Antibodies in Primates to the Marburg Virus* (33476)

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The recent outbreak of human disease occurring among laboratory and hospital personnel in Germany and Yugoslavia associated with handling of tissues and blood of African green monkeys has been well documented (1–5). Characterization and electron microscopy of the agent has recently been reported by Kissling *et al.* (5). Unfortunately, still not resolved is the epidemiology of this disease, although it is accepted that African green monkeys (*Cercopithecus aethiops*) were the vector responsible for this outbreak. Of importance, therefore, is the need to ascertain the source and reservoir of this agent in order to institute more stringent precautions to insure the safety of laboratory workers.

Materials and Methods. Sera. Primate sera were obtained from the following sources: man—Africans (Kenya) and laboratory personnel at the Southwest Foundation for Research and Education (SFRE); gorilla (*Gorilla gorilla*)—Yerkes Primate Center; chimpanzee (*Pan* sp.)—Yerkes and Delta Primate Centers, SFRE; orangutan *Pongo pygmaeus*)—Yerkes Primate Center; baboon (*Papio* sp.)—SFRE; African green (*Cercopithecus aethiops)*—SFRE and Institute Merieux; talapoin *(Cercopithecus talapoin)* —San Diego Zoo; patas *(Erythrocebus patas)* —Delta Primate Center and Institute Merieux; gibbon (*Hylobates* sp.)—Yerkes and Delta Primate Centers and National Institutes of Health; rhesus *(Macaca mulatta)*— SFRE, Delta Primate Center and School of Aerospace Medicine, Brooks A.F.B., Texas; cynomolgus *(Macaca fascicularis)*—Institute Merieux; and marmoset (*Sanguinus fuscicollis* and *S. nigricollis*)—St. Luke's Presbyterian Hospital. Details concerning collection of these sera have been described elsewhere (6).

These sera were maintained frozen at —20° from time of collection until used in these studies. All sera were inactivated at 56° for 30 min prior to use and tested at a single dilution of 1:10.

Antigen. Due to the high infectivity of this virus, inactivated CF antigen prepared by Dr. R. Kissling, National Communicable Disease Center (NCDC), was obtained. This antigen, prepared from infected guinea pig spleens, was inactivated with 1:2000 β-propriolactone and supplied to us frozen. A "checkerboard" titration with guinea pig antisera indicated a working titer of 1:16 which was in agreement with that obtained by Dr. Kissling. In spite of the demonstration that this antigen had been inactivated by failure to produce evidence of infection in guinea pigs, all precautions were taken to prevent possible laboratory infection.

Complement fixation test. The microtiter procedure as described by Sever (7) was employed for this survey.

Results. Figure 1 presents the findings as obtained by testing the various listed sera. As shown, the two different groups of human sera—i.e., from Africans living in Kenya and laboratory personnel at SFRE, as well as gorilla, orangutan, gibbon, and marmoset sera—demonstrated no evidence of CF antibody to the Marburg virus antigen. Two of the cynomolgus monkey and one of the rhesus monkey sera showed antibody. All the other sera also indicated some antibody to varying degrees: chimpanzee, baboons, African greens, patas, and talapoins. Interestingly, baboons and chimpanzees born in captivity (SFRE and Yerkes) did not have any antibody by this technique.

Discussion. Occurrence of a highly fatal disease in handlers of tissues and blood derived from African green monkeys has resulted in understandable concern among individuals employing nonhuman primates for various biomedical studies. It would be of obvious assistance if information was available relative to the specific animal source of

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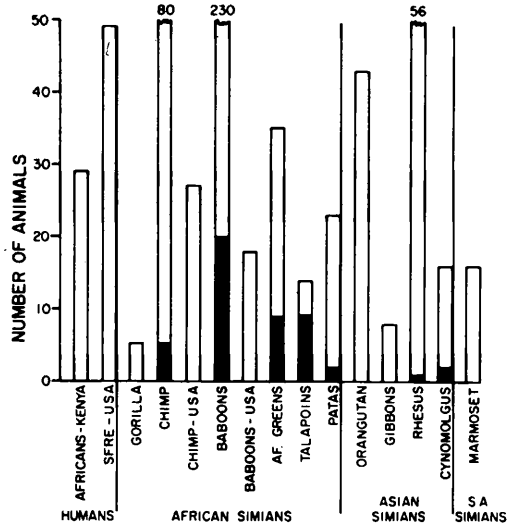

FIG. 1. Number of sera with complement fixing antibody to the Marburg virus.

this virus. Various considerations have been given to the possible relationship of this virus to one or another simian. However, little, if any, clear-cut pertinent data are available. For example, Simpson *et al.* (4) examined, with negative results, 201 vervet monkey sera collected in Uganda as well as the one langur with which the original monkeys were in contact. Kissling (personal communication), employing the same antigen as reported herein, found extensive evidence of antibody in practically all species of simians tested. Stojkovic (personal communication) found 90% of 41 surviving African green monkeys to have CF antibody to this agent. These African green monkeys had come in the original shipment and started dying several weeks after arrival in Yugoslavia.

Two questions still remain unresolved at this time. Perhaps the most important concerns the failure to see this clinical picture prior to 1967 in spite of thousands of African green monkeys sacrificed. Also of importance is the failure of Simpson and his coworkers (4) to detect CF antibody in the vervet monkeys studied by them. A possible explanation, but still in need of study, may reside in preparation of the antigens by the different laboratories. Also disconcerting was the extremely high mortality found in experimentally inoculated monkeys (8). Results such as these may be interpreted as overwhelming infection with no antibody development.

The results reported herein at first appeared to be somewhat at odds with those obtained by Kissling. However, when the NCDC test was adjusted (in terms of serum dilution used) to compare with that of SFRE, relatively good agreement was noted. Furthermore, if the results obtained in this study can be confirmed, it sould appear that this agent is associated with African animals. Three Asian animals, *i.e.*, two cynomolgus monkeys and one rhesus monkey, had antibody, and Kissling found antibody in orangutans (personal communication). Unfortunately, complete histories on these positive non-African primates are not available. It is known that both the cynomolgus and rhesus monkeys as well as the orangutans had previous contact with African simians. Further support to the suggestion that this virus has an African source may be the failure to find antibody in chimpanzees and baboons born in the U.S. Additional studies relative to these findings are in progress.

Summary. A serologic (complement fixing) survey of various primate sera for antibodies to the Marburg agent has demonstrated that the majority of positives were obtained from nonhuman primates born in Africa.

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Growth of High Titered Rubella Virus in Roller Bottle Cultures of Vero Cells* (33477)

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Attempts to isolate and fully characterize the structural components of rubella virus (RV) and associated virus specified nonstructural proteins have been seriously restricted by the relatively low yields of virus obtained from infected tissue cultures. Suspended cultures of BHK-21 cells which have yielded infectivity titers as high as $10^{7.5}$ ID_{50}/ml have been the most efficient producers of rubella virus available to date (1).

Replication of RV with concomitant production of cytopathic effects (CPE) in Vero cells (continuous African green monkey kidney) was reported in a previous publication (2). Though the data presented in that study dealt primarily with the use of these cells for primary isolation and direct assay of RV infectivity, it was shown that they supported the growth of RV to relatively high titers. The present communication describes a method of growing RV in roller bottle cultures of Vero cells which yields 1–2 liter quantities of virus suspension titering in excess of 10^9 $TCID_{50}$/ml. The procedure provides in addition a correspondingly large yield of hemagglutinin (HA) and complement fixing (CF) antigen.

Materials and Methods. Cylindrical bottles 10 cm in diameter × 40 cm in length (840 cm^2 surface area)[2] were seeded with 3 × 10^7 Vero cells in 150 ml of prewarmed HMEM–5% CS[3]. The bottles were placed in a roller apparatus[2] housed in a 37° warm room and rolled at 0.5 rpm. After 2 or 3 days the medium was replaced with EMEM–5% CS medium (3).[3] The monolayers were confluent and ready to use 2–3 days later. At this stage the average number of cells per bottle was 2 × 10^8. Before virus inoculation, growth medium was removed and the cell sheet was washed with 100 ml of balanced salt solution. The RV inoculum (10–15 ml) was allowed to adsorb to the monolayer on the roller apparatus for 60–90 min. The unadsorbed inoculum was then removed and replaced with 50 ml of prewarmed EMEM containing 5% kaolin treated newborn agamma calf serum. At 24-hr intervals the culture fluid was harvested and replaced with 50 ml of fresh prewarmed medium. Daily harvests were continued for up to 10 days. Culture fluids were assayed for HA (4) and infectivity by CPE in Vero cells and by interference in primary African green monkey kidney cultures (2). For extraction of HA and CF antigen from the infected monolayer, 10 ml of 0.1 *M* glycine buffer, pH 9.0, containing 0.4% bovine serum albumin (4) was added to the culture bottle after the final daily harvest of infectious fluid was removed. The bottle was then rolled at 37° for periods of 8 or 30 hr. The extract was freed of gross

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[1] Liebhaber, H., Pajot, T., to be published.

[2] Purchased from Bellco Glass Co., Vineland, New Jersey.

[3] (MEM) minimal essential medium; (H) indicates Hanks balanced salt solution; (E) indicates Earles balanced salt solution; 5% CS indicates 5% calf serum.