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USE OF MAC-ELISA FOR EVALUATION OF YELLOW FEVER VACCINATION

Rita M. Ribeiro NOGUEIRA(1), Hermann G. SCHATZMAYR(1), Marize P. MIAGOSTOVICH(1), Silvia M. B. CAVALCANTI(1) & Ricardo de CARVALHO(2).

SUMMARY

An evaluation of the IgM antibody immune response against yellow fever using strain 17D was carried out by MAC-ELISA and PRNT. The results showed an agreement of 97% between both tests and the authors conclude that MAC-ELISA can be used as a specific and sensitive asssay to replace the PRNT for detecting yellow fever antibodies in human sera, after vaccination programs.

KEY WORDS: Yellow fever vaccinated; MAC-ELISA;PRNT

INTRODUCTION

The laboratory diagnosis of yellow fever is based on the patient immune response, virus isolation or/and on histopathological findings in liver specimens[8].

The immune response can be evaluated by different techniques like neutralization (NT), hemagglutination inhibition (HI), complement fixation (CF) and more recently introduced immunoenzimatic tests (ELISA).

For the evaluation of the immune response the NT test has been more widely applied[7, 10, 15] due to its sensitivity but, particularly to the specificity. However, it is a complex test and requires the use of tissue culture which is troublesome to deal with a large number of serum samples.

On the other hand, ELISA is presently preferred for the evaluation of the immune response in viral infections by allowing not only the population study, but also, the results are provided in short time[14].

MAC-ELISA was first applied in 1985 for the diagnosis of Japanese B encephalitis and dengue[1]. Since then this approach has been used by different investigators[5, 9]with minor modifications.

There is a large endemic area for yellow fever in Brazil. Active vaccination programs has been maintained aiming to immunize all persons living or working in these area. Thus, in the present paper, MAC-ELISA was evaluated in comparison with a plaque reduction neutralization test (PRNT) studying the immune response of subjects who received the yellow fever vaccine strain 17D. The specificity of the test was assessed using dengue virus type 1 and Rocio virus antigens.

MATERIALS AND METHODS

Serum samples - Group 1: Serum specimens were collected from 140 military personnel vaccinated against yellow fever, strain 17D, before and 28 days after vaccination.

Group II: Sera were collected from yellow fever suspected patients, one from a fatal case occurred in Bolivia, in 1988, and 5 from Angola during the outbreak occurred in 1988.

Group III: 138 sera from dengue cases of our collection, received during the outbreak at Rio de Janeiro, 1986-1987, in which IgM anti-dengue virus were detected.

Antigens - Yellow fever (Asibi strain) and Rocio virus antigen were supplied by CDC (Atlanta) and by Adolfo Lutz Institute (São Paulo), respectively. Dengue type 1 (Mochizuki) antigen was prepared by intracerebral inoculation of suckling mice, and extracted by acetone/saccharose method according to CLARKE & CASALS[3].

(1) Instituto Oswaldo Cruz, Departamento de Virologia, Fundação Oswaldo Cruz, Caixa Postal 926, 21040 Rio de Janeiro, RJ, Brasil.
(2) Bio-Manguinhos, Fundação Oswaldo Cruz.

Peroxidase conjugate - Monoclonal antibody (6B6C - 1) reacting with flavivirus group[8] and coupled with peroxidase was commercially purchased (Jackson Immunoresearch Laboratories, USA).

PRNT - This test was carried out as described by LOPES et al.[7].

IgM capture (MAC-ELISA) - It was performed according to KUNO et al.[4].

RESULTS

Table 1 shows the yellow fever antibody response of Group I by MAC-ELISA in relation to PRNT. In 4 cases, the antibody to yellow fever was only detected by MAC-ELISA. Two cases had 10, one, 50 and one, 250 titers. Fifty-seven sera were titrated by MAC-ELISA and showed titers ranging from 50 to 10,000, with a geometric mean titer of 1,387.

Table 1
Comparison of results obtained in the study of 140 sera by PRNT and MAC-ELISA for detection of antibody after yellow fever (17 - D strain) vaccination.

MAC-ELISA	PRNT	
	Positive	Negative
Positive	136	04
Negative	0	0

When dengue type 1 and Rocio antigens were used in a MAC-ELISA for the same sera (Group I), no antibodies could be detected for Rocio virus and only one case showed titer (10) for dengue.

Table 2 shows results of MAC-ELISA in confirmed cases of dengue fever using yellow fever and Rocio antigens in order to check the specificity of the reaction. In 138 cases of dengue, 94 (68.5%) show positive results for yellow fever antigen and only 3 out of 29 (10.3%) reacted with the Rocio antigen.

Table 2
Cross-reactivity of dengue confirmed cases with yellow fever and Rocio antigens by MAC-ELISA.

Virus	Positive	Negative	Total	%
YF	94	44	138	68.1
Rocio	03	26	29	10.3

In Group II, the fatal case from Bolivia showed IgM titers of 20,000 and 50, to yellow fever and to dengue 1 antigens, respectively. No cross reactivity with dengue type 1 antigen was observed among Angola cases.

DISCUSSION

The flavivirus infection in many areas of our country makes the interpretation of serological test results sometimes difficult, specially by the use of hemagglutination inibition test only, since paired sera can rarely be obtained.

The NT test which is accepted as having higher specificity, on the other hand is limited to be carried out in well equipped laboratories.

The experience gained in our laboratory with MAC-ELISA for dengue diagnosis with clear cut results is confirmed here for yellow fever in the evaluation of the antibody response in vaccinees.

An agreement of 97% between both tests was observed on Group I where MAC-ELISA was able to detect yellow fever IgM antibodies in all specimens collected 4 weeks after vaccination. These results seems to allow the use of this method for yellow fever antibodies studies in substitution of the NT test. In four cases in which yellow fever antibodies were detected only by MAC-ELISA, the titers ranged from 10 to 250, confirming the low levels not detected by NT method. It should be pointed out that no heterologous response was observed with dengue type 1 and Rocio antigens both also present in the country[6,16]. Immunoglobulin M antibody persistence in yellow fever could not be determinated but, high titer (10,000) was detected four weeks after vaccination. In a volunteer however, IgM was detected 12th day after vaccination and persisted until 60th day. It should be pointed out that the vaccination of the group I was carried out before the dengue outbreak in the country which occurred by April, 1986.

The complete specificity of MAC-ELISA for yellow fever patients was described[5, 11]. Association of dengue virus infection in the patient with yellow fever (Group II) from Bolivia which

showed IgM titer of 50 to dengue type 1 antigen could not be ruled out because dengue epidemic occurred in that country.

In the Group III (dengue patients) however, a cross reactivity between yellow fever and Rocio antigens could be observed in MAC-ELISA. Heterologous response in cases of dengue infections using encephalitis Japanese B antigen was reported by BURKE & NISALAK[2].

Our findings associated to the experience in Africa [11] indicates that MAC-ELISA could be applied for active epidemiological surveillance of yellow fever in Brazil. However, in endemic area of dengue all suspected cases of yellow fever should be tested by both antigens. According to LHUILLIER et al.[5] the histopathologic lesion may not be characteristic enough to diagnose yellow fever, and IgM detection may be useful to assure the diagnosis. Another approach would be the use of a direct immunofluorescence assay to the search for viral antigens [12].

MAC-ELISA is a rapid and relatively inexpensive test and could be performed in laboratories adequately supplied. The specificity of MAC-ELISA in yellow fever vaccinees, utilizing a commercially available monoclonal antibody makes this method applicable to the vaccination control programs for yellow fever.

RESUMO

Uso do MAC-ELISA para avaliação de vacinados contra febre amarela.

Foi avaliada a resposta imune de anticorpos IgM em vacinados contra febre amarela usando a amostra 17D empregando-se provas de captura de anticorpos IgM (MAC-ELISA) e teste de neutralização por redução de placas (PRNT). Os resultados mostraram uma concordância de 97% entre ambas as provas e os autores concluem que o MAC-ELISA pode ser utilizado como um método específico e sensível para substituir o teste de neutralização para detecção de anticorpos IgM em vacinados contra febre amarela.

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