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# The causes of false-positives encountered during the screening of old-world primates for antibodies to human and simian retroviruses by ELISA

Niels C. Pedersen[1], Linda Lowenstine[2], Preston Marx[3], Joanne Higgins[4], Jean Baulu[5], Michael McGuire[6] and Murray B. Gardner[7]

*Departments of [1]Medicine, [2]Pathology, [4]Surgery, [3]California Primate Research Center, School of Veterinary Medicine and [7]Department of Human Pathology, School of Medicine, University of California, Davis, CA 95616, U.S.A., [5]Barbados Primate Research Center and Wildlife Reserve, Bathsheba, Barbados, and [6]Department of Psychiatry and Behavorial Sciences, University of California, Los Angeles, CA, U.S.A.*

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Sera from 526 old-world primates representing 50 different species were screened by ELISA for antibodies to human T-lymphotropic viruses I and III, and simian retrovirus type 1 (SRV-1). About one-fourth of the sera were positive by ELISA. There was a tendency, however, for the same sera to be positive for all three human and simian retroviruses. Only about one in five of the ELISA antibody-positive sera were confirmed to be positive by Western blotting. False-positive ELISA antibody tests were particularly common among sera from mandrills, crab-eating macaques, lion-tailed macaques, African green monkeys, and DeBrazza's and moustached guenons. Sera that were falsely positive in ELISA antibody tests to the three human and simian retroviruses were found to contain antibodies that reacted at comparable intensity with feline leukemia, infectious peritonitis and panleukopenia viruses. The false anti-viral activity of these sera was found to be due to antibodies that reacted with non-viral proteins that were copurified with all five virus preparations. These proteins were present in normal cat and human cells used to grow the various viruses and in gelatin. The implications of nonspecific cell-protein antibodies in primate sera were discussed in the light of this and previous seroepidemiologic studies of man and old-world monkeys.

primate, retrovirus, ELISA, false-positive

## Introduction

We have reported in a companion paper on the serologic screening of 526 old-world primates of 50 different species for antibodies to human T-lymphotropic virus, types I and III (HTLV-I, III) and simian retrovirus type 1 (SRV-1) (Lowenstine et al., 1986). The preliminary screening of these animals was conducted with an enzyme-linked immunosorbent assay (ELISA). A large number of positive reacting monkeys were identified by ELISA, most of which were negative when tested

by more specific procedures, such as Western blotting. False positive ELISA antibody tests, while sporadically encountered among most of the 50 species of old-world primates, were especially prevalent in *Mandrillus sphinx* (mandrills), *Macaca fasicularis* (crab-eating macaques), *Macaca silensus* (lion-tailed macaques), *Cercopithecus aethiops* (African green monkeys), *Cercopithecus neglectus* (De-Brazza's guenons), *Cercopithecus cephus* (moustached guenons) and *Miopithecus talapoin* (talapoins). Talapoins and mandrills were somewhat unique, however, in that they also possessed a considerable amount of true seropositivity that was being masked by the high background of false-reacting antibodies (Lowenstine et al., 1986).

With the world-wide search for animal reservoirs for human retroviruses, it is natural for investigators to concentrate on man's closest relatives, the old-world primates. Serologic screenings of old-world primates for other virus infections in the past have also yielded confusing results, and several investigators have cryptically mentioned the problems of false positive-reacting antibodies (Kalter et al., 1969; Kalter, 1971; Strickland-Cholmley and Malherbe, 1971). The cause of false positive serologic tests in these other virus systems was not determined, however. Considering the importance of old-world primates as potential reservoirs for human pathogens, and the number of investigators doing serologic studies with these animals, we felt that it was important for us to spend some time on the causes of the false positive serologic reactions that we had encountered. In this paper, therefore, we will present evidence that a small proportion of monkeys of some species, and a large percentage of monkeys in others, possess serum antibodies that cross-react with a common cellular protein or proteins that are copurified with most virus antigen preparations.

## Materials and Methods

*Serum samples*

Sera from 413 old-world primates comprising 50 different genera and species were obtained from six different zoos located across the United States. Sera from 105 African green monkeys originating from the Caribbean Islands of St. Kitts, Nevis and Barbados were obtained from the Delta Regional Primate Center, Covington, LA, from the vivarium of the University of California, Los Angeles, CA and from the Barbados Primate Research Center and Wildlife Reserve, Barbados. An additional eight sera from African green monkeys from eastern Africa were kindly provided by Dr. D.E. Redman, Yale University School of Medicine, Yale University, New Haven, CT.

*Preparation of antigens for serologic testing*

Simian retrovirus type 1 (SRV-1) isolated at the California Primate Research Center was grown in human Raji cells (Daniel et al., 1985; Marx et al., 1984). Human T-lymphotropic virus type III (HTLV-III), provided by Dr. R.C. Gallo (NIH), was grown in H9 cells. LAV provided by Dr. F. Barre-Sinoussi (Pasteur Institute)

was grown in HUT-78 cells. Human T-cell leukemia virus (HTLV-1) and permissive in MT-2 cells were provided by Dr. R.C. Gallo (NIH). Feline leukemia virus (FeLV) was grown in FL74 cells (Theilen et al., 1969). Procedures used for the purification of retroviruses have been described elsewhere (Higgins et al., 1986). Briefly, virus was concentrated by ultracentrifugation from cell culture fluid, clarified by low-speed centrifugation and filtration. Virus was further concentrated by sucrose gradient centrifugation. Fractions with a refractive index corresponding to a density of 1.16–1.18 g/ml in sucrose and containing the peak $Mg^{2+}$-dependent reverse transcriptase (RT) activity were collected by tube puncture, diluted with Tris-NaCl buffer, and pelleted by ultracentrifugation. Virus pellets were resuspended in the Tris-NaCl buffer and the protein concentration determined by the method of Bradford (1976). This antigenic preparation was used for ELISAs, Western blots and viral adsorption studies.

The UCD1 strain of FIPV was grown on Fcwf-4 feline cell cultures (Pedersen et al., 1981). The purification of FIPV was carried out using the procedure of Boyle and coworkers (1984). Feline panleukopenia virus was grown on Crandell feline kidney cells and the virus purified from culture supernatants by low-speed centrifugation to remove cell debris, ultracentrifugation to pellet virus, and continuous CsCl gradient separation (Mathys et al., 1983). Cellular debris was obtained from the culture supernatants of HUT-78 human T-lymphoblastoid cell cultures. Culture supernatants were clarified of cells by low-speed centrifugation. Subcellular debris was pelleted by ultracentrifugation and the pellets washed once in Tris-EDTA saline buffer, pH 7.4.

*Enzyme-linked immunosorbent assay (ELISA)*

Techniques for ELISA have been previously described (Lutz et al., 1983). Briefly, microELISA plates (Dynatech, Alexandria, VA) were coated with 400 ng of purified virus per well. After washing the plates, monkey sera diluted 1:100 were added to duplicate wells. After incubation and washing, rabbit anti-cynomologous monkey IgG conjugated to horseradish peroxidase and diluted 1:500 was added to each well. Anti-cynomologous monkey IgG reacts well with IgG from all species of cercopithecine monkeys and pongid and hylobatid apes (Damian and Greene, 1972). After incubation, all trays were washed and substrate added to each well. The reaction was stopped after 1 h and colorometric reactions read in a Dynatech ELISA reader. Positive and negative control sera were used on every plate. A positive reaction was considered to be any corrected optical density (OD) reading times 1000 that was greater than 115. This was usually 30–40% of the values obtained from positive control sera.

*Western blotting procedure (WB)*

Purified virus was disrupted in 0.05% SDS and separated on 8% polyacrylamide gels according to the procedure of Laemmli (1970). One lane of each gel contained the molecular weight standards. The protein bands were electrophoretically transferred to nitrocellulose paper by the technique of Tsang and coworkers (1983). Unbound protein-binding sites were blocked with 3% gelatin in ELISA dilution

TABLE 1

Results of ELISA and Western blot testing of old-world primates for antibodies to human and simian retroviruses, FeLV, and cell debris.

| Genus and species | ELISA antibody-positive/total animals tested (Western blot+) | | | | |
|---|---|---|---|---|---|
| | HTLV-I | HTLV-III | SRV-1 | FeLV | Cell debris |
| Apes | | | | | |
| *Gorilla gorilla* | 0/26 | 0/26 | 0/26(0) | 0/26 | 0/26 |
| *Pongo pygmaeus* | 1/19(0) | 2/19(0) | 2/19(0) | 2/19(0) | 2/19 |
| *Pan troglodytes* | 1/2(1) | 0/2 | 0/2 | 0/2 | 0/1 |
| *P. paniscus* | 1/6(2) | 0/6 | 0/6 | 0/6 | 0/6 |
| *Hylobates lar* | 0/7 | 0/7 | 1/7(0) | 0/7 | 0/7 |
| *H. concolor* | 2/11(0) | 1/11(0) | 2/11(0) | 2/11 | 2/11 |
| *H. agilis* | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| *H. agilis* × *hoolock* | 0/2 | 0/2 | 2/2(0) | 1/2(0) | NT |
| *Hylobates sp.* | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| *Symphalangus syndactylus* | 0/10 | 0/10 | 2/10(2) | 1/10(0) | 0/10 |
| Baboons | | | | | |
| *Papio papio* | 0/10(0) | 1/10(0) | 1/10(0) | 1/10(0) | 0/10 |
| *Mandrillus sphinx* | 8/23(6) | 11/23(4) | 11/23(0) | 9/23(0) | 3/14 |
| *M. leucophaeus* | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| *Theropithecus gelada* | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| Macaques | | | | | |
| *Macaca fasicularis* | 3/23(1) | 4/23(0) | 8/23(1/7)* | 7/23 | 5/23 |
| *M. silensus* | 17/26(1) | 17/26(0) | 17/26(0) | 20/22(0) | 8/8 |
| *M. maurus* | 2/5(2) | 0/5 | 0/5 | 0/5 | 0/5 |
| *M. niger* | 3/12(2) | 0/12 | 0/12 | 0/12 | 0/12 |
| *M. tonkeana* | 6/21(6) | 1/21(0) | 7/21(6) | 0/5 | 0/5 |
| *M. nemestrina* | 1/6(1) | 0.6 | 0/6 | 0/6 | 0/6 |
| *M. sylvanus* | 0/12 | 0/12 | 2/12(0) | 1/12(0) | 1/12 |
| *M. mulatta* | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| *M. radiata* | 1/1(1) | 0/1 | 0/1 | 0/1 | 0/1 |
| Mangabys | | | | | |
| *Cercocebus atys* | 3/7(2/4) | 2/7(4/4) | 6/7(0/4) | 0/7 | 0/7 |
| *C. torquatus, torquatus* | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| *C. torquatus, lunulatus* | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| *C. albigena* | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| *Erythrocebus patas* | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| Guenons | | | | | |
| *Cercopithecus aethiops* | 55/116(5) | 61/116(0) | 55/116(0) | 81/116(0) | 53/116 |
| *C. albogularis kolbi* | 5/11(4) | 1/11(2) | 1/11(0) | 0/11 | 0/11 |
| *C. diana* | 2/8(0) | 2/8(0) | 2/8(0) | 2/8(0) | NT |
| *C. neglectus* | 1/4(0) | 2/4(0) | 2/4(0) | 3/4(0) | 1/2 |
| *C. hamlyni* | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| *C. nictitans* | 1/7(0) | 1/7(0) | 2/7(0) | 1/7(0) | 1/7 |
| *C. mona* | 1/1(0) | 1/1(0) | 1/1(0) | 1/1(0) | 1/1 |
| *C. campbelli lowei* | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| *C. cephus* | 4/5(1) | 4/5(0) | 4/5(0) | 4/5(0) | 4/5 |
| *C. mitis stuhlmani* | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| *Allenopithecus nigroviridis* | 1/8(0) | 1/8(0) | 1/9(0) | 3/8(0) | NT |
| *Miopithecus talapoin* | 8/15(4) | 9/15(11) | 13/15(10) | 9/15(0) | 9/15 |
| Colubids | | | | | |
| *Colubus quereza* | 0/14 | 1/14(0) | 2/14(0) | 1/14(0) | 0/10 |
| *C. guereza kukuyuensis* | 1/24(0) | 1/24(2) | 0/24 | 1/24(0) | 0/24 |

| Genus and species | ELISA antibody-positive/total animals tested (Western blot+) | | | | |
|---|---|---|---|---|---|
| | HTLV-I | HTLV-III | SRV-1 | FeLV | Cell debris |
| *Presbytis entellus* | 0/5 | 0/5 | 1/5(0) | 0/5 | 0/5 |
| *P. obscurus* | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 |
| *P. senex* | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| *P. francoisi* | 0/7 | 0/7 | 0/7 | 0/7 | 0/7 |
| *P. cristata* | 0/12 | 0/12 | 2/12(0) | 0/12 | 0/12 |
| *Pygathrix nemaeus* | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| *Nasalis larvatus* | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| *Rhinopithecus rosellane* | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| Total | 124/526(39) | 123/526(23) | 149/519(17) | 150/508(0) | 90/488 |

Number of animals with true serum antibody reactions to test virus by Western blotting in parentheses.
*(No. positive/No. tested) in Western blots.

buffer. The nitrocellulose sheet was then cut into strips; molecular weight standards were stained with amido black.

Strips were incubated with monkey sera diluted 1:100 in ELISA dilution buffer for 2 h at 37°C. Strips were then washed several times in ELISA washing buffer and reacted with rabbit anti-monkey IgG conjugated to horseradish peroxidase for 1 h at 37°C. The strips were then washed and incubated with substrate solution (25 mg diaminobenzidine, 0.1 M Tris, 0.01% $H_2O_2$, pH 7.4) for 5–20 min. Strips were washed several times with distilled water and air dried.

## Results

*Antibody testing of old-world primates*

ELISA screening of 526 old-world primate sera demonstrated a high incidence of positive reactions to one or more of the three principal screening retroviruses, HTLV-I, HTLV-III and SRV-1 (Table 1). One hundred twenty-four out of 526 sera from 21 species of monkeys were ELISA-positive for HTLV-I antibodies, 123/526 sera from 18 species were positive for antibodies to HTLV-III, and 149/519 sera from 24 species had antibodies to SRV-1. The high incidence of ELISA-positive reactions to HTLV-I, HTLV-III and SRV-1 was surprising and led us to question the specificity of the ELISA antibody procedure. Therefore, the same sera were retested by Western blotting. A presumptive positive reaction was only scored on sera reactive by this procedure. Antibodies to HTLV-I, confirmable by Western blotting, were detected in 1/2 *Pan troglodytes* (chimpanzees), 2/6 *Pan paniscus* (pygmy chimpanzees), 6/23 *M. sphinx* (mandrills), 1/23 *M. fasicularis* (crab-eating macaques), 1/26 *M. silensus* (lion-tailed macaques), 2/5 *M. maurus* (Moor macaques), 2/12 *M. niger* (black macaques), 6/21 *M. tonkeana* (Tonkeana macaques), 1/6 *M. nemistrina* (pigtail macaques), 1/1 *M. radiata* (bonnet macaques), 2/7 *Cercocebus atys* (sooty mangabys), 5/116 *C. aethiops* (African green monkeys), 4/11 *Cercopithecus albogularis kolbi* (Kolb's guenons), 1/5 *C. cephus* (moustached guenon), and 4/15 *M. talapoin* (talapoins). Specific antibodies to HTLV-III were found in 23 monkeys, 4/7 sooty mangabys, 11/15 talapoins, 2/11

TABLE 2

The results of HTLV-I, HTLV-III and SRV-1 ELISA antibody in sera from African green monkeys and lion-tailed macaques.

| Primate species | ELISA antibody (P:N) to: | | |
|---|---|---|---|
| | HTLV-I | HTLV-III | SRV |
| African green monkeys | | | |
| 1 | 7.1 | 8.4 | 4.8 |
| 2 | 5.2 | 4.4 | 3.8 |
| 3 | 6.9 | 7.3 | 4.7 |
| 4 | 4.1 | 5.3 | 3.1 |
| 5 | 3.3 | 3.0 | 3.0 |
| 6 | 5.1 | 5.1 | 3.8 |
| 7 | 5.4 | 5.3 | 4.0 |
| 8 | 1.9 | 3.2 | 1.9 |
| 9 | 6.6 | 8.0 | 4.4 |
| 10 | 1.5 | 2.6 | 1.7 |
| 11 | 2.1 | 3.8 | 2.0 |
| 12 | 1.0 | 1.0 | 1.0 |
| 13 | 8.1* | 2.4 | 2.3 |
| 14 | 1.0 | 1.2 | 1.6 |
| 15 | 8.0* | 1.1 | 1.7 |
| 16 | 7.6* | 1.0 | 1.4 |
| Lion-tailed macaques | | | |
| 1 | 1.0 | 1.4 | 1.0 |
| 2 | 4.7 | 6.3 | 3.0 |
| 3 | 1.0 | 0.7 | 1.0 |
| 4 | 19.3 | 23.6 | 11.6 |
| 5 | 7.9 | 8.9 | 4.5 |
| 6 | 16.4 | 18.0 | 10.0 |
| 7 | 11.6 | 15.8 | 8.5 |
| 8 | 15.3 | 18.8 | 10.0 |

ELISA values expressed as ratio of positive to negative (control) readings.
* Confirmed positive by Western blotting.

Kolb's guenons, 2/24 kukuyu colobus and 4/23 mandrills. Antibodies to SRV-1, confirmable by Western blotting, were detected in 11 monkeys, 1/23 crab-eating macaques and 10/15 talapoins. Out of 396/1571 serologies that were positive for one of the three test viruses by ELISA, only 79/396 (20%) were confirmably positive by Western blotting.

False-positive ELISA antibody reactions to one or more viruses were found in a low proportion of individuals from many different species of old-world primates (Table 1). Six species in particular, mandrills, crab-eating macaques, lion-tailed macaques, African green monkeys and DeBrazza's and moustached guenons, were often falsely positive for all three viruses (Table 1). We were interested, therefore, in determining why sera from these six species of monkeys possessed such high levels of nonspecific reacting antibodies.

One explanation for the high incidence of false-positive ELISA antibody tests was that the positive to negative ratio (P:N) that we used as a cutoff value was too low. In these studies, we assumed that a P:N ratio of 2.0 or greater was positive.

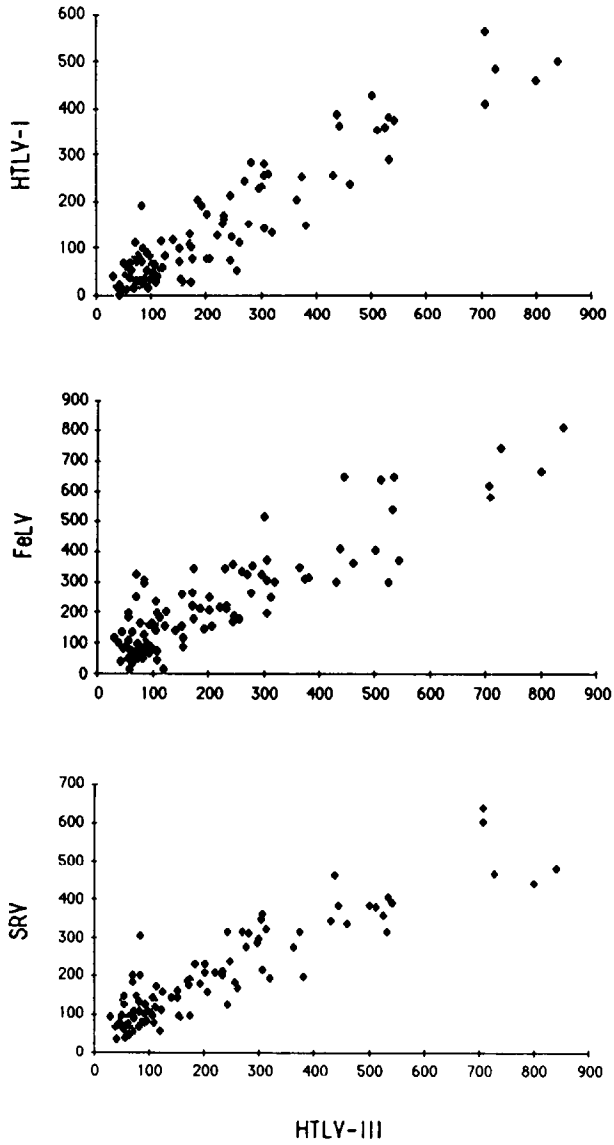


Fig. 1. Plots made from graphing OD values of African green monkey sera to HTLV-I (upper), FeLV (middle) or SRV-1 (lower) against OD values obtained when the same sera were tested against HTLV-III.

Non-specifically positive ELISA tests (ELISA positive, Western blot negative) in langurs (*Presbytis entellus* and *P. obscurus*), silver-leaf monkeys *(Presbytis cristata)*, spotnose guenons *(Cercopithecus nictitans)*, colobus, baboons, siamangs *(Symphalangus syndactylus)*, and orangutans *(Pongo pygamaeus)* were almost always in the range of 2.5–4.0 (data not shown). Exceptions were found, however,

in sera from the six problem species that were antibody-positive across the board and in a high proportion of animals in each group. Table 2 shows the P:N ratios of representative monkeys from two of these six species, African green monkeys and lion-tailed macaques. False positives in these species frequently had P:N ratios above 5. It was concluded, therefore, that raising the P:N ratio to 4.0 would eliminate false-positive reactions in some monkeys, but not in the six problem species.

*Causes of false-positive ELISA antibody reactions*

In a further attempt to identify the causes of the nonspecific ELISA antibody reactions that we had observed, we decided to concentrate the remainder of our studies on sera from African green monkeys. The pattern of false-positive reactions was identical in all six of the problem species, so we felt that African green monkey sera contained similar false-reacting factors. African green monkey sera were also much easier to obtain in sufficient number and quantity.

African green monkey sera that were positive for HTLV-III antibodies by ELISA were usually positive for HTLV-I and SRV-1 as well. In fact, plots graphing the optical density (OD) values of the HTLV-III antibody assay against the OD values for HTLV-I, FeLV and SRV-1 antibodies were virtually linear, with correlation coefficients greater than or equal to 0.88 (Fig. 1). This type of reactivity made us question whether we were even dealing with an antibody-mediated reaction. The anti-viral activity of the positive sera, however, could be isolated to the globulin component and comigrated with the IgG fraction obtained by Biogel 1.5 M column chromatography (Fig. 2).

The retrovirus specificity of ELISA-positive and -negative African green monkey sera was next studied. Sera were reacted against two nonretroviruses: (1) feline infectious peritonitis virus (FIPV), a coronavirus, and (2) feline panleuko-

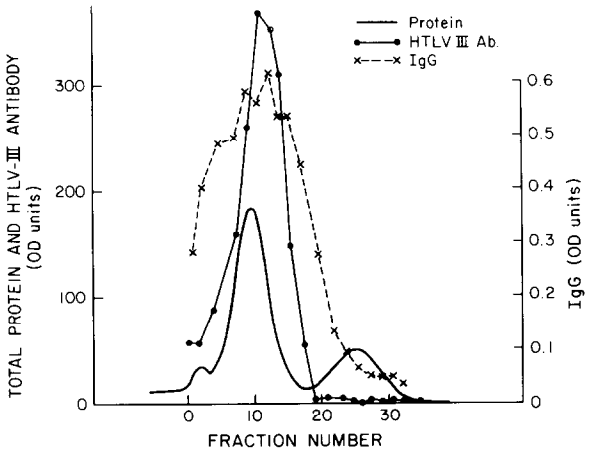


Fig. 2. Biogel 1.5 M separation of African green monkey serum containing high levels of ELISA-reacting antibody to HTLV-III. HTLV-III antibody activity resided in the IgG-containing fractions.

penia virus (FPV), a parvovirus. Sera that were positive against HTLV-III also reacted strongly against each of these non-retroviruses. The values for the antibody reactions to the two viruses, when plotted against the OD values for HTLV-III antibodies, were also linear, with correlation coefficients greater than 0.88 (Fig. 3).

At this point, it was doubtful whether the ELISA antibody reactivity contained in African green monkey sera was even viral specific. In our final test, mock pur-

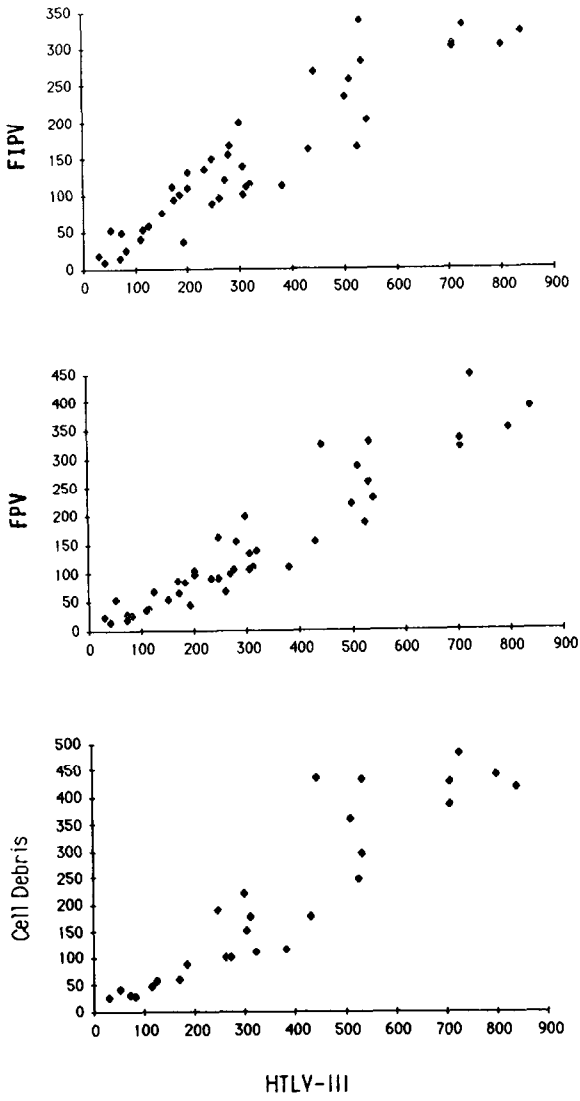


Fig. 3. Plots made from graphing OD values of African green monkey sera to feline infectious peritonitis virus (FIPV) (upper), feline panleukopenia virus (FPV) (middle) and HUT-78 cell debris (lower).

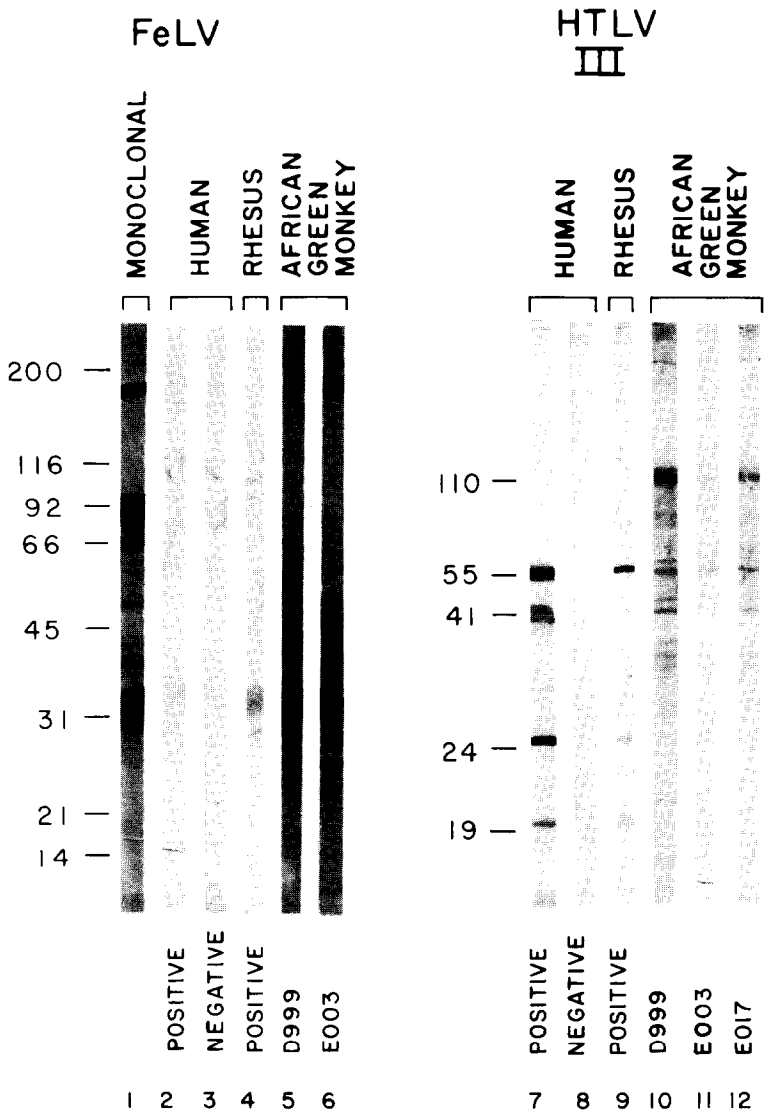


Fig. 4. Western blots of various antisera that were reacted against FeLV or HTLV-III. Western blots of FeLV were run against mouse monoclonal anti-gp70 and -27 (lane 1), HTLV-III antibody-positive and -negative human sera (lanes 2,3), HTLV-III antibody-positive rhesus monkey serum (lane 4), and two African green monkey sera with false ELISA-positive antibodies to HTLV-III (lanes 5,6). HTLV-III blots were tested against HTLV-III-positive human sera (lane 7), HTLV-III-negative human sera (lane 8), HTLV-III antibody-positive rhesus monkey sera (lane 9), false positive (lane 10), negative (lane 11) and false positive (lane 12) African green monkey sera. Molecular weight standards in kilodaltons are indicated to the left of each series of gels.

ified subcellular debris from non-infected HUT-78 cells was used as the test antigen. The same sera that reacted positively against HTLV-I, HTLV-III, SRV-1, FeLV, FIPV and FPV also reacted positively at the same proportionate intensity with the cell debris (Fig. 3).

*Nature of cellular antigen*

In an attempt to identify the cellular antigen present in all of our virus preparations against which the African green monkey sera reacted, ELISA-positive and -negative sera were reacted in Western blots against gradient purified virus, cell lysates of virus-infected cells, and cell lysates of non-infected cells. None of the ELISA-positive or -negative African green monkey sera reacted positively against specific viral bands of HTLV-I, SRV-1, FIPV or FPV in Western blots (data not shown). However, antibody activity of ELISA-positive sera was strongly directed against the envelope proteins of HTLV-III and FeLV in Western blots (Fig. 4). Interestingly, whether identifiable bands were present or not in the blots, ELISA-positive sera tended to produce a much more intense overall background stain than negative sera (data not shown). This reaction could be eliminated by blocking the strips with bovine serum albumin rather than gelatin (data not shown). A group of 10 ELISA-positive and -negative African green moneky sera were also reacted in Western blotting against infected and non-infected cell lysates. None of the positive or negative sera demonstrated strong reactions against any single protein found in lysates of infected cells used to propagate HTLV-III (HUT-78 cells) or SRV-1 (Ragi cells) (data not shown).

Similar to Western blots prepared from whole virus, there was a pronounced tendency for positive sera to produce a greater diffuse background staining than negative sera (data not shown). This background was proportional in intensity to the reactivity of the sera in ELISA against whole virus. It also could be eliminated by blocking the strips with bovine serum ablumin rather than gelatin.

The fact that ELISA-positive African green monkey sera reacted equally with a number of different virus and cell protein preparations suggested that they recognized a common antigen(s). This was confirmed by doing competition ELISAs. HTLV-III antibody-positive African green monkey sera, when preabsorbed with FeLV, no longer reacted with HTLV-III, SRV-1 or FeLV (Table 3). Antibody could not be absorbed out with fetal bovine serum, indicating that the antibodies present in the sera were specific for cellular proteins. Similarly, HTLV-III positive serum preabsorbed with SRV-1 reacted much more weakly with HTLV-III, SRV-1 and FeLV than nonabsorbed serum (Table 3). In contrast, human or rhesus monkey sera that were specifically positive for HTLV-III still reacted at the same relative intensity after absorption with FeLV or SRV-1 (Table 3).

*Incidence of anti-cell protein antibody*

Studies with African green monkey sera established the cause of false-positive ELISA antibody tests in this species; sera appeared to contain a specific antibody or antibodies that was directed against cell-associated protein(s) that were co-purified with the various virus preparations. This protein was present in several di-

TABLE 3

Removal of HTLV-III binding activity from sera of African green monkeys by absorption with fetal bovine serum unrelated retroviruses.

| Serum | Unabsorbed | Serum HTLV-III ELISA antibody titer after absorption with: | | |
|---|---|---|---|---|
| | | Fetal bovine serum | FeLV | SRV-1 |
| Positive human | 0.397±0.80 | 0.508±0.21 | .299±.002 | 0.265±.019 |
| African green | | | | |
| High positive | | | | |
| D999 | 0.344±.009 | 0.301±.016 | 0.010±.001 | 0.069±.019 |
| E022 | 0.303±.003 | 0.255±.005 | 0.010±.003 | 0.043±.001 |
| Medium positive | | | | |
| E006 | 0.145±.012 | 0.118±.005 | 0.012±.002 | 0.015±.004 |
| Low positive | | | | |
| E023 | 0.083±.002 | 0.081±.010 | 0.012±.012 | 0.018±.001 |
| Rhesus positive | 0.205±.013 | 0.225±0.35 | 0.156±.001 | 0.142±.003 |

All sera were diluted to half-maximal binding capacity. Maximal binding occurred at 1:100 dilution for each serum, while half-maximal binding occurred at 1:400.

TABLE 4

Correlation between ELISA antibody seropositivity to FeLV or cell-debris and to false-positive reactions to HTLV-I, HTLV-III, or SRV-1.

| Genus and species | Positive to FeLV or cell-debris, or both | FeLV- and cell debris-positive sera that were also false-positive to one or more of the test viruses, HTLV-I, III, SRV-1 |
|---|---|---|
| *Pongo pygmaeus* | 2/19 | 2/2 |
| *Hylobates concolor* | 2/10 | 2/2 |
| *Mandrillus sphinx* | 9/24 | 8/9 |
| *Macaca fasicularis* | 7/22 | 4/7 |
| *Macaca silensus* | 20/22 | 18/20 |
| *Cercopithecus aethiops* | 82/116 | 82/82 |
| *Cercopithecus neglectus* | 3/4 | 2/3 |
| *Cercopithecus cephus* | 4/5 | 4/4 |
| *Miopithecus talapoin* | 9/16 | 4/9 |

verse species of animal cells and appeared to be present at low levels in gelatin, a complex animal protein. The possibility that this antibody was directed to glycoproteins was suggested by Western blot studies.

As a final experiment, we retested most of the old-world primate sera for ELISA antibodies to FeLV and to cell debris. Both of these antigen preparations, but in particular FeLV, seemed to identify sera that contained the anti-cell protein antibody. We found that 150/508 old-world primate sera reacted with FeLV in ELISA and 90/488 with HUT-78 cell debris (Table 1). There was a high correlation between seropositivity to FeLV or cell debris and false-positive ELISA antibody re-

actions to HTLV-I, HTLV-III or SRV-1, especially in the six problem species that were identified earlier in the study (Table 4).

## Discussion

The influence of nonspecific antibody reactivity on seroepidemiologic studies was apparent from this study. About one-fourth of the monkeys that we tested were positive for one or more of the human and simian retrovirus antigen preparations that we used. However, only about one in five of these ELISA antibody-positive sera was confirmed to be positive by Western blotting. The cause of these false-positive ELISA reactions was found to be a serum antibody that reacted with cellular proteins that were co-purified with our viral antigen preparations. Such contaminating proteins are virtually impossible to remove from preparations of viruses made from cell-culture fluid or infected tissues. The propensity of this antibody to react with cellular and viral membrane proteins, and its seeming lack of host specificity, indicated that it was directed against glycoproteins or carbohydrate moieties of glycoproteins. Antibodies to Forssman's protein, blood group substances, and histocompatibility antigens occur naturally in many animal sera and might have been involved in the phenomenon that we observed. The fact that certain species of monkeys possessed this antibody to a much higher degree than others indicated that it was not due just to environmental exposure. It is common knowledge, however, that many sera from African humans and primates tend to be 'sticky' in antibody assay procedures and some researchers believe that this might be due to the high degree of exposure to infectious diseases (Biggar et al., 1985).

The problem of nonspecific antibody binding is intrinsic to ELISA antibody procedures. The appearance of color in the reaction mixture indicates only that antibody has been bound to the plastic well. Whether or not this binding is specific cannot be accurately answered without appropriate controls. This is not as great of a problem with Western blots, which measure antibody binding to characteristic bands of viral protein. Complement fixation tests suffer from the same problems as ELISA procedures. If the complement fixating antigen is impure, and antibodies bind to antigens other than the ones being tested, then a nonspecific reaction occurs.

This study was not the first to indicate that nonspecific antibody-binding reactions might be a problem in primate seroepidemiologic studies. Kalter and colleagues (1969) reported that a large proportion of simians gave strong complement fixation reactions to Marburg virus-positive guinea pig antigen prepared by the Communicable Disease Center (CDC). Strickland-Cholmley and Malherbe (1971), however, indicated that such reactions might have been nonspecific. They found that positive CDC antigen reacting sera were usually negative when tested in parallel with a monkey liver antigen preparation. These investigators postulated that the CDC antigen contained a second and stronger non-Marburg virus antigen, and that this antigen accounted for the high number of simian reactors tested with this antigen. Strickland-Cholmley and Malherbe (1971) also observed that many ba-

boon and African green monkey sera reacted strongly in complement fixation tests with uninfected guinea pig and monkey control liver antigen. They postulated that these 'autoantibodies' might have resulted from previous liver damage. A more likely explanation, however, was that these sera contained antibodies that reacted specifically or nonspecifically with normal cellular proteins present in their liver preparations. Kalter (1971), after an exhaustive seroepidemiologic search for antibody to Marburg virus in African green monkeys, concluded that "it is obvious that a larger number of sera were either nonspecific, anticomplementary, or both...". Kalter (1971) also found, after examination of Japanese macaque sera for antibody to a number of agents, "...that antibody to an uncommonly large number of antigens are detected by CF" and HI procedures. At this time it is difficult to evaluate these findings. It would appear that these animals have a rather high incidence of 'antibody' as a result of (1) numerous contacts with various agents other than Marburg virus, (2) a nonspecific inhibitor in the sera of this species of Macaca resulting in a high incidence of 'seropositives'...". Kalter went on to state that "...testing old-world African simians for antibody continued to indicate a large number of serologic positives, with the talapoin stil showing the greatest incidence." It is noteworthy that in our study several species of macaques and talapoins also possessed high levels of nonspecific cell protein antibodies.

The problem of nonspecific antibodies in sera, especially when assayed by ELISA, is not unique to primates. ELISA antibody screening of human sera for HTLV-III is most accurate when high-risk candidates are tested. Nonspecific positives are a much bigger problem, however, when the test is used for screening of low-risk groups (Carlson et al., 1985a). Many researchers believe, therefore, that all HTLV-III ELISA positives should be confirmed by other procedures, such as Western blotting (Carlson et al., 1985b). In fact, some investigators state that even Western blotting may not be accurate with freeze-thawed and hypergammaglobulinemic sera, and that radioimmunoprecipitation assays should be the standard for specificity (Brun-Vezinet et al., 1986). False-positive HTLV-III antibody reactions can also be a problem in populations considered to be at high risk. Biggar and associates (1985) found that from 12 to 25% of 250 Zairian people reacted positively in ELISA to HTLV-I, II and III. There was a strong correlation between the levels of these antibodies and the levels of antibodies to *Plasmodium falciparum*. One of their possible explanations for this phenomenon was "false-positive reactivity in the ELISA assay due to cross-reactive antibodies or other unknown factors." Biggar (1986) has also eluded to false-positive reactions in sera from African children tested against HTLV-III (Epstein et al., 1985; Sainger et al., 1985). False-positive reactions to human retroviruses have also been described by Snyder and associates (1976) and Snyder and Fox (1978).

These studies bring in the question of using ELISA antibody tests to screen large populations of animals or people for viral antibodies. In most species, however, ELISA antibody tests have proven fairly accurate for screening purposes. We were able to concentrate almost all of our 'true' positives into a smaller number of sera, on which we could concentrate our more specific testing. The only notable exception was in the case of sooty mangabys and Kolb's guenons; only 3/8 were positive

for HTLV-III antibodies by ELISA, while 6/15 that were tested showed characteristic HTLV-III core protein bands on Western blots. A lentivirus was readily isolated from the blood of the sooty mangabys (Lowenstine et al., 1986). Care should also be taken not to eliminate sera that appear to be reacting falsely. Talapoins and mandrills in our study were found to have a very high incidence of nonspecific FeLV and cell debris antibodies, but this was masking a large number of HTLV-I, III and SRV-1 specific reactors (Lowenstine et al., 1986).

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