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Serological Relationship of the Tacaribe Complex of Viruses to Lymphocytic Choriomeningitis Virus

WALLACE P. ROWE, WENDELL E. PUGH, PATRICIA A. WEBB, AND CLARENCE J. PETERS

Laboratory of Viral Diseases and Middle America Research Unit, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

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By means of the indirect fluorescent-antibody test, cross serological reactivity was demonstrated between lymphocytic choriomeningitis (LCM) virus and the viruses of the Tacaribe complex. Antisera to all members of the Tacaribe complex reacted with LCM virus; LCM antisera gave significant staining of Amapari virus, but minimal or inconsistent reactions with Tacaribe virus, and no reaction with two other viruses of the Tacaribe complex. A low level cross-reaction was observed in complement fixation tests of Machupo and Pichinde antisera against LCM antigen. Immunization with Tacaribe and Amapari viruses did not protect mice against challenge with LCM virus. Because of the identical appearance of the virions, the sharing of antigens, and the many biological similarities between LCM and the Tacaribe complex viruses, it is proposed that they be considered as constituting a new taxonomic group of viruses.

Lymphocytic choriomeningitis (LCM) virus, a chloroform-sensitive ribonucleic acid (RNA) virus, has not been assignable into the presently established virus groups, and no serological relationship to other viruses has been described. Electron microscopic visualization of LCM virus has confirmed its uniqueness; the virions are enveloped, roughly spherical particles, ranging in size from 50 to 300 nm in diameter and containing one or more RNA-containing granules resembling ribosomes (1, 5).

Recent observations by Murphy et al. (14) have shown that viruses of the Tacaribe complex have a similar ultrastructural appearance, and these authors have suggested that LCM and the Tacaribe complex viruses be considered a new morphological group. The Tacaribe complex of viruses includes the etiologic agents of Argentinian (15) and Bolivian (10) hemorrhagic fevers (Junin and Machupo viruses, respectively) as well as a number of viruses of unknown pathogenicity (6, 17; C. H. Calisher et al., Amer. J. Trop. Med. Hyg., *in press*; P. A. Webb, K. M. Johnson, and M. L. Kuns, *in preparation*). The members of the complex share complement-fixing (CF) and fluorescent-antibody (FA) stainable antigens (2; W. D. Hann et al., Bacteriol. Proc., p. 185, 1969) but are antigenically distinct by neutralization tests.

In addition to the similarity of appearance, the Tacaribe complex viruses share a number of biological properties with LCM virus, such as production of persistent tolerant infections in rodents (11) and dependence of the mechanism of pathogenesis on the integrity of the thymus-dependent lymphoid system (7, 9, 18-21).

Because of these similarities, a study was undertaken to detect a possible serological relationship between LCM and the Tacaribe complex viruses.

MATERIALS AND METHODS

Viruses. The Fo-2 (12) and CA1371 (20) strains of LCM virus were used; the stocks were prepared in mice before any virus of the Tacaribe complex was introduced into the laboratory.

Two sublines of the prototype strain Tacaribe virus were used. One was obtained from the American Type Culture Collection, and the other was virus maintained in the Middle America Research Unit (MARU) laboratory. The latter had been triply plaque purified in monkey cell culture. No differences were seen in results obtained with the two sublines. Amapari virus was from MARU and had also been plaque-purified. The other Tacaribe complex viruses were prototype strains carried at the MARU laboratory; many had been passed only in hamsters.

Sera. The LCM guinea pig antiserum has been described (22). Hyperimmune mouse LCM antiserum was prepared by R. E. Wilsnack, and hamster LCM antiserum was prepared by John C. Parker from animals bearing transplants of an LCM-contaminated hamster tumor. All immunization was with live virus.

Antiserum to Tacaribe complex viruses was of two types. Hyperimmune polyvalent Tacaribe complex mouse ascitic fluid was obtained from the Reference Reagents Branch of the National Institute of Allergy and Infectious Diseases; this reagent was prepared by

immunization with Tacaribe, Junin, Amapari, Pichinde, and Tamiami viruses. As a control, group A arbovirus grouping mouse ascitic fluid was used; this was prepared in the same laboratory during the same month as the Tacaribe grouping immune ascitic fluid. Hamster antisera to all members of the Tacaribe complex were prepared at MARU; LCM virus has never been knowingly introduced into this laboratory, and serological testing of the mouse and hamster colonies over a 2-year period failed to show LCM antibody. Furthermore, adult mice from the MARU colony were susceptible to intracerebral challenge with LCM virus. The hamsters were immunized by 6 weekly intraperitoneal injections of infected hamster brain, the first 4 being with complete adjuvant.

Mice. Animals used for virus production and immunity tests were of the National Institutes of Health (NIH) strain of Webster Swiss, obtained from the Animal Production Section of NIH.

Tissue culture. Antigens for use in the FA test were prepared in tissue culture. LCM virus was grown in 3T3 (mouse) or Vero (African green monkey kidney) continuous cell lines grown on cover slips; 3T3 cover slips were fixed at 2 or 3 days, and Vero cells were fixed at 4 to 6 days after infection. Tacaribe complex viruses were grown in Vero cells, and cover slips were harvested when cytopathogenicity was first seen, usually at 4 to 6 days. Cover slips were fixed in cold acetone, air dried, and stored at −20 C.

Serological procedures. FA tests were done by the indirect procedure, by using fluorescein isothiocyanate conjugated anti-species globulins and lissamine-rhodamine-bovine serum albumin counterstain. The sources of the anti-globulins were as follows: goat anti-guinea pig, Microbiological Associates, Bethesda, Md.; horse anti-mouse, Progressive Laboratories, Baltimore, Md.; and goat anti-hamster and goat anti-human, Roger E. Wilsnack, Huntington Research Laboratory, Baltimore, Md. The hamster antisera were heated at 56 C for 30 min, whereas the other antisera were used unheated.

CF tests were done in the microtiter system, with 1.8 full units of complement. LCM antigens were cell packs of infected 3T3 and monkey kidney tissue cultures and hamster tumor extracts. Tacaribe and Amapari antigens were 10% suckling mouse brain extracts.

RESULTS

Serological tests. Table 1 summarizes the patterns of results obtained by cross FA testing. All of the Tacaribe complex antisera stained LCM-infected cells and gave no staining of uninoculated control cells; none of the control sera reacted. The LCM antisera stained Amapari-infected cells and gave variable results with Tacaribe virus. The LCM guinea pig serum was also tested

TABLE 1. *Fluorescent antibody tests of LCM and Tacaribe complex reagents*

Antiserum		Infected cells						
		3T3 cells		Vero cells				
Immunizing virus	Species	LCM	Control	LCM	Machupo	Tacaribe	Amapari	Control
LCM	Guinea pig	++(640)[a]	−	++	−	±	±-+(100)	−
	Hamster	++	−	−	−	±	+	−
	Mouse	++	−	−		±	+	−
Polyvalent Tacaribe complex	Mouse	++(100)	−	++(30)		++(300)	++(3,000)	−
Tacaribe	Hamster	++	−	+				−
Amapari	Hamster	++(30)	−	+(10)		++(100)	++(1,000)	−
Machupo	Hamster	++	−	+	++(16)			−
Junin	Hamster	+	−					
Parana[b]	Hamster	+	−					
Pichinde[c]	Hamster	++	−					
Tamiami	Hamster	++	−					
Latino[b]	Hamster	+	−					
None	Guinea pig	−	−		−	−	−	
Normal hamster brain	Hamster	−	−	−	−	−	−	−
Arbovirus group A	Mouse	−	−		−	−	−	−
Murine viruses[d]	Mouse	−	−					
SV5	Guinea pig	−	−		−			

[a] Intensity of staining with 1:10 serum, on a scale of − to ++. FA titer of serum (reciprocal) is indicated in parentheses.

[b] P. A. Webb, K. M. Johnson, and M. L. Kuns, *in preparation*.

[c] A new member of the Tacaribe complex isolated in Cali, Colombia (H. Trapido and C. San Martin, *in preparation*).

[d] Specific antisera for mouse hepatitis, polyoma, Theiler's GD7, Sendai, and K virus.

against Vero cells infected with Pichinde and Tamiami viruses, with completely negative results.

The cytological pattern of FA staining of LCM by the Tacaribe complex antisera resembled closely that seen with LCM antisera (22), that is, cytoplasmic granules and amorphous masses; generally, the cytoplasmic granules were smaller and less abundant with the Tacaribe complex antisera. The staining of Amapari-infected cells by the LCM antisera was chiefly of large disclike cytoplasmic masses, which were also a major feature of the homologous staining patterns.

Tests with human sera confirmed the cross relationship (Table 2). One of two LCM cases showed a clear-cut FA response to Amapari, and one of two Machupo cases developed antibody reactive with LCM-infected cells.

The FA studies thus indicate that there is a serological relationship between the Tarcaribe complex viruses and LCM virus, the reaction being most marked in the direction of Tacaribe complex antisera reacting with LCM antigen.

The cross serological reactivity seen by FA testing generally was not reflected in the CF test. All of the Tacaribe complex antisera listed in Table 1 were tested against 4 to 8 units of LCM CF antigen with completely negative results; these sera had high CF antibody titers to homologous antigens and to other members of the Tacaribe complex. When 32 to 64 units of LCM antigen prepared in 3T3 cells was used, the Machupo and Pichinde hamster antisera reacted to titers of 1:20 and 1:40, respectively (homologous antibody titers were 1:128 and 1:8,192, respectively); these sera gave no reaction with control 3T3 cells. LCM guinea pig, mouse, and hamster antisera were completely negative against potent Tacaribe and Amapari virus antigens when tested at serum dilutions containing 32 to 64 units of LCM antibody.

TABLE 2. *Development of immunofluorescent stainable antibody in humans infected with LCM and Machupo viruses*

Human serum[a]		FA staining[b]					
Infection	Case	Time serum taken	LCM	Machupo	Tacaribe	Amapari	Control
LCM	WP	Preillness	−		−	−	−
		1 month	++		−	−	−
	CS	Preillness	−		−	−	−
		4 months	++		Trace	+	−
Machupo	KJ	Preillness	−	−	−	−	−
		3.5 months	−	++	+	+	−
	RL	7 days	−	−	−	−	−
		4 months	+	++	+	++	−

[a] Sera were tested at 1:10 dilution.

[b] Intensity of staining graded on a scale of − to ++.

The Tacaribe, Machupo, and Tamiami hamster antisera were tested at 1:4 dilution for neutralizing antibody to LCM, by using the mouse footpad technique (8). These tests were done by Sue S. Cross and John C. Parker of Microbiological Associates, Inc., on contract PHS-SA-43-67-700. All were completely negative.

Failure of Tacaribe and Amapari viruses to immunize against LCM infection. Cross immunity testing is a useful method of identifying LCM virus strains; weanling mice are immunized by intraperitoneal or subcutaneous infection and challenged intracerebrally 2 to 3 weeks later. Homologous immunity is absolute in the sense that massive challenge doses produce no signs of illness; however, the virus does replicate to high titer (17).

Weanling NIH mice were immunized by intraperitoneal inoculation with Tacaribe, Amapari, LCM, or normal mouse brain; in addition, some of the Tacaribe immune mice received a second injection of the same virus given intracerebrally. Two to three weeks later they were challenged by intracerebral inoculation of approximately 10^4 LD_{50} of LCM virus. All of the mice pretreated with Tacaribe (52 mice), Amapari (16 mice), or normal mouse brain (37 mice) died, whereas all 34 LCM immune mice survived.

These findings indicate that the LCM reactive antibody in antisera to viruses of the Tacaribe complex neither inhibits the growth of LCM virus nor desensitizes against the cellular immune response to LCM which produces the fatal convulsions. They also provide strong evidence that the Tacaribe and Amapari viruses were not contaminated with LCM virus.

To evaluate whether LCM contamination could be detected in a Tacaribe virus pool, LCM virus was serially diluted in a 1% brain extract of Tacaribe virus-infected mice and in diluent with normal mouse brain extract. The dilutions were inoculated intraperitoneally into groups of 10 mice; 14 days later, 6 mice per group were challenged with LCM virus intracerebrally, and at 32 days the other 4 were tested for CF antibody to LCM. The titer of LCM virus producing immunity to challenge was $10^{4.9}$ ID_{50}/0.1 ml in the presence of Tacaribe virus and $10^{4.6}$ in the control diluent; the titer by induction of CF antibody was $10^{4.5}$ in both groups. Thus, even trace amounts of LCM virus contaminating the Tacaribe virus could have been detected by either the challenge or serological tests.

DISCUSSION

The data presented here justify the conclusion that LCM virus shares one or more antigens with the viruses of the Tacaribe complex. The hy-

pothesis that contamination of the Tacaribe agents with LCM virus was responsible for the observed FA reactions is untenable for several reasons. The failure of Tacaribe and Amapari viruses to immunize against LCM, the absence of LCM neutralizing antibody or high-titer CF antibody in the Tacaribe complex hamster antisera, and the FA responses in the human infections constitute strong evidence in this regard.

It is not clear why the FA cross-reactivity was not reflected in the CF test. The indirect FA test for LCM antibody is generally more sensitive than the CF test as judged by serum antibody titers (3) and is also able to detect a small amount of antigen per cell. It is also possible that different antigens are detected by the two tests; in studies of LCM-infected tissue cultures, we have occasionally observed discrepancies between the amount of CF antigen and the intensity and amount of FA-stainable antigen.

The serological relationship of LCM and Tacaribe complex viruses confirms a relationship strongly suggested by comparison of their biological properties. They resemble one another in the morphology of the virion, formation of antigen in the cell cytoplasm, lack of hemagglutinin, natural occurrence in rodents, production of chronic carrier states in rodents, a mechanism of pathogenesis in mice which requires thymus-dependent lymphocytes for disease production, and lack of a clear-cut role of arthropods in transmission. Also, all available evidence indicates that they are RNA viruses (4, 5, 13, 16). Additional similarities observed in the course of the present experiments are that LCM virus produces cytopathic effects in Vero cells identical to those produced by Tacaribe and Amapari viruses, and that the disease produced in weanling NIH mice by intracerebral inoculation of Tacaribe virus is indistinguishable in latent period and symptomatology from the classical LCM disease.

It thus appears useful to consider LCM and the Tacaribe complex viruses as constituting a new virus group, with the Tacaribe complex a subgroup sharing CF antigens.

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