

THE ISOLATION OF ILHÉUS VIRUS FROM MAN IN PANAMÁ

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Since the initial isolation of Ilhéus encephalitis virus from mosquitoes in Brazil in 1944,¹ a number of other isolates have been obtained in Brazil,² Trinidad,^{3, 4} Colombia,⁵ Panamá,^{6, 7} and Central America.^{8, 9} Most of these isolates were obtained from mosquitoes and birds.^{10, 11} Although antibody rates to Ilhéus virus were high in human beings in much of northern South America, identifiable disease was not often seen. So far two Ilhéus isolates in Brazil² and three in Trinidad²² have been secured from man. No isolations of this virus have been made from wild mammals.

This report describes the isolation of Ilhéus virus from man, representing the first case to be reported from Panamá and gives the results of an antibody survey in the human population from the same area where the case in a human being occurred.

Isolation of Virus

One of our staff members (C.M.) was collecting amphibians and reptiles in the northwestern part of Panamá in November 1964. After 3 weeks in the field, he returned to the city with general malaise. High fever with pronounced headache developed 3 days later and lasted for about a week. Physical examination revealed no evidence of involvement of the central nervous system and no abnormalities other than a fever of 101°F. A blood count at this time showed 7,800 leukocytes per cmm with 88% neutrophils, 11% lymphocytes, and 1% monocytes. Three days later, the white count was 4,950 per cmm with 31% lymphocytes. A blood sample drawn 3 days after onset of illness was centrifuged and serum inoculated intracerebrally (i.c.) into a group of seven 3-day-old Swiss mice and into two tubes of hamster-kidney tissue culture (HKTC). All inoculated mice survived during a 2-week observation period, but cytopathogenic effects were detected in the HKTC. Fluid from the HKTC was then inoculated i.c. into a group of seven suckling mice, and two of these became sick 8 days later. Brain suspension from the affected mice was passed to another group of suckling mice. All inoculated mice were ill on the 4th day,

and a stock virus was prepared from their brains. This agent was designated F 267 A.

Virus Identification

Prototype strains of group B arboviruses used in the identification of the newly isolated strain were as follow: Ilhéus (BT 3875),⁶ St. Louis encephalitis (BV-7) (both isolated in Panamá), yellow fever (French neurotropic), Bussuquara (BeAn 4116), received from Belém Virus Laboratory, and Dengue type II (tr 1751) obtained from the Communicable Disease Center, Atlanta, Georgia.

Immune sera for each of these type strains, as well as the test strain, were prepared by immunizing adult mice with two intraperitoneal injections, 10 days apart, of 10% infected mouse-brain suspensions. Bleeding was done 10 days after the second inoculation. Hyperimmune mouse sera prepared from different viruses of groups A, B, C, and others were also used in screening tests of the new isolate antigen.

Sucrose-acetone extracted antigen¹² of the new isolate, F 267 A, prepared from suckling-mouse brain gave hemagglutinating (HA) titer of 1:320. Hemagglutination-inhibition screening tests of this new isolate showed negative results with hyperimmune mouse sera of groups A, C, Bunyamwera, Guamá, California, Turlock, and Icoaraci viruses. This hemagglutinin was highly reactive when tested with group B immune mouse serum. Cross-HI tests were performed with this isolate and with various members of group B arboviruses. The results, shown in Table 1, indicate that it was closely related to Ilhéus virus strain BT 3875 isolated from mosquitoes in Panamá in 1958.⁶ Confirmation of these results by cross-CF test (Table 2) demonstrated that the virus strain F 267 A isolated from man was indistinguishable from Ilhéus virus.

Validity of Isolate

On attempts at reisolation from the original F 267 A human serum 3 months after storage at -65°C, both inoculated mice and HKTC appeared normal during a 14-day period of observa-

TABLE 1

HI tests of the virus isolate with group B arboviruses

| Serum | Antigen | | | | | |
|-----------------|---------|--------|-----|-----|------|-------|
| | F 267A | Ilhéus | YF | SLE | D II | Buss. |
| F 267 A | 320* | 320 | 40 | 20 | 20 | 20 |
| Ilhéus | 320 | 320 | | | | |
| YF | 0 | | 320 | | | |
| SLE | 20 | | | 320 | | |
| D II | 0 | | | | 80 | |
| Bussu- quara | 20 | | | | | 80 |

* Reciprocal of serum titer, 0 = less than 1:20.

tion. Material from HKTC from the 6th day of this period, repeatedly frozen and thawed, was also inoculated into a group of suckling mice with negative results. However, serum obtained from the patient 3 weeks after the onset of illness, during convalescence, showed a CF titer of 1:32 to Ilhéus antigen, whereas the sample taken during the acute stage of the illness was negative in the same test. In addition, no Ilhéus virus was handled in the laboratory during the period when this isolation was made.

Serum Survey

Human sera collected in 1964 at Almirante in the northwestern part of Panamá, where the patient might have contracted the infection, were tested with certain members of group B arboviruses in HI tests and with Ilhéus virus in neutralization tests. Of 348 sera from all age groups tested, 94 samples were positive in HI

tests with Ilhéus or with other group B antigens included in the tests (SLE, YF, Bussuquara and Dengue type II), or with both. In the *in vivo* neutralization tests of 70 randomly selected samples, 19 were positive when tested with approximately 100 LD₅₀ doses of Ilhéus virus. Results in HKTC neutralization tests indicated that 72.7% of 154 group B HI-positive samples were capable of neutralizing 100 TCD₅₀ doses of Ilhéus virus. Thus, infection of man with Ilhéus virus was not uncommon in the Almirante area during 1964, or earlier.

DISCUSSION

Results of the isolation of Ilhéus virus from blood serum of a febrile patient, initially obtained by tissue-culture host system but not by suckling mice, suggest that HKTC is more susceptible to Ilhéus virus of low concentration during the late acute phase of the illness. However, titration of Ilhéus virus does not differ much in these two host systems under our test conditions. The difficulties in recovering the virus from the patient's blood serum in this study are correlative with the fact that only this single isolate of Ilhéus was made from more than 400 cases of fever of unknown origin studied in Panamá since 1961,^{14, 15} although neutralizing antibodies to this virus were found in a high percentage of the Panamanian population.

SUMMARY

The first case in man of Ilhéus-virus infection in Panamá was detected in November 1964. This strain of virus was shown to be closely related to

TABLE 2

*CF tests of the virus isolate with group B arboviruses**

| Serum | Antigen | | | | | | | |
|-----------------|---------|--------|----|-----|------|-------|---------|-----|
| | F 267A | Ilhéus | YF | SLE | D II | Buss. | Powass. | NMB |
| F 267 A..... | 32† | 32 | 0 | 4 | 0 | 4 | 0 | 0 |
| Ilhéus..... | 64 | 32 | 4 | 4 | 0 | 4 | 0 | 0 |
| YF..... | 0 | | 64 | | | | | 0 |
| SLE..... | 4 | | | 64 | | | | 0 |
| D II | 0 | | | | 32 | | | 0 |
| Bussuquara..... | 0 | | | | | 64 | | 0 |
| Powassan..... | 0 | | | | | | 32 | 0 |

* Test done with 2-4 units of antigen.

† Reciprocal of serum titer, 0 = less than 1:4.

Ilhéus virus isolated earlier from birds and mosquitoes in Panamá. Neutralizing antibodies to Ilhéus virus were demonstrated in the human population from the same area.

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