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EARLY DIAGNOSIS OF VIRAEMIA IN SOME EXPERIMENTAL ARBOVIRUS INFECTIONS IN MICE

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Summary. — Viraemia was demonstrated by the indirect haemagglutination (IHA) test in newborn white mice infected with dengue type 1 and 2 and Japanese encephalitis viruses but not with Chikungunya virus. The IHA allowed the detection of viraemia in the flavivirus infections before the appearance of clinical symptoms of the disease and was strictly specific.

Key words: Flavivirus; viraemia; early diagnosis; indirect haemagglutination test

Introduction

The laboratory diagnosis of arbovirus infections has been aimed at the detection of specific antibody by various serological tests and makes impossible a diagnosis in the first days of the disease. Since arbovirus infections are, as a rule, accompanied by viraemia, their early diagnosis could be based on virus detection in the blood. But isolation and identification of virus is a time- and labour-consuming process; the development of rapid diagnostic methods has, therefore, attracted the attention of many research workers. Attempts were made to use several reactions for this purpose: the complement fixation reaction in tick-borne and Japanese encephalitis (Drobyshevskaya, 1954; Ilienko, 1954; Smorodintsev *et al.*, 1954) and dengue fever (Brandt *et al.*, 1970); the haemagglutination (HA) test in Sindbis and West Nile (Melnikova *et al.*, 1972), Venezuelan equine encephalomyelitis (Agrba, 1969), and yellow fever (Porterfield, 1954) virus infections; and the agar gel diffusion and precipitation test in Semliki Forest, Pixuna, Venezuelan equine encephalomyelitis and Uukuniemi virus infections (Gaidamovich *et al.*, 1970). But a vireamia could be detected by these methods only when the viruses reached high titres in the blood. Therefore the search for a more sensitive and readily reproducible test remained urgent. In view of its high sensitivity demonstrated previously with arboviruses belonging to various antigenic groups (Gaidamovich *et al.*, 1974*a*, *b*; Klisenko *et al.*, 1978), we used the indirect haemagglutination (IHA) test to viraemia in some arbovirus infections.

Table 1. Viraemia in suckling mice inoculated with dengue type 1 and 2, JE and Chik viruses

Virus		Day (hr) of examination						
		12 hr	1	2	3	4	5	6
Dengue 1	I	nd	nd	2.9	3.3	3.7	5.0	3.2
	II	nd	nd	—	—	40	10	—
Dengue 2	I	nd	—	—	2.0	2.0	3.5	D
	II	nd	—	—	—	10	20	
JE	I	nd	nd	nd	5.75	6.25	D	
	II	nd	nd	—	80	160		
Chik	I	5.0	6.1	7.8	D			
	II	—	—	—				

Notes: nd = not done; — = negative result; D = death. I — Virus titres in log LD_{50}; II — titre in IHA test.

Materials and Methods

Dengue type 1 (Hawaii strain) and type 2 (strain 23 085) and Japanese encephalitis (JE; P_I strain) virus (genus *Flavivirus*) and chikungunya (Chik; Ross strain) virus (genus *Alphavirus*) were used. To produce viraemia, 2-3-day-old white mice were inoculated intracerebrally with the viruses in doses = from 1000 — 10, 000 LD_{50}/0.01 ml. The animals inoculated with dengue and JE viruses were bled daily up to their death, those infected with Chik virus at 12, 24, and 36 hr post inoculation (p.i.). The sera were examined by IHA and titrated for virus in suckling mice (dengue) and 5-6 g mice (JE and Chick viruses). Before examined by IHA, the sera were heated at 65 °C for 20 min and absorbed with sheep erythrocytes to remove heteroagglutinins.

The IHA test was performed with immunoglobulin erythrocyte diagnostic preparations consisting of sheep erythrocytes sensitized with antibodies to the respective virus. For specificity control the test was inhibited in each case with a homologous and heterologous 0.05 % gamma-globulin. The method of erythrocyte sensitization and the IHA technique were described previously (Gaidamovich *et al.*, 1974*a*, *b*).

Results

The results of comparative detection of viraemia by infectivity assay and IHA are summarized in Table 1.

Dengue type 1 virus infection was accompanied by a quite long viraemia: the virus could be demonstrated in the blood of baby mice from the 2nd to the 6th day p. i. The virus titre increased slowly, reached the peak on the 5th day and declined on the 6th day (at the peak of the disease). The period of antigenaemia was shorter: the antigen was detectable by IHA only at 4 and 5 days p. i.

Dengue type 2 virus was demonstrated by infectivity assays later, beginning since the 3rd day p. i.; viraemia persisted till the death of the animals at 5 days and was less intensive than with dengue-1 virus. The specific antigen was demonstrable by IHA at the same intervals as with dengue-1 virus.

JE virus titres in the blood of the infected suckling mice were rather high: 5.75 and 6.25 log LD_{50} at 3 and 4 days p. i., respectively. JE viral antigen was also found in higher titres than dengue antigen and was demonstrable at the same intervals as the infectious virus.

Table 2. IHA specificity in detecting dengue type 1 and 2 and JE-viruses in the blood of infected suckling mice

Virus	Serum dilution reciprocals					
	10	20	40	80	160	320
Dengue 1						
IHA	4	4	3	2	—	—
IHA inhibition with IgG to viruses						
Dengue 1	—	—	—	—	—	—
TBE	4	4	3	2	—	—
Ťahyňa	4	4	3	2	—	—
Chik	4	4	3	2	—	—
Dengue 2						
IHA	4	3	2	—	—	—
IHA inhibition with IgG to viruses						
Dengue 2	—	—	—	—	—	—
TBE	4	3	2	—	—	—
Ťahyňa	4	3	2	—	—	—
Chik	4	3	2	—	—	—
JE						
IHA	4	4	4	4	3	2
IHA inhibition with IgG to viruses						
JE	—	—	—	—	—	—
TBE	4	4	4	4	3	2
Ťahyňa	4	4	4	4	3	2
Chik	4	4	4	4	3	2

2-4 = degree of agglutination; — = no agglutination. Controls were invariably negative.

In Chick virus infection, viraemia was detected as early as 12 hr p. i., virus titres increased rapidly and by the time of death of the animals (36 hr p. i.) reached 7.8 log LD_{50}. IHA revealed no virus antigen in any serum specimen.

Discussion

We showed that IHA may detect flaviviruses in the blood of acutely infected suckling mice. Although the antigen could be demonstrated by IHA, as a rule, slightly later p. i. than infectious virus by infectivity assay. IHA allowed the diagnosis of viraemia before the onset of the clinical symptoms of the disease. This took only a few hours whereas virus detection by inoculation of animals and subsequent identification require at least 2 weeks under optimal conditions. A significant advantage of the IHA test over CF and HA tests is the fact that no special treatment of the serum is required for demonstration of the antigen. The presence of virus in the blood was demonstrated most regularly, and the reaction proved to be strictly specific as it was inhibited by homologous antibodies only (Table 2).

The detection of antigen in our experiments did not always correlate with infectious virus titres. Despite the fact that Chik virus multiplied in the animals and was present in the blood in high titres, no antigen could be

detected by the IHA test. But after inoculation with dengue viruses the antigen was demonstrable by IHA even at virus titres in the blood as low as 2.0—3.5 log LD_{50}. This appears to be associated with features of the pathogenesis of infection, since after inoculation with Chik virus the infection develops very swiftly and the animals die as early as 36 hr p. i. Further studies are required to elucidate this problem.

The present data indicate that the threshold sensitivity of IHA is much higher than that of the serological methods previously used to detect viraemia; in the latter methods, viruses in the blood have to be present in titres of at least 7.0—8.0 log LD_{50}.

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