Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 83(2): 161-164, abr./jun. 1988

JATOBAL VIRUS ANTIGENIC CHARACTERIZATION BY ELISA AND NEUTRALIZATION TEST USING EIA AS INDICATOR, ON TISSUE CULTURE

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A virus antigenic characterization methodology using an indirect method of antibody detection ELISA with virus-infected cultured cells as antigen and a micro virus neutralisation test using EIA (NT-EIA) as an aid to reading were used for antigenic characterization of Jatobal (BeAn 423380). Jatobal virus was characterized as a Bunyaviridae, Bunyavirus genus, Simbu serogroup virus. ELISA using infected cultured cells as antigen is a sensitive and reliable method for identification of viruses and has many advantages over conventional antibody capture ELISA's and other tests: it eliminates solid phase coating with virus and laborious antigen preparation; it permits screening of large numbers of virus antisera faster and more easily than by CF, HAI, or plaque reduction NT. ELISA and NT using EIA as an aid to reading can be applicable to viruses which do not produce cytopathogenic effect. Both techniques are applicable to identification of viruses which grow in mosquito cells.

Key words: virus classification – ELISA – neutralization test – *Bunyavirus* – Simbu Serogroup

In the last 12 years enzyme-linked immunosorbent assay (ELISA) has been shown to be applicable to antibody detection of Togaviridae (Voller & Bidwell, 1976), Flaviviridae (Aaskov, 1983), and Bunyaviridae (Niklasson et al., 1984) agents. Based on ELISA using virus infected tissue culture (Anderson & Rowe, 1982; Figueiredo & Shope, 1987; Smith, 1985; Yong-he et al., 1984), we show a virus antigenic characterization methodology using an indirect method of antibody detection with virus-infected cultured cells as antigen and a micro virus neutralisation test using enzyme immunoassay (NT-EIA) as an aid to reading.

Jatobal virus (BeAn 423380) was isolated from the blood of a *Nasua nasua* (coati), carnivore collected in Tucuruí, Pará State, Brazil, in 1985.

MATERIAL AND METHODS

Preparation of viruses – Virus from newborn mouse brain tissue was inoculated into Vero, CER and Aedes albopictus (C6/36 clone) cell cultures. At second passage after a 5 days infection period (IP), the infected cell cultures were scraped in the nutrient medium and disrupted by freezing and thawing. Material was clarified by centrifugation for 10 minutes 10000 x g and the supernatant fluids aliquoted and stored at – 70°C as virus stocks.

Mouse imune serum (MIS) and ascitic fluid (MIAF) – Immunizing antigens for preparation of MIS and MIAF were prepared from infected newborn mouse brain tissue. Immunization was made following a procedure described by Shope & Sather (1979).

Cell cultures – C6/36 cells were grown in Leibowitz L15 medium (GIBCO – New York, USA) containing 10% heat-inactivated FBS, 10% triptose phosphate broth, 100 U/ml of penicillin and 100 μg/ml of streptomycin. C6/36 cells were maintained at 28°C in a humidified atmosphere.

Viral infections and antigen processing – C6/36 cells were added to 96-well cluster dishes (Corning 25860, New York, USA) in 150 μl of growth medium at a density of 2 x 10^4 cells per well. After 24 hours the cells were infected with 50 μl of 50 to 200 $TCID_{50}$ of virus for ELISA or virus-MIAF mixtures for neutralization tests. Alternate columns of wells were left uninfected. After a 5 days IP, all the wells

This research was carried out at Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 0651 and was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil fellowship – 20.0035/85-BM.

Received June 18, 1987.
Accepted October 8, 1987.

received 100 μl of neutral buffered formalin (NBF) pH 7 (37-40% formaldehyde 100 ml, sodium phosphate dibasic-anhydrous 6.5 g, sodium phophate monobasic 4.0 g, and distilled water 900 ml) and were held overnight at 4 °C. Within 18-24 hours of fixation, the cells were washed twice with phosphate buffered saline (PBS). Some microplates were processed immediately for ELISA. Others were air dried, put in plastic bags and stored at – 70 °C.

NT – The NT with cell cultures was done as by inhibition of cytopathogenic effect (CPE) using serial two fold serum dilutions and a constant amount of virus (between 50 and 200 $TCID_{50}$). After 1 hour incubation period at 37 °C the virus-serum mixtures were added to microplates. Titrations of 10 fold dilution of virus were processed at the same assay (Hsiung, 1982). Microplates were processed as for ELISA after a 5 days IP.

ELISA – The ELISA was performed by adding 100 μl of antiserum (MIS or MIAF) diluted in PBS containing 0.5% bovine serum albumin (BSA) to the microplate wells. The antisera for processing NT-EIA were specific for the virus inoculum. After 1 hour at 37 °C and 3 washes with PBS, horseradish peroxidase-conjugated to goat anti-mouse IgG (diluted 1/2000; TAGO Inc., Burlingame, California, USA) in 0.5% BSA-PBS was added to the wells. Microplates were maintained for 1 hour at 37 °C and washed 5 times in PBS. Results were read visually and spectrophotometrically (414 nm) 20 to 30 minutes after the addition of ABTS substrate (Kirkegaard and Perry, Gaithersburg, MD, USA) to the microplate wells.

Calculations – Optical Density (OD) values were considered significant if they exceeded by 3 SD the mean value for control wells in the same assay. Virus titers were calculated by the method of Reed & Muench (1938).

Antisera – Three kinds of tests were performed in order to find antigenic relationships among viruses: initial ELISA screening, ELISA cross-testing and NT-EIA. For each virus, initial ELISA screening was carried out using a battery of 23 polivalent antibodies: Alphavirus MIAF (National Institute of Allergy and Infectious Diseases, Maryland 20014, USA (NIAID)-G-209-701-567), Flaviviridae MIAF (NIAID G-216-701-567), Group C MIAF (NIAID G-201-701-567), Bunyamwera MIAF (NIAID G-205-701-567), Bwamba MIAF (NIAID G-212-701-567), California MIAF (NIAID G-206-701-567), Polyvalent Groups Anopheles A, B and Turlock (NIAID G-211-601-567), Phlebotomus fever MIAF (NIAID G-208-701-567), Simbu MIAF (NIAID G-203-701-567), Guama MIAF (NIAID G-202-701-567), Polyvalent Congo (NIAID G-221-601-567), Capim (NIAID G-215-701-567), Polyvalent Patois (NIAID G-213-601-567), Polyvalent Rabies (NIAID G-236-601-567), Tacaribe MIAF (NIAID G-207-701-567), Vesicular Stomatitis MIAF (NIAID G-204-701-567), Polyvalent Quaranfil (NIAID G-210-601-567), Polyvalent 2 (NIAID G-223-601-567), Polyvalent 5 (NIAID G-226-601-567), Polyvalent 6 (NIAID G-227-601-567), Polyvalent 7 (NIAID G-228-601-567), Polyvalent 8 (NIAID G-229-601-567), Polyvalent 9 (NIAID G-230-601-567). All antisera were used at starting dilution 1/50.

ELISA cross-testing and NT-EIA were carried out with antigen related serogroup viruses. The following viruses (and antisera) were used for Jatobal (BeAn 423380) virus ELISA cross-testing and NT-EIA; Oropouche (strain BeAn 19991), Utinga (strain BeAn 84785), Simbu (strain SaAr 53), Manzanilla, Sathuperi (strain 87603), Ingwavuma, Mermet, Inini (strain Ca o 1093a), and Button Willow. Assays were done using C6/36 cells and microplates were processed after a 5 day IP.

RESULTS

Screening ELISA results showed a positive reaction with Simbu pool at 1/50 dilution. Simbu serogroup viruses cross-reacted extensively and with very high antibody titers by ELISA. It was also demonstrated that Jatobal virus is distinct but related to all the selected Simbu viruses (Table I).

NT-EIA results showed that Jatobal virus is antigenically distinct from the other Simbu serogroup selected viruses. Simbu serogroup viruses did not cross-react by NT with the exception of Inini and Mermet (Table II).

TABLE I

Results of ELISA with selected Simbu serogroup viruses

Virus	Antibody							
	BeAn 423380	ORO	UTI	MER	INI	SAT	ING	MAN
BeAn 423380	80000	400	100	1000	1000	3200	200	200
Oropouche	51200	3200	50	1000	200	1600	NT	NT
Utinga	3200	<50	400	50	100	NT	NT	NT
Mermet	800	<50	<50	12600	3200	NT	NT	NT
Inini	800	<50	<50	3200	51200	NT	NT	NT
Sathuperi	3200	<50	NT	NT	NT	51200	NT	NT

NT – Not Tested.

TABLE II

Results of NT-EIA with selected Simbu serogroup viruses

Virus	Antibody				
	BeAn 423380	ORO	UTI	MER	INI
BeAn 423380	1280	<40	<40	<40	<40
Oropo	<40	80	NT	NT	NT
Utinga	<40	<40	40	<40	<40
Mermet	<40	<40	<40	2560	1280
Inini	<40	<40	<40	320	1280

BeAn 423380 – $10^{3.9}$ $TCID_{50}$/ml; Oropouche – $10^{5.3}$ $TCID_{50}$/ml; Inini – $10^{4.7}$ $TCID_{50}$/ml; Mermet – $10^{5.9}$ $TCID_{50}$/ml; Utinga – 10^{6} $TCID_{50}$/ml.
NT – Not Tested.

DISCUSSION

Jatobal (BeAn 423380) virus belongs to the family Bunyaviridae, genus *Bunyavirus*, Simbu serogroup. Jatobal virus can be antigenically differenciated by ELISA and NT-EIA from other Simbu serogroup viruses. ELISA results showed a close antigenic relationship among Jatobal, Oropouche and Utinga viruses (Manzanilla complex). Oropouche, and Utinga were distinct by ELISA. These 2 viruses also occur in the Brazilian Amazon area where Oropouche virus causes large epidemics in human populations (Pinheiro, 1980). Antigenic cross-relationships detected by ELISA within Simbu serogroup viruses are comparable to these reported by Takahashi et al. (1968) using CF. The findings of Kinney & Calisher (1981) showing that most viruses of the Simbu serogroup do not cross-react by NT are confirmed by our NT-EIA results. Mermet and Inini viruses (Manzanilla virus subtypes) were exceptions. Our results showed a very close antigenic relationship between Mermet and Inini viruses (Tables I and II).

ELISA's are sensitive tests based in part, on the amplification factor due to the nature of enzyme-substrate reactions (Kurstak et al., 1984). ELISA using infected cultured cells as antigen is a sensitive and reliable method for identification of viruses and has many advantages over conventional antibody capture ELISA's and other tests: it eliminates solid phase coating with virus and laborious antigen preparation; it permits screening of large numbers of virus antisera faster and more easily than by CF, hemagglutitination-inhibition (HAI) or plaque reduction NT; 3.3% formalin inactivates viral infectivity, thereby reducing the potential hazard of laboratory infection (Yong-He et al., 1984; Figueiredo & Shope,

1987); cell cultures infected with viruses and fixed with formalin in microplates can be stored for at least 3 months at – 20°C (Figueiredo & Shope, 1987). ELISA and NT using EIA as an aid to reading can be applicable to viruses which do not produce cytopathogenic effect. Both techniques are applicable to identification of viruses which grow in mosquito cells. NT in cell culture using ELISA as indicator is reliable, simple and specific.

RESUMO

Caracterização antigênica do vírus Jatobal utilizando ELISA e neutralização lida por método imuno-enzimático, efetuados em cultura de tecidos – A caracterização antigênica do vírus Jatobal (BeAn 423380) foi efetuada utilizando uma técnica de ELISA para detecção de anticorpos que utiliza culturas celulares infectadas como antígeno e um micro teste de neutralização para vírus que utiliza o método imuno-enzimático como auxiliar para a leitura dos resultados (NT-EIA). O vírus Jatobal foi caracterizado como um Bunyaviridae, gênero *Bunyavirus*, pertencente ao sorogrupo Simbu. A técnica de ELISA, utilizando culturas celulares infectadas como antígeno, trata-se de método sensível e confiável na identificação de agentes virais, possuindo muitas vantagens sobre ELISAs convencionais e outros testes: elimina a preparação laboriosa de antígenos para o revestimento em fase sólida; permite que se teste de forma mais rápida e fácil que por CF, HAI e neutralização por redução de plaques um grande número de antisoros de vírus. ELISA e NT-EIA podem ser utilizados para a classificação de vírus que não produzem efeito citopático e podem ser aplicáveis na identificação de vírus que crescem em células oriundas de mosquitos.

Palavras chave: classificação de vírus – ELISA – teste de neutralização – *Bunyavirus* – soro-grupo Simbu

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Robert E. Shope, head of the Yale Arbovirus Research Unit for providing reagents as well as much useful advice.

REFERENCES

AASKOV, J., 1983. Rapid sero-diagnosis of human arbovirus infection. *Proceedings of the International Conference on Dengue/Dengue Haemorrhagic Fever.* Pathmanathan, Kuala Lumpur. p. 457-465.

ANDERSON, J. & ROWE, L. W., 1982. The use of an Enzyme-Labelled assay as an Aid to Reading Micro Virus-Neutralization Tests. *J. Immunol. Methods, 53:* 183-186.

FIGUEIREDO, L. T. M. & SHOPE, R. E., 1987. An Enzyme Immunoassay for Dengue virus antibody detection using infected cultured cells as antigen. *J. Virol. Methods* (In press).

HSIUNG, G. D., 1982. *Diagnostic Virology*, 3th edition, New Haven, Yale University Press, p. 25-34.

KINNEY, R. M. & CALISHER, C. H., 1981. Antigenic Relationships among Simbu Serogroup (Bunyaviridae) Viruses. *Amer. J. Trop. Med. Hyg., 30:* 1307-1318.

KURSTAK, E.; TIJSSEN, P. & KURSTAK, C., 1984. Enzyme Immunoassays Applied in Virology: Reagents Preparation and Interpretation. p. 479-503. *Applied Virology*, Academic press.

NIKLASSON, B.; PETERS, C. J.; GRANDIEN, M. & WOOD, O., 1984. Detection of Human Immunoglobulins and M antibodies to Rift Valley Fever Virus by Enzyme-Linked Immunosorbent Assay. *J. Clin. Microbiol., 19:* 225-229.

PINHEIRO, F. P., 1980. Situação das Arboviroses na região Amazônica. *Internacional Simposium on Tropical Aboviruses and Haemorrhagic Fevers*, Belem, Brazil. p. 27-48.

REED, L. J. & MUENCH, H., 1938. Simple method of estimating fifty percent endpoints. *Amer. J. Hyg., 27:* 493-497.

SHOPE, R. E. & SATHER, G. E., 1979. Arboviruses. p. 767-814. In: E. H. Lennette and N. J. Schimidt, eds., *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, 5th edition Amer. Publ. Health Ass., Washington, DC.

SMITH, A. L., 1985. An Enzyme Immunoassay for Identification and Quantification of Infectious Murine Parvovirus in Cultured Cells. *J. Virol. Methods, 11:* ü21-327.

TAKAHASHI, K.; OYA, A.; OKADA, T.; MATSUO, R.; KUMA, M. & NOGUSHI, H., 1968. Aino virus, a new member of Simbu Group of arbovirus from mosquitoes in Japan. *Jap. J. Med. Sci. Biol., 21:* 95-101.

VOLLER, A. & BIDWELL, D. E., 1976. Enzyme-immunoassays for antibodies in measles, cytomegalovirus infections, and after rubella vacination. *Br. J. Exp. Pathol., 57:* 243-247.

YONG-HE, Z.; WEN-FANG, T.; ZHONG-WEN, G.; JI-QIAN, C.; QIAN-SHENG & YI-MIN, 1984. Simplified New Method for the Identification of Arboviruses: Virus detection using Enzyme Immunoassay on Cultured Cells. *J. Virol. Methods, 9:* 45-51.