Wyomyia Subgroup of Arbovirus: Isolation from Man

Abstract. An agent, serologically identical to a Wyomyia virus obtained from mosquitoes, was isolated from a worker on the inter-American highway project in Darien Province in eastern Panama. He experienced a mild febrile illness with recovery. A significant rise in antibody titer to this virus was demonstrated in his serum during convalescence. Neutralizing antibodies to this newly isolated strain were found in 10 of 59 blood samples from inhabitants of Darien Province. The virus is designated the Darien strain.

The arthropod-borne Wyomyia virus was first isolated in 1940 from Wyomyia melanocephala in Colombia (1) and has since been classified as belonging to the Bunyamwera group (2). For many years there were no further reports of its occurrence, but recently new strains have been isolated repeatedly from mosquitoes in Brazil (3), Trinidad (4), Colombia (5), and Panama (6, 7). Serological differences have been demonstrated among these various strains which are now regarded as forming a subgroup, called the Wyomyia complex (8). We report the first isolation of a virus strain of this subgroup from a vertebrate host. It is proposed to call this strain Darien for the place where the patient worked.

During the course of a preliminary survey of diseases along the proposed Darien Province section of the inter-American highway in eastern Panama, an adult male worker of the road surveying company was seen at the El Real field station of the Gorgas Memorial Laboratory. Physical examination showed no abnormal signs or symptoms other than a low-grade fever. Malaria parasites were not found in the blood smear; the white cell count was 3800 per cubic millimeter with 73 percent neutrophils, 23 percent lymphocytes, 3 percent monocytes, and 1 percent eosinophils. The red blood count was 4.12 million per cubic millimeter and the hemoglobin was 13 g per 100 ml.

Blood serum from this patient was inoculated intracerebrally into a litter of suckling mice. One mouse was found dead on each of days 10, 11, and 13 after inoculation; the fourth mouse was sick on the 11th day. Brain suspension from the sick mouse was passed to another litter of suckling mice, and all of these were either dead or sick by the 5th day. Stock virus was prepared from a 20 percent suspension of suckling mouse brain in buffered saline containing 0.75 percent bovine albumin. The brain tissue was obtained from mice during the third passage, when all of them were sick after a 4-day incubation period. This preparation had a log10 titer of 6.4 LD50 (lethal dose, 50 percent effective) per 0.02 ml, determined by intracerebral inoculation in suckling mice.

An antigen of this virus, extracted with sucrose and acetone (9), did not agglutinate blood cells of geese, and had a high titer in the complement-fixation test. A screening test was done with mouse antisera to ten different types of arboviruses including Wyomyia; the antigen reacted only with the antiserum to Wyomyia virus. Comparison by checker-board titration in the complement-fixation test indicated that the new strain was closely related to Wyomyia virus strain BT 219, isolated from mosquitoes in Panama at the Middle America Research Unit (6).

Because the Wyomyia virus is classified as a member of the Bunyamwera group, complement-fixation tests were done with other members of this group (Table 1). Strains from Cache Valley and Maguari have been shown to be closely related (8). Antigen from the Darien strain reacted with antisera to the Cache Valley (6V-633), Maguari (BeAn 7272), and Guaroa (BT 1122) strains, but not with antiserum to the Kairi (Tr 8900) strain. On the other hand, in the complement-fixation test, serum of adult mice immunized with three successive intraperitoneal injections of the Darien strain produced heterologous reaction only to the Maguari strain.

Neutralization tests were carried out in suckling mice inoculated intracerebrally with the Darien and BT 219 Wyomyia strains and their antisera from hyperimmune guinea pigs. Both strains were neutralized to the same degree by homologous and heterologous antisera, an indication that these two strains were closely related, if not identical.

The original serum was stored for 6 months at -65°C. The first group of mice inoculated from this material appeared normal during a 15-day observation period. Ten days after inoculation, passage was made from the brains of two of these mice to two other groups of suckling mice. This second group was either sick or died within 7 days after inoculation, which indicated a successful reisolation. All mice inoculated in the third passage were sick within 4 days, and the antigen was prepared from these brains. Identity of the reisolated virus has been confirmed by the complement-fixation test.

A comparison of samples of the patient's serum (Table 1) showed that there were no antibodies to any of the Bunyamwera-group viruses that were tested in the serum obtained during the acute phase of the disease. The samples that were collected 4 and 5 months later during convalescence showed a
significant rise in complement-fixation titers to Darien and BT 219 Wyeomyia strains as well as to Cache Valley and Maguari viruses. This rise indicated that infection with another virus in this group might have occurred not long before or after the Wyeomyia infection. Neutralization tests in mice with the Darien strain and all of the serum samples showed a 2.1 log_{10} rise in the antibody titer. No neutralizing antibodies to Cache Valley and Maguari viruses were demonstrated in the blood sample collected 5 months after the patient's acute phase of infection.

In an effort to determine the prevalence of infection by Wyeomyia virus in human beings, two areas in Panama were selected for antibody surveys. A small number of sera collected from the native population was tested against the Darien strain by neutralization tests. Each undiluted serum was tested with approximately 100 LD_{50}’s of the virus dilution in a group of suckling mice inoculated intracerebrally. Positive reactions are considered as those in which six or seven out of seven inoculated mice were protected. Ten of 59 samples collected from Darien Province gave positive results. In western Panama, where Wyeomyia virus has been isolated from mosquitoes, 60 serums were tested. Only five sera showed neutralizing antibody to this virus.

The recovery of the Darien strain represents the first isolation of a Wyeomyia-complex virus from man, 23 years after the first isolation from mosquitoes. Although only a single isolation from man has been made, the limited antibody surveys indicate its presence in two widely separated areas of Panama.

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References and Notes

5. C. Sanmartin, personal communication.
8. L. Whitman, personal communication.
10. We thank M. Palau for assisting in the clinical study and L. Whitman for suggestions.

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