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Evaluation of a cell enzyme-linked immunosorbent assay for the detection of Borna disease virus antibodies in experimentally infected animals

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보르나 바이러스를 실험감염시킨 동물에서 항체검출에 대한 세포효소면역반응법의 평가에 대한 연구

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초록 : 보르나병 바이러스 혈청학적 진단법에 있어서 새로 개발된 세포효소면역법의 항체검출에 대한 평가를 위하여 지금까지 주로 사용되어진 간접형광항체법과 네 종류의 실험동물에서 서로 비교하였다.

모든 동물에서 간접형광항체법의 역가와 세포효소면역반응치 사이에 상관계수가 모두 0.8 이상으로 고도의 유의성이 인정되었고 두 진단법의 일치율은 한 희석단계 이하로서 재현성이 아주 좋았다. 세포효소면역반응법은 보르나병 바이러스의 혈청역학적 조사 및 병인기전 연구에 유용하게 이용될 수 있을 것으로 생각된다.

Key words : indirect immunofluorescense antibody test, cell enzyme-linked immunosorbent assay, Borna disease virus

Introduction

Borna disease(BD), which occurs naturally in horses and sheep, and causes encephalomyelitis,[1~2] has been grouped with the slow virus infections.[3]

The clinical course differs in several naturally and experimentally infected animal species.[4~8] Animal models are extensively used for the study of Borna disease(BD) virus.[9] In such studies, the serological response is usually assessed using the indirect immunofluorescence antybody test(IFA). However, this test needs fluorescence microscope. Recently new serological test has been introduced for the measurement of antibody to BD virus, including cell enzyme-linked immunosorbent assay (CELISA).[10]

In comparison with IFA and CELISA, they were both sensitive and specific in detecting BD viral antibodies in rabbit sera. In laboratory the measurement of serum antibodies in animal studies have been routinely carried out using the IFA test, and I report here the value of CELISA as a possible replacement for the IFA in the determination of serum antibody responses to BD virus in animal models. The correlation of CELISA with IFA antibody levels in sera from four animal species was determined.

Materials and Methods

Viruses : The strain V of BD virus, which was originally isolated from a brain homogenate of a naturally diseased horse and rabbit adapted, was used.[11, 12] The virus was pathogenic for rabbits when inoculated intracerebrally. The infected brain was homogenized in minimal essential medium(MEM) for 45sec by ultra-sonication to obtain a 10% (W/V) suspension and centrifuged for 10 mins at 300rpm. The supernatant served as inoculum.

Serum samples : Serum samples were obtained from inoculated intracerebrally with BD virus 0.02㎖ for rats, mice and chickens, and 0.1㎖ for rabbits, at 15 days post-infection.

Cell cutures : Rabbit embryo brain cells were prepared according to Herzog and Rott.[13] For antibody titraions the second passage of newborn rabbit brain cells were used.

Cell enzyme-linked immunosorbent assay : Embryonic brain cells from rabbits were seeded in 96-well microtitre plates(NUNC) and one day later inoculated with BD virus. In the case of antibody titeration, 20 ~ 50 focus forming units(ffu) of the virus per well were inoculated. The investigation of a variety of fixation and staining resulted in the following scheme, which gave optimal results : Medium poured off, cells in the plates were fixed with 3% formaldehyde in PBS(pH 7.5) at 4℃. When required for use, poured off the fixative, washed once and incubated with Triton X-100 in PBS for 30min at room temperature, then twice washed with PBS plus 1% foetal calf serum(fcs) and incubated with geometrically diluted serum samples for 1 hr at room temperature. Thereafter twice washed with PBS/FCS and incubated with appropriately diluted anti-immunoglobulin antibodies coupled to horseradish peroxidase for 1 hr at room temperature. Again three washes with PBS/FCS followed by incubation with hydrogen peroxide(H_2O_2) activated 3-amino-9-ethyl-carbazol till foci turned reddish brown, and the reaction stopped by washing with tap water. Cell foci easily be evaluated.

Indirect immunofluorescence antibody test : The IFA assay was performed by a previously described method.[14] As second antibody used was fluorescein isothiocyanate (FITC) coupled goat anti-rabbit IgG(Nordic, Bochum).

Results

Reproducibility of the IFA test and CELISA : The reproducibilty of the IFA test and CELISA was assessed using six sera from each of the four animal species tested. Each serum was tested in five replicates on a different plate and days. The value of antibodies is assessed using logarithmic value, exponent, to easy comparison. From the range of zone areas found for each serum, the standard deviation between the lowest and the highest values was calculated(Table 1). The results show that mean of standard deviations were 0.76 and 0.85 by IFA and CELISA, respectivly.

Correlation of IFA and CELISA : Correlation between IFA and CELISA was assessed using regression analysis. In the studies using sera from 114 rabbits, 197 rats, 46 mice, and 21 chickens, a relationship was found between IFA and CELISA antibodies titres in all species.

The results obtained for rabbits are shown in Fig 1 and good correlations was observed($r=0.855$, $p<0,001$).

Table 1. Intraplate variation of sera from different animal species tested by IFA and CELISA

Species	Seurm No.	Antibody titers	±SD* (log^2)
		IFA	CELISA
Rabbit	1	8.8±0.8	7.2±0.8
	2	10.0±1.0	9.0±0.7
	3	4.8±0.8	5.0±0.7
	4	8.4±0.5	7.4±0.5
	5	7.8±0.8	6.2±0.8
	6	5.0±1.0	5.0±1.0
Rat	1	1.0±0.0	1.4±0.5
	2	4.2±0.4	3.0±0.7
	3	6.0±0.7	3.6±0.5
	4	7.0±0.7	4.6±0.8
	5	6.6±0.5	3.8±0.4
	6	6.8±1.6	6.2±0.8
Mouse	1	4.8±0.8	3.6±0.9
	2	9.2±0.8	5.2±0.8
	3	7.0±0.7	6.2±0.8
	4	5.2±0.8	4.2±0.8
	5	6.4±0.9	5.6±0.5
	6	6.0±1.0	5.0±1.0
Chicken	1	5.8±0.4	4.8±0.4
	2	6.0±0.7	4.0±0.4
	3	5.6±0.9	4.8±0.8
	4	6.0±1.0	4.6±0.5
	5	0.6±1.0	5.0±1.0
	6	6.4±0.5	5.2±0.8
Mean value of S.D.*		$\bar{X}=0.762\pm0.3$	$\bar{X}=0.854\pm0.7$

* S.D. : Standard deviation

Table 2. Correlation of IFA and CELISA tests on animal sera tested against BD virus

Species	Number of sera tested	Correlation coefficient(r)	Level of significance
Rabbit	114	0.855	$p<0.001$
Rat	197	0.845	〃
Mouse	46	0.928	〃
Chicken	21	0.839	〃

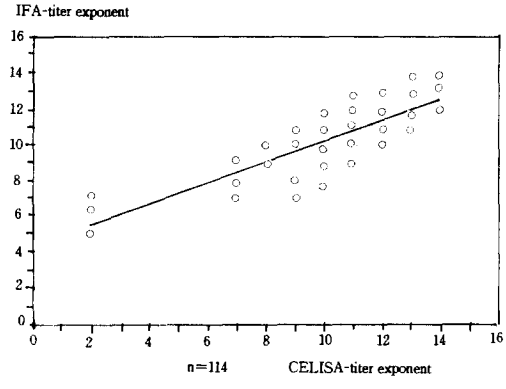

Fig 1. Correlation between IFA and CELISA titers obtained by testing rabbits sera for the presence of antibody to Borna-disease virus($r=0.855$, $p<0.001$).

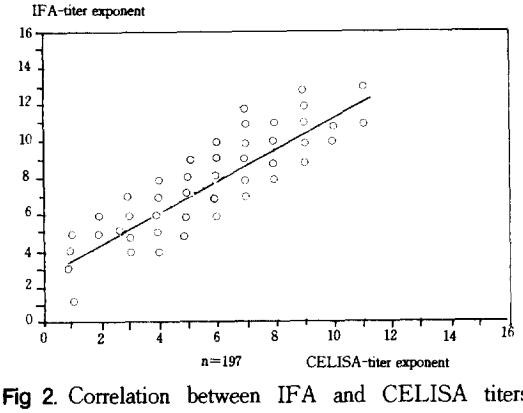

Fig 2. Correlation between IFA and CELISA titers obtained by testing rats sera for the presence of antibody to Borna-disease virus($r=0.845$, $p<0.001$).

The following regression equation was derived : log(IFA)=4.4+0.65log(CELISA). A comparison of IFA and CELISA titers in sera of rats, mice, and chickens showed also good correlation(all $r = 0.8$, $p < 2$ 0.001), when a logarithmic regression analysis was performed-(Fig 2, 3, 4) (Table 2). The regression equation was derived respectively:for rats it was log(IFA) = 2.6 + 0.86 log(CELISA), for mice it was log(IFA) = 2.3 + 1.1 log(CELISA), and for chickens it was log(IFA) = 3.3 + 0.8 log(CELISA).

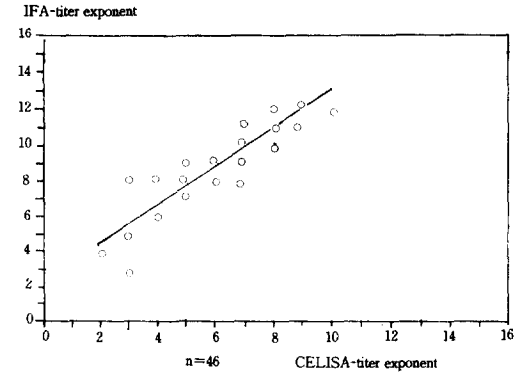

Fig 3. Correlation between IFA and CELISA titers obtained by testing mouse sera for the presence of antibody to Borna-disease virus($r=0.928$, $p<0.001$).

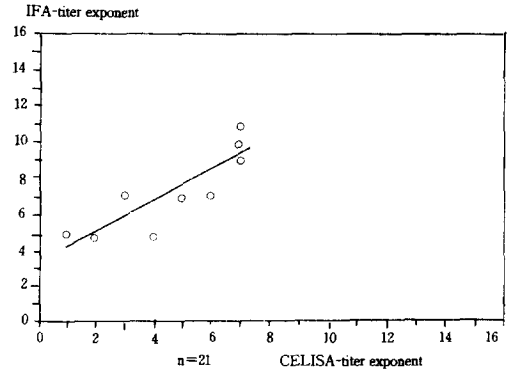

Fig 4. Correlation between IFA and CELISA titers obtained by testing chickens sera for the presence of antibody to Borna-disease virus($r=0.839$, $p<0.001$).

Discussion

The studies described above were carried out to assess the value of the CELISA test for measuring serum antibody levels to BD virus in animal models.

In assessing CELISA for use in animal studies it was necessary to ensure that reliable and reproducible results could be obtained. The variability of the test was investigated by determining the zone area increase corresponding to a significant increase in antibody. For the all species sera tested this figure was less than one diluent step. It means that the reproducibility was good for both tests.

In the present studies, a good correlation between CELISA and IFA antibody levels was observed in sera from all species infected with BD virus. The results presented strongly suggest that the CELISA system assaye-

d is an adequate approach to circumvent some limitations of the methods currently used to detect BD virus antibodies. The high specificity and sensitivity of CELISA test, in addition to the simplicity of its performance, make this method suitable for BD virus diagnosis, seroepidemiological surveys, and may be applied with advantage to testing lage numbers of sera for BD virus antibody.

The most prominent advantage of this test is in screening of monoclonal antibodies.[10] Experience shows that conventional ELISA techniques result in selection of clones producing antibodies against serum proteins even when gradient-purified virus preparations are used as antigens. For this reason it is often necessary to select antiviral monoclonal antibodies by fluorescent antibody methods. However, CELISA was modified of the usual ELISA. CELISA is performed directly on infected and fixed cells. cultured in microtitre plates. It shows clearly whether antibodies bind to infected cells or to uninfected antigen-free cells growing in the same or other wells and serving as internal controls.

Summary

The value of the cell enzyme-linked immunosorbent assay as a possible replacement for the indirect immunofluorescence antibody test for the estimation of antibodies against BD virus was assessed in four animal models.

The serum antibody response was measured by both assay systems;the variability of both tests was less than one diluent step, and correlation of the two tests was assessed using regression analysis. The study showed that the all four animal models gave satisfactory correlation of CELISA and IFA. There, CELISA is acceptable for use in mouse, rabbit, chicken and rat models.

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