brasiliensis was determined by indirect hemagglutination assay. Sera from 744 persons in seven tribes were tested. Positive IHA titers against falciparum were found in 67%, and against vivax and brasiliensis in 51 an 53% respectively. Positive reactions against all three types were less frequent in children less than 6 years old, but there was little age correlation above that. The prevalence of high falciparum titers ($\geq$4096) ranged from 2.3% in tribe W to 20.0% in tribe J but these frequencies did not correlate with the prevalence of Retroviral antibody (Table). Many of the malaria antibody assays used sera of different dates from those used for Retrovirus tests; however 398 used the same specimens. The mean P. falciparum titer in 6 Retrovirus positive specimens was 572; the mean titer in the negative sera was 286. Only one Retrovirus positive specimen had a titer >4086, but 43 negative specimens had this titer. Neither was there any correlation in these sera bewteen Retrovirus positivity and high P. vivax or P. brazilensis titer. Thus,we were not able to demonstrate any association between malaria antibody and the Retroviral reactions either in tribal frequencies or in individual sera. The study of Biggar etal. (Lancet 1985;ii:520) which best describes this phenomonon, was carried out in "healthy" persons in an area that has only since been recognized as having an extremely high prevalence of HIV1 infections.

Table 4. Proportion of Population with High Titer IHA Antibody to P. falciparum

Tribe	Number Tested	Percent with Titer >4096	Percent with Positive HIV1 ELISA
D	183	4.4	0.66
G	106	18.9	4.63
J	25	20.0	6.45
N	91	18.7	3.44
R	136	19.1	1.31
T	39	7.7	8.33
V	131	2.3	3.17

Coef. Corr. = .124; P = > .05.

AD________

Sylvatic HTLV Related Viruses in South America

Final Report

Francis L. Black, Ph.D.

March 11, 1988

Supported by

U.S. ARMY RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-87-C-7065

Yale University School of Medicine
New Haven CT 06510

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited.

The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SUMMARY

Ninety-one of 2441 serum specimens (3.7%) from persons in 18 of 25 Amerindian tribes distributed across the length and breadth of South America reacted positively in HIV1 ELISA tests. None of 148 specimens from non-Indians living near one tribe were positive. Twenty-seven, mostly different individuals, of 1084 tested (2.5%) reacted positively in an HTLV-I ELISA test. Twelve sera, that were positive in one or both of the ELISA tests, reacted in Western Blot tests with proteins of HIV1, but only one of these gave the pattern of bands usually encountered in HIV1 infection. Similar partial reactions were obtained in Western Blot tests with SIV_{MAC} and HIV2 proteins. The positive sera included, more frequently then by chance, marital couples, sibs and mother-child pairs.

Eighteen of 387 serum specimens (4.6%) from Platyrrhine monkeys caught in various parts of the Amazon basin were positive by a protein A ELISA test for HIV1. The positive specimens included the genera Alouatta, Aotus, Ateles, Cebus and Sanguinus. Fourteen of 30 ELISA positive sera were also positive for one or more proteins by HIV2 Western blot. One, a Cebus, positive for P18,24,34,110 and 140, fulfilled the criteria for specificity.

Some of the positive results may be non-specific and due to reactions with falciparum malaria antibody. However, the pattern of distribution does not follow that of malaria and the Western Blot reactions suggest the existence of one or more viruses related to, but different from, that of AIDS in the Amazon area. Regardless of the cause, false positive serological reactions, complete with Western Blot confirmation, are to be encountered in South America. The distribution of these reactions is very wide both geographically and in terms of host species.

They react with HIV1, HIV2 and HTLV-I, but more strongly with HIV2 than HIV1 and more strongly with HIV1 than HTLV-I.

FOREWORD

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR56.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Serological reactions to Human Immunodeficiency Virus (HIV) have been reported in sera collected more than ten years ago from three Venezuelan (1) Indian populations. There is also a negative report based on sera from three Brazilian tribes (2). The Venezuelan study obtained positive results with 9 of 224 sera by HIV immunofluorescence, Western Blotting (WB) and radio-immuno precipitin methods. It also reported reactions with HTLV-I antigens. The negative study involved only 167 sera. In the present study we have examined more than 2400 sera collected from 25 tribal groups living in areas from $9^{\circ}30'$N to $38^{\circ}20'$S and from $46^{\circ}25'$ to $71^{\circ}20'$W (Figure 1). Most of the tribes studied are very isolated and have escaped infection with many agents common in the cosmopolitan populations around them (3). No clinical sign of AIDS infection has been recognized in these people, and the chance of their having been involved in the current AIDS pandemic, especially at the time the earlier serum samples were collected, is exceedingly small.

Several Lentiviruses have been isolated from Old World monkeys. The best studied of these, SIV_{MAC} cross reacts with HIV1 and HIV2, but more strongly with HIV2 (4). It shares 65% base sequence homology with the latter agent. Antibody to this virus has not been sought in wild macaques, but it is found in wild-caught

African Green monkeys although the only viruses isolated from green monkeys are quite different. Another retrovirus, Squirrel Monkey Retrovirus, has been isolated from a New World primate, but this is a distinct D-type virus not closely related to HIV or HTLV-I (5). In the present study we have examined nearly 400 sera from wild caught New World monkeys and found a pattern of HIV and HTLV-I positive reactions similar to that observed in humans indigenous to the same area. We note that the people of all but one of the human tribes we found positive commonly hunt, butcher and eat monkeys, and that many keep wild-caught monkeys as pets.

Because of the small size of the tribes, presentation of the human data by tribal name might affect individual rights and we will refer to specific tribes only by code and by approximate map location.

TABLE OF CONTENTS

METHODS

Specimens. Human serum samples were collected between 1972 and 1987 from tribal populations in South America. The majority of these societies were small - less than 1000 persons - and lived in the forest, isolated from cosmopolitan communities (6). Except the southernmost tribe, where specimens were collected at health clinics and schools, each collection comprised the nearly complete population of one or more villages, except small infants. Often, however, specimens from children less that two years old were under-represented. Between collection and testing, the sera were stored at -20°C. The older specimens, but not the 1987 collections, had been thawed several times in the interval.

Monkey serum samples were from two collections. One is held by the Instituto Evandro Chagas in Belem and consists of sera from a variety of species collected from several sites in Para and Goias states at various times over the last 15 years. Most of these specimens were collected in conjunction with studies of outbreaks of Yellow fever and other Arboviruses. The other collection is held at the Universidade Federal do Para and consists mostly of specimens collected from animals trapped on islands formed as the Tocantins reservoir filled in 1984-5. These are almost all Alouatta belzebub (Howler monkeys).

ELISA Tests on Human Sera. HTLV-I ELISA tests used commercially available kits from either Cellular Products or Dupont. The two products yielded very similar results and have not been distinguished in the analyses. HIV direct ELISA tests used kits from four lots manufactured by Electronucleonics Inc. Three lots gave quite comparable results, but the fourth gave a substantially higher antibody prevalence rates. This fourth test was used mostly with relatively fresh 1987 serum specimens, but it also gave positive reactions in older sera that were not picked up by the other lots. Because the different HIV ELISA lots were used with different tribes, direct comparisons of the proportion positive in different tribes may be misleading. The tests for both HTLV-I and HIV yielded some high absorbancy values, but the distribution of absorbancies formed a continuous curve with no node separating positive from negative values. For comparison of data from tests done with different lots, we used a cutoff in the less sensitive tests at two times the mean of the negative controls. Sera with absorbancy values above this level were termed "reactive". The term "positive" has been reserved for results that met the manufacturer's criteria for positivity, and gave a value at least at the reactive level if retested. The numbers of reactive sera permit comparisons between tribes, but the reactive tests cannot be considered individually significant.

The "Wellcozyme" competitive test, available only outside the USA, was also used to test a number of Brasilian Indian specimens. This test is commonly viewed as more specific than the direct tests.

ELISA Tests on Monkey Sera The standard ELISA test depends on a enzyme-linked goat or sheep anti-human IgG. This reagent reacts with Ateles monkey serum in a Ouchterlony test, but with a prominent spur. Others have used it in testing Old World monkey sera for simian immunodeficiency virus (SIV) but estimated that the sensitivity was 1/5 that of human sera (7). We used this test with 78 monkey sera and found 2 reactors (Table 7). Because the New World monkeys are genetically even more different from humans than the old world animals studied by Kanki et al, we concluded that the method was inappropriate for our studies and that this number was probably erroneously low. Because we wished to survey a variety of species, it was not practical to prepare enzyme tagged goat antibodies specific for each species. Instead we chose to use peroxidase tagged Staphylococcal protein A. This has a specificity for the IgG of a very wide variety of mammalian species (8). The method was tested on sera from Cebus and Alouatta monkeys collected before and after immunization with measles vaccine with measles antigen in the ELISA. Strong positive reactions were observed with the post vaccination specimens but not with the pre specimens.

Western Blot Tests. Nitrocellulose strips for HIV Western blot analysis were purchased from Bionetics Ltd and used according to the manufacturer's instructions. Monkey sera were also taken to the Pasteur Institute, Paris where Western blot tests were carried out with HIV2 strips prepared in the laboratory of Dr. L. Montagnier. Other sera were sent to Dr. Phyllis Kanki at Harvard and tested with proteins of SIV. (Then referred to as HTLV-IV).

RESULTS

A. Human Sera

Distribution of Direct ELISA Reactions. To check reproducibility of the HIV ELISA test, 94 initially HIV positive sera were retested (Table 1). Sixty-three (67%) were fully positive on retest and 85 (90%) at least reactive. Five other initially positive specimens were not retested because they were only recognized in the last batch of tests. They are, nevertheless, tabulated with the positives. A test of the reproducibility of the HTLV-I test gave a slightly lower concordence; 27 specimens initially positive for HTLV-I were retested and 15 (56%) confirmed.

To test persistence of the reaction in the same individuals over an extended time period, HIV ELISA tests were carried out on 38 pairs, and HTLV-I tests on 28 pairs, of sera collected seven to eleven years apart. Most of these specimens came from tribe "D" which has one of the higher positivity rates. The correlation between pairs was not significant for either serological test (Table 2).

The ELISA results are shown distributed by tribe in Table 3, and by sex and age in Table 4. The earliest date at which a positive sample was collected was 1976. Both positive HIV and positive HTLV-I ELISA reactions were widely distributed geographically, in both sexes, and in all age groups. The youngest HIV positive individual was one year old and the youngest positive for HTLV-I was two. The overall proportion positive was small, but not in comparison to the prevalence of these antibodies in other populations. The prevalence of HTLV-I antibody increased with age, but HIV antibody prevalence decreases slightly between ages 10 and 50. The sexes did not differ significantly in prevalence of either antibody.

There was little correlation between tests for antibody to the two viruses. Although more values were positive by both tests than expected by chance, this correlation was not significant (Table 5).

Among the 23 adults positive to HTLV-I, there were 4 conjugal couples. Among the 88 persons from family studies who were reactive to HIV1, there were 9 couples, 12 mother-child pairs, 8 father-child pairs, and 21 pairs of siblings. It is difficult to determine what numbers in these relationships are to be expected positive by chance, because both proportion positive, and proportion of relatives in the sample, vary between tribes. However, if the positive tests had been uniformly distributed by tribe and every adult in the sample had his or her spouse also included, one would have 0.7 chance of finding one HIV positive husband-wife pair and 0.4 chance of finding one HTLV-I positive couple. Similarly, if the mother of every child were included in the sample, and positives were uniformly distributed, one would expect to find 1.5 mother-child pairs positive to HIV and 0.2 positive to HTLV-I.

As a test of the ethno-cultural specificity of these reactions, we tested 148 specimens that had been collected from Caucasians and mixed race persons living in the same valley as tribe Y and stored with the Indian sera more than ten years. None of these specimens was positive.

Indirect ELISA Tests. Fewer sera were positive by the indirect ELISA test (Table 1). The direct and indirect tests did not coincide to confirm positivity in particular individuals. Persons positive by this test were again widely distributed by tribe (Table 3), but the mean age was higher (35 years) than those positive by the direct test (19 years). The youngest positive individual was 15.

Three of the seven positive were women.

Western Blots. Twelve sera, seven positive for HTLV-I, four for HIV, and one positive for both, were tested for antibody to HIV proteins by WB. Ten of the twelve reacted with at least three of the major viral proteins, but reactions with P31 were rare and none reacted with GP120 (Table 6 and Figures 2 and 3). The manufacturer's criterion for positivity required reactions with P24, 31 and either GP41 or 120; only D269, the doubly ELISA positive sample, fulfilled this criterion. The HIV ELISA positive sera gave stronger P24 reactions than the HTLV-I specimens, but otherwise there did not seem to be a significant difference between the two sets. It was striking that some of the most consistent reactions were with minor protein bands. These bands are not usually considered in evaluation of HIV reactions, being relatively weak on the positive control strips. Nevertheless, they were absent from the negative control and consistently placed on the test strips. There was a general parallel between absorbancy in the ELISA test and the intensity of the WB bands.

Confirmatory Tests and Extension Studies by Others.

Nineteen sera selected to have a variety of HIV ELISA reactions were sent blind to Dr. Judith Britz at Electronucleonics Inc. for independent testing. With one exception, the correspondence between the two tests was close [illegible].

Thirty-nine sera were sent to Dr. Phyllis Kanki in Boston for testing against SIV in WB tests. Three of these sera were HIV ELISA positive and four reactive. Four sera reacted positively with SIV P24; two of these also reacted with the polymerase band, and one weakly with the envelope protein. These positive reactions did not, however, coincide with the ELISA results, only one being ELISA reactive.

B. Monkey sera.

Using the Protein A-ELISA HIV procedure, 387 monkey sera were tested. Eighteen (4.7%) were found positive (Table 7). The positive specimens included 5 of 9 species tested. Ten of 246 Alouatta were positive and 3 of 82 Cebus. A parallel test using the protein A method with Dupont's HTLV-I ELISA wells gave only two positives in 170 monkey sera. The 18 HIV1 positive specimens, as well as 60 negative sera, were retested by the Wellcozyme indirect test. None was positive.

Twelve ELISA positive specimens were tested against HIV1 proteins by WB without protein A. Six gave positive reactions with one to five protein bands, but generally the reactions were weak and none fulfilled the manufacturer's criterion for HIV1 positivity described above.

Twenty eight specimens that were either positive or borderline by HIV ELISA were taken to Dr. Montagnier's 's laboratory in Paris and tested by HIV2 WB and RIPA. Nineteen were positive by WB for one or more protein bands, but the specific band reacting was variable, and only one, a Cebus, was positive for all critical proteins. The same serum, and only this serum reacted positively in the HIV2 RIPA. It was one of only three Cebus specimens to give a full positive reaction in the ELISA test. It and several other specimens included in the series came from animals captured in 1978 near the Trombetas river (1^{o}S, 57^{o}W).

DISCUSSION.

Antibody to falciparum malaria has been reported to give false positive HIV1 ELISA reactions(14) and may explain some of the observed patterns. It is known to have occurred recently in tribe B and probably D but it is by no means ubiquitous in the area (15). It may be resonsible for some of the observed reactions, but it would be difficult to attribute the Western Blot reactions to it and the pattern of these reactions would not fit the total pattern observed.

Several trivial explanations for the data have been considered and excluded. First is the possibility that the reactions were totally non-specific. This is made improbable by the reproducibility of the ELISA patterns and the sharpness and precise locations of the WB bands as well as by the FA and RIPA confirmations. Also, the same sera have been used in a wide variety of virological serology tests without yielding comparably strange results (eg ref. 6).

Some of the sera had been held long periods in storage, as had all the sera reported on by Rodriguez et al. (1). Degeneration might have given rise to unusual reactions. These would, however, probably be non-specific and excluded by the considerations above. More definitively, a significant number of fresh sera were also tested and gave essentially the same patterns.

We also considered the possibility that the reactions were specific for HLA antigens acquired from the cultures in which the virus antigens were grown. However, the HLA proteins band at locations different from the viral proteins in WB and could not easily have been confused in that test. Two different cell lines were used: H9 in the Electronucleonics tests and HUT78 in Dr Kanki and Dr

Montagnier's confirmatory tests. The HLA antigens of these lines are not well defined because they are not clearly expressed, but it unlikely that they would have given the same pattern. Finally, men and children are usually only induced to produce HLA antibody by transfusion or transplantation. None had been so treated, and yet nearly as many of them were positive as women. Even for the women reactors, HLA antibodies do not provide a valid explanation because, for unknown reasons, Amerindian women rarely produce antibody to the HLA antigens of their children (9).

The low frequency of strong positive serological reactions, lack of specificity for any one member of a virus group, and fluctuation in titer over time, are in fact, the usual pattern when one tests for antibody with antigen from a related, but immunologically distinct, virus. This kind of variability of specificity toward different viruses of one taxonomic group is typical of the patterns observed in some of these (6) and other 10) sera with Flaviviruses and Alphaviruses. There, too, infection with one or two members of the group elicited unpredictable patterns of reaction with other members of the virus group. It is also typical of HTLV-I reactions in areas where HIV-1 is common (11), and of HIV-1 reactions where HIV2 is common (12). This irregularity is to be expected, if the humans and monkeys had been infected with some Retrovirus other than those for which we have tests.

The similarity of the WB reactions with sera reacting to HIV and to HTLV-I suggests that the two ELISA tests may be detecting the same immunogen. Similarly, the parallels observed in distribution of positive tests in human and monkey sera permits the hypothesis that one virus is infecting both man and animals. It seems clear that the agent detected in this study is not HIV. The total absence of antibody to HIV GP120 in the samples tested by WB and rarity of antibody to P31 contra-indicate HIV as the immunogen. The absence of clinical signs of AIDS also suggests that HIV1 is not present. Neither, does it seem to be one of the other well characterized human retroviruses. The HIV1 Elisa test gave more positive reactions than the HTLV1 and the HIV2 Western blot reacted with more bands than HIV1. If the infecting agent were HTLV-II, the HTLV-I ELISA test would be expected to pick up more positives than HIV ELISA. The large proportion of weak reactions, and the absence of any clear division between positive and negative results, reenforce the impression that the immunity is not the result of infection with one the viruses tested.

On the basis of this evidence that the serological reactions we have observed are not due to infection with HIV1, we must conclude that false positive Western Blot confirmable, serological reactions to the AIDS virus are to be encountered in humans living in the New World. We must also conclude that false positive reactions to HIV2 occur in new world monkeys. It is probable the false positive HIV2 reactions will also be encountered in humans, although that has not been demonstrated.

Two substansive hypotheses concerning the epidemiology of the infecting agent are possible and set out below. A choice between these alternatives will require isolation of the agent and tests with the specific antigens. The first possiblity would hypothesize that the reactions are caused by a relatively infrequent infection, as indicated by the small fraction of the sample positive. Low, widely distributed virus antibody prevalence has, heretofore, only been observed in these Amerindian populations with zoonotic agents that persist in a reservoir in the forest fauna (6). Diseases that are transmissible directly from human to human have always been found to elicit very high antibody prevalence rates when they gain access to these tightly integrated communities. The presence of antibody patterns in monkey sera suggests that monkeys may be the reservoir host. This hypothesis would explain the slightly elevated HIV ELISA frequencies found in young girls who commonly carry monkey pets and in women who do most of the butchering. It would not explain the three positive results in members of the southernmost tribe where there are no monkeys. Those few results may be have some trivial explanation, or there may be more than one reservoir, or more than one virus.

The alternative hypothesis would suppose that the tests are only measuring the strongest and broadest reactions to a rather common immunogen. This would explain the absence of any node in frequency of absorbance values that might divide positive from negative reactors. It would also be consonant with the finding of reactions to HIV1, HIV2, HTLV-I, and SIV_{MAC} in similar patterns of distribution, but different individuals. It would permit postulation of direct human to human transmission and thus more readily explain the concordance of reactions in spouses, mother-child pairs, and sibs. The absence of similar reactions in Caucasians living with the Southernmost tribe suggests an ethnic factor influences the pattern. This hypothesis, then, looks very much like the postulated pattern of HTLV-I in Japan. There, sexual and mother-child transmission has been observed, and it has been suggested that the the virus persists in the aboriginal Ryukan and Ainu races without having reached a comparable prevalence in the predominant Japanese stock during the 2647 years tradition says they have lived in the archipelago (13).

Regardless of the explanation of the antibody in humans, the evidence of retroviral antibodies in New World Monkeys, if confirmed as evidence of retrovirus infection, these findings will add a major new dimension to our knowledge of distribution of retroviruses in Primates (Table 8).

REFERENCES

1. Rodriguez L, Dewhurst S, Sinagil F, Merino F, Godoy G, Volsky DJ. 1985. Antibodies to HTLV-III/LAV among aboriginal Amazonian Indians in Venezuela. Lancet ii:1098-1100.

2. Galvao-Castro B, Pereira MS. 1986. HTLV-III antibody in Brazilian Indians. Lancet i:976-77.

3. Black FL, Hierholzer WH, Pinheiro FP, et al. 1974. Evidence for the persistence of infectious agents in isolated populations. Am J Epidemiol 100:230-250.

4. Kestler HW III, Li Y, Naidu YM, et al 1988. Comparison of simian immunodeficiency virus isolates. Nature 331:619-22.

5. Barker CS, Pickel J, Tainsky M, et al, 1986. Molecular cloning of the Mason-Pfizer virus genome: biological characterization and molecular comparisons with other retroviruses. Virology 153:201-14.

6. Black FL, 1984. Interrelationships between Amerindian tribes of lower Amazonia as manifest by HLA haplotype disequilibria. Am J Hum Genet 36:1318-1331.

7. Kanki PJ, Barin F, M'Boup S, et al, 1986. New human T-lymphotropic Retrovirus related to Simian T-lymphotropic virus Type III ($STLV\text{-}III_{AGM}$). Science 232:238-43.

8. Goding JW, 1978. Use of Staphylococcal Protein A as an immunological reagent. J Immunol Methods 20:241-53.

9. Kostyu DD Amos DB, 1981. Mysteries of the Amerindians. Tiss Ags 17:111-23.

10. Shope RE, Andrade A, Bensabath G, et al, 1966. The epidemiology of EEE, WEE, SLE and Turlock viruses with special reference to birds in a tropical rain forest near Belem, Brazil. Am J Epidemiol 84:467-77.

11. Weiss PJ, Chiengsong-Popov R, Clayden S, et al, 1986. Lack of HTLV-I antibodies in Africans. Lancet i:794-95.

12. Montagnier L. Presentation at W.H.O. conference on newly identified HIV-related viruses. Geneva Feb 12-12 1987.

13. Ishida T, Hinuma Y, 1986. The origin of Japanese HTLV-I. Nature 322:504.

14. Biggar RJ, 1986. Possible nonspecific association between malaria and HTLV-III/LAV. New Engl J Med 315:457-8.

15.Salzano FM and Calligari-Jaques S. South American Indians: A Case Study in Evolution. Oxford University Press, Oxford 1987.

Table 1. Repeated tests on initially positive specimens

Initially	Positive	Repeat Test Result Reactive	Negative	Not retested
HIV Positive	63	23	8	5
HTLV-I Positive	15	-	12	12

Table 2. Tests on Paired Samples Collected more than 8 Years Apart.

	Initial HIV ELISA			Initial HTLV-I ELISA	
	Positive	Reactive	Negative	Positive	Negative
Second test Positive	1	3	7	0	1
Negative	1	3	25	2	25

Table 3. ELISA test results by tribe. Tribes in North to South Order.

				HIV					HTLV-I		
	Direct ELISA Test					Competitive ELISA			ELISA Test		
Tribe	No.	+	%	Reactive	%	No.	+	%	No.	+	%
A	148	0	<0.7	2	1.4	113	2	1.8	146	0	<0.7
B	152	2	1.3	14	9.2	37	1	2.7	59	2	3.4
C	17	0	<5.9	7	41.1						
D	222*	27	12.2	53	23.1	77	0	<1.3	195	12	6.2
E	100	3	3.0	5	5.0	55	0	<1.8	92	1	1.1
F	185	3	1.6	4	2.2	61	0	<1.6	1	0	
G	103	5	4.9	17	16.3	57	0	<1.8	101	0	<1.0
H	31	1	3.2	3	9.7						
I	20	0	<5.0	5	25.5	10	0	<10.0			
J	48	0	<2.1	4	6.1	14	0	<7.1			
K	44	0	<2.3	1	2.3	42	0	<2.4			
L	87	3	3.4	11	12.6	47	0	<2.1	39	0	<2.4
M	13	1	7.7	1	7.7						
N	66	0	<1.5	4	6.1	77	0	<1.3			
O	31	2	6.5	10	32.3	8	0	<12.5	2	0	
P	121	3	2.5	12	9.9	61	0	<1.6	92	9	10.9
Q	153	2	1.3	24	15.7	73	2	2.7	108	3	2.8
R	196	0	0.5	23	11.7	62	0	<1.6			
S	45	1	2.2	3	6.7	24	2	8.3	1	0	
T	12	1	8.3	4	33.3						
U	189	7	3.7	15	7.9						
V	77*	14	18.2	18	22.1	41	0	<2.4	67	0	<1.5
W	78*	8	10.2	8	10.2				77	0	<1.3
X	108*	5	4.6	5	4.6				108	0	<0.9
Y	195	3	1.5	11	5.6						
All	2441	91	3.7	264	10.8	859	7	0.8	1084	27	2.5

* Include tests done with more sensitive fourth HIV kit.

Table 4. Distribution of ELISA Positive Tests by Age and Sex.

Age	HIV Positive	HIV Tested	HIV Percent	HTLV-I Positive	HTLV-I Tested	HTLV-I Percent
0–4	13	334	3.9	3	106	2.8
5–9	18	432	4.2	1	182	0.5
10–14	16	345	4.6	1	129	0.8
15–19	9	289	3.1	3	116	2.6
20–29	18	433	4.2	6	201	3.0
30–39	8	309	2.6	5	147	3.4
40–49	4	189	2.1	4	83	4.8
50–59	3	87	3.4	3	34	8.8
60 +	2	45	2.2	1	15	6.7
Male	41	1212	3.4	17	559	3.0
Female	50	1229	4.1	10	525	1.9

Table 5. Correlation between HIV and HTLV-I Elisa Positives.

	HIV Status Positive	HIV Status Reactive	HIV Status Negative
HTLV-I Positive	1	4	22
HTLV-I Negative	74	60	923

Table 6. Protein Specific Antibody Detected by Western Blot in ELISA Positive Sera.

Band Mobility in kilo-Daltons. Major viral proteins underlined.

Bands seen: Serum	Positive to:	17	24	31	33	41	44	51	55	60	66	70	74	83	90	120	160
D219	HTLV-I		+		+	+							+	+			+
D242	"					+			+			+	+	+	+		+
D278	"	+	+		+	+	+			+		+	+	+	+		+
P001	"	+			+			+			+	+	+	+			+
P002	"	+	+		+	+						+	+	+			+
P 26	"	+	+		+	+	+	+	+		+	+	+	+	+		+
P 27	"		+										+	+			+
D269	HTLV-I and HIV	+	+	+	+	+	+		+		+	+	+	+	+		+
D423	HIV				+	+						+					+
D438	"					+					+	+	+				+
P090	"	+		+			+		+			+	+				+
P122	"	+				+	+		+			+	+	+	+		+

Table 7. Retrovirus ELISA Tests on South American Monkey Sera.

Genus	HIV1 ELISA Using Anti-human Sera		HIV1 ELISA Using Staph Protein A			HTLV-I ELISA Using Staph Protein A	
	Tested	Positive	Tested	Positive	%	Tested	Positive
Alouatta	23	1	246	10	4.1	154	1
Aotus	2	0	7	1	14.3	1	0
Ateles	10	1	17	3	17.6	4	0
Callicebus	0	-	1	0	0	0	-
Cebus	38	0	82	3	3.6	9	1
Chiropotes	4	0	21	0	0	1	0
Pithecia	0	-	1	0	0	0	-
Saimiri	0	-	4	0	0	0	-
Sanguinus	1	0	8	1	12.5	1	0
Total	78	2	387	18	4.6	170	2

Table 8. Relationships of Primates with Evidence of Retroviruses.

Family	Genus	Viruses Isolated	This Study
Hominidae	Homo	HIV1,HIV2, HTLV-I,-II,-V	+
Pongidae		None	
Hylobatidae		None	
Cercopithecidae	Macaca	SIV(MAC)	
	Papio	SIV(MND)	
	Cercocebus	SIV(SM)	
	Cercopithecus	SIV(AGM)	
Colobidae	Presbytis	PO-1-Lu	
Callithricidae	Sanguinus		+
Callimiconidae		None	
Cebidae	Aotes		+
	Callicebus		-
	Pithecia		-
	Chiropotes		-
	Alouatta		+
	Saimiri	SMRV, M543	-
	Cebus		+
	Ateles		+
Old World Prosimians			

Legends for figures.

Figure 1. Northeastern South America showing geographic distribution of South American Indians samples tested by HIV ELISA . Number positive over number tested. Results published by others are in brackets.

Figure 2. Western Blot test with HIV proteins and HIV ELISA positive sera. Strips 1 and 2 are strong and weak positive controls; 3 is a negative control. Strip 23 carries the serum positive for both HIV and HTLV-I.

Figure 3. As in figure 2 but with sera positive by ELISA for HTLV-I only.

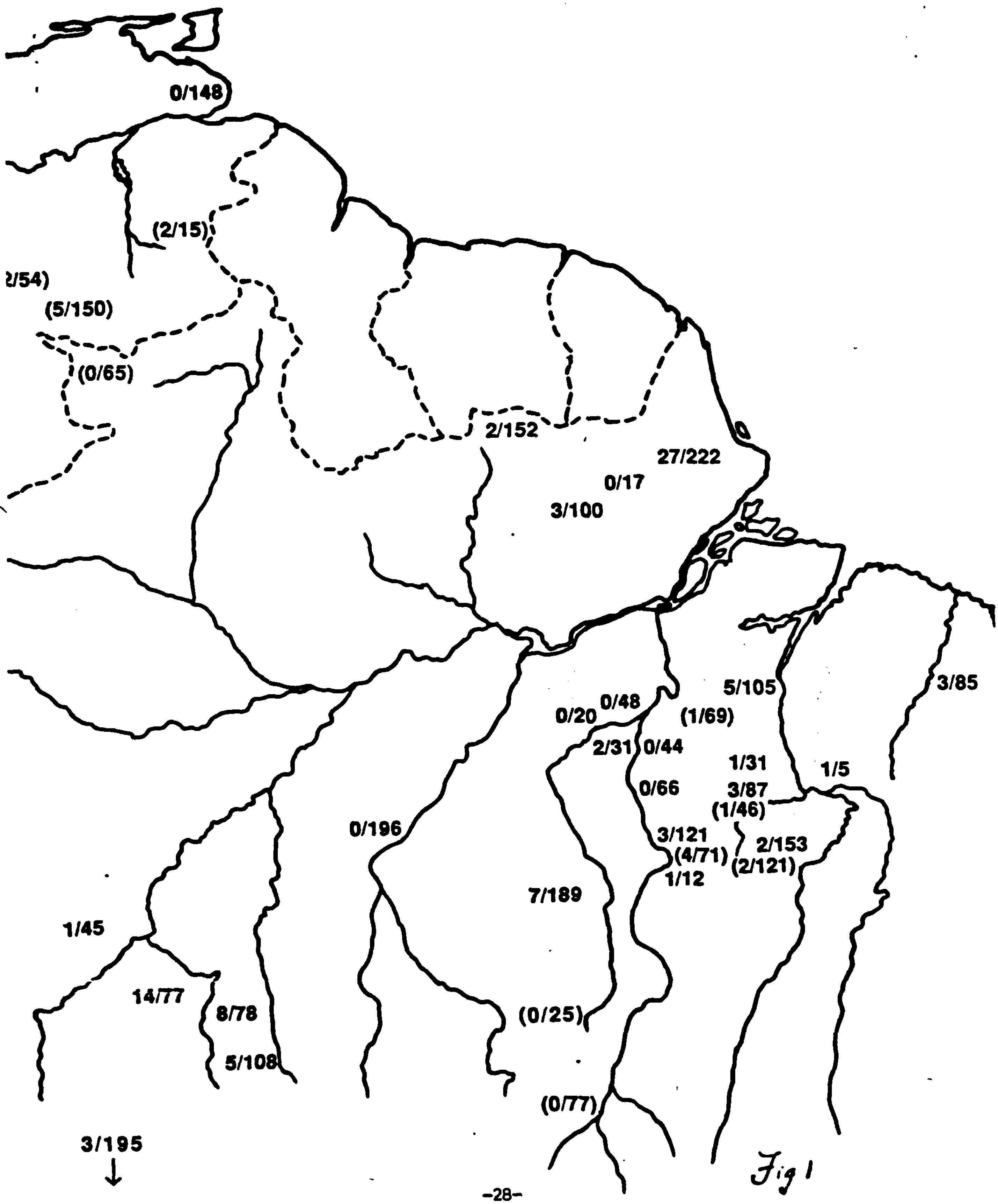
0/148
(2/15)
2/54)
(5/150)
(0/65)
2/152
27/222
0/17
3/100
5/105
3/85
0/48
0/20
(1/69)
2/31
0/44
1/31
1/5
0/66
3/87
(1/46)
0/196
3/121
(4/71)
2/153
(2/121)
1/12
7/189
1/45
14/77
8/78
5/108
(0/25)
(0/77)
3/195

Fig 1

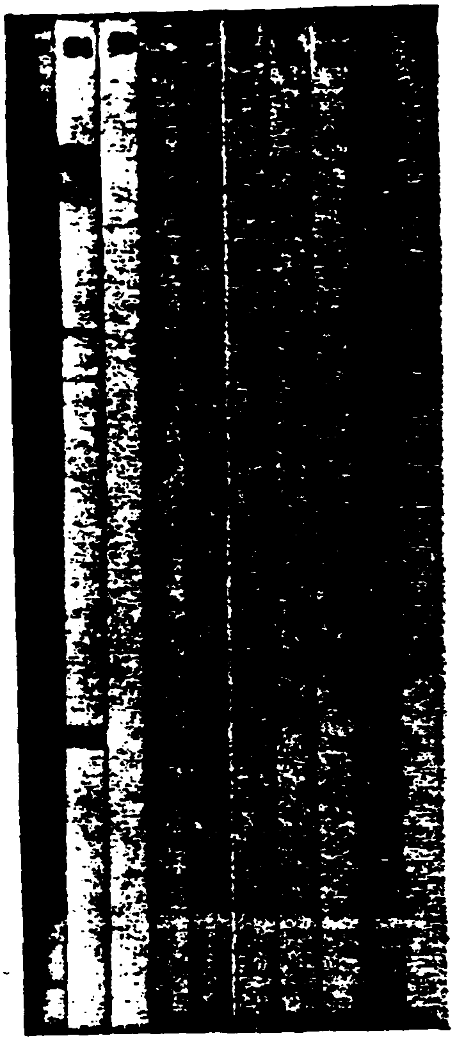

Fig 2.

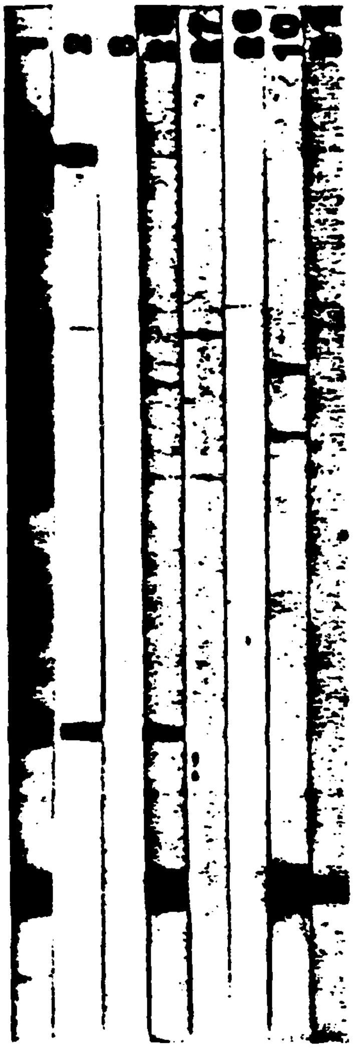

Fig 3.