Enzyme-Linked Immunosorbent Assay for the Detection of Infectious Bronchitis Virus (IBV) Antigen with Monoclonal Antibody

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(Received 28 August 1989/Accepted 18 January 1990)

Jpn. J. Vet. Sci. 52(3): 657-659, 1990

KEY WORDS: ELISA, IBV, monoclonal antibody.

Enzyme-linked immunosorbent assay (ELISA) has been employed as a rapid method for the detection of IBV antibody [3, 5, 7, 8]. Recently, Yagyu and Ohta [9] reported the detection of IBV antigen with polyclonal antibody in allantoic fluid of infected embryonated chicken eggs and tracheal emulsions of infected chickens. Further, monoclonal antibodies (Mabs) to IBV were isolated and characterized in some laboratories [2, 4, 6]. We also isolated Mabs directed to IBV strain Beaudette 42 (B42) [5]. In this paper, we described the Mab application directed for N protein to the detection of IBV antigen by ELISA.

The IBV strains used were B42, Gray, A5968, Iowa 609, ON, KH, Nerima, SS, Takeshima, Shizuoka, S4W and 0353. These viruses were propagated and purified as previously described [5].

Gradient-purified B42 virus [5] was disrupted in 0.1% Triton X-100 and used for the inoculum to mice. Hybridoma cell was prepared from the immunized BALB/c mice as described [1] and there were established five hybridoma cell lines secreting antibodies against spike (S2), N or matrix (M) protein of strain B42 (Table 1). Mabs secreted by these cell lines were tested for cross-reactivity with other strains in an ELISA [5]. Mabs 1F and 3H reacted with all of the strains tested (Table 1). Mab 1F was employed for following experiments because of its higher ELISA value (1.98) than Mab 3H (0.58).

ELISA was performed according to the method of Yagyu and Ohta [9] with a slight modification. Briefly, 1% Nonidet P-40 (Sigma, U.S.A.) treated and 10-fold diluted samples were added to chicken anti-B42 IgG-coated ELISA plates (Sankojunyaku, Japan) and incubated at

37°C for 1 hr. After washing, Mab 1F was added to the plate and incubated at 37°C for 1 hr. Following the addition of HRPO-rabbit anti-mouse IgG conjugate to the wells, the plates were incubated again at 37°C for 1 hr, and then washed. Peroxidase binding was visualized by 2-2'-azinobis-3-ethylbenzthiazoline sulfonic acid (ABTS; Sigma, U.S.A.) at 25°C for 10 min and the reaction was stopped by the addition of 0.1 ml of 0.1 N NaOH. Absorption was measured at 405 nm in a microplate photometer (Corona electric, Japan). The recognizable absorbance was employed for the ELISA value.

The allantoic cavities of SPF eggs were inoculated with 10^2 50% egg infectious dose (EID_{50}) of IBV strain B42 or A5968. The allantoic fluids were harvested 5 hr, 1, 2, 3 and 4 days after inoculation with five eggs each. These samples served for both ELISA and virus titration in chicken embryo kidney (CEK) cell cultures.

SPF chickens were inoculated intranasally with 10^3 EID_{50} of IBV strain M41 or A5968. The respiratory tracts were harvested from three chickens each once a day for a week. These respiratory tracts were homogenized to 10% (w/v) emulsions with PBS. The samples served for both ELISA and virus titration in embryonated eggs.

To determine the negative threshold line, ELISA values of allantoic fluid collected from 95, 14-day-old, SPF embryonated chicken eggs were measured. The mean value ($\bar{x}$) of the samples was 0.17 and the standard deviation (SD) was 0.03. Therefore, the negative threshold line was put at 0.26 as a 1% probability of misclassification ($\bar{x} + 3SD$). Infectious IBV was recovered from embryonated eggs inoculated with strain B42 or A5968 one day after inoculation. Titers of the embryonated eggs infected with strain B42 declined slightly at three days and those with strain A5968 did not change throughout the observation period. The ELISA values of the samples also became positive one day after inoculation. There was no significant fluctuation

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Table 1. Cross-reactivity of monoclonal antibodies to IBV-B42 with other IBV virus isolates

Virus	Monoclonal antibody (Protein specificity) ^{a)}				
	3G (S2)	1F (N)	1E (M)	3H (M)	1C (M)
B42	+++ ^{b)}	+++	+++	+++	+++
Gray	—	++	+++	+	—
A5968	—	++	++	+++	—
Iowa 609	++	++	—	++	—
ON	+++	+++	+++	+++	+
KH	+++	+++	+	++	—
Nerima	+++	+++	+++	+++	+++
SS	+++	+++	+++	+++	+++
Takeshima	+++	+++	+	++	—
Shizuoka	++	+++	—	++	—
S4W	++	++	—	++	—
0353	+	++	—	+	—

a) Analysed by western blotting.

b) Twice the amount of monoclonal antibody giving maximal binding to IBV-B42 was used. Binding to the heterologous virus is expressed as the percentage of the absorbance obtained with IBV-B42. +++: 75–100%, ++: 50–75%, +: 25–50%, and —: less than 25%.

in ELISA values of either strain (Fig. 1).

Similarly, the ELISA values of tracheal emulsions of 10 two-week-old SPF chickens were measured. From the result, the negative threshold line was put at 0.24. Strain M41 was employed as inoculum since strain B42 was no longer infectious to chickens and strain M41 and cross-reactivity with strain B42 by neutralization test [5]. Infectious IBV was recovered from chickens inoculated with strains M41 and A5968 two days after inoculation. The titers of strain M41 and A5968 reached the peak four to five days and three to four days after inoculation, respectively. ELISA values of chickens inoculated with strains M41 and A5968 became positive four to five days and three to four days after inoculation, respectively. ELISA values of chickens inoculated with strains M41 and A5968 became positive four to five days and three to four days after inoculation, respectively, which coincided with the peak of virus titers of each strain (Fig. 1).

The properties of Mabs to IBV strain B42 and ELISA system were tested for the detection of IBV antigen with Mab 1F directed against N protein. Mab 1F directed to N protein reacted with all of IBV strains tested, which coincided with the results of Koch *et al.* [2]. The result

Fig. 1. Virus detection from SPF embryonated chicken eggs infected with IBV-B42 or A5968 and from respiratory tracts of chickens infected with IBV-M41 or A5968. Samples were tested with ELISA (●) and virus titration (▲). A straight line (at point of 0.26 and 0.24) is the negative-threshold lines estimated with ELISA values of allantoic fluids from 95 non-infected SPF eggs and of respiratory tracts of 10 non-infected chickens, respectively.

suggests that N protein may be highly conserved.

Mab 3G directed to peplomer (S2 protein) had wide-range reactivity to IBV strains in ELISA. This suggests that at least one epitope on S2 protein may be conserved well among IBV strains. Koch *et al.* [2] also reported that some Mabs to S2 protein had an ability to react with many IBV strains, whereas the other to the S2 protein reacted with a few strains. This meant that there were conserved region(s) and variable region(s) on S2 protein of IBV.

Yagyu and Ohta [9] reported that IBV antigen was detected from strain M41-infected SPF embryonated eggs by ELISA three days after inoculation. IBV antigens were detected from embryonated eggs inoculated with strain B42 or A5968 one day after inoculation. This discrepancy regarding the day of antigen detection between our results and those of Yagyu and Ohta [9] is considered to result from the difference in virus growth curve.

In this ELISA system, the limit of virus titer detectable was calculated as approximately $10^{6.0}$ TCID₅₀ in allantoic fluids or $10^{6.0}$ EID₅₀ in respiratory tracts. The results show the possible application of the ELISA to diagnosis of field samples. Field trials are in progress.

ACKNOWLEDGEMENTS. This work was supported in part by the Japanese Association of Veterinary Biologists.

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要 約

単クローン抗体を用いた酵素抗体法による鶏伝染性気管支炎ウイルス抗原の検出(短報): 長野秀樹・土本まゆみ・宝達 勉・山上 正・井出誠弥・永口良雄・田中義夫・山岸郭郎(北里研究所附属家畜衛生研究所)——鶏伝染性気管支炎ウイルス(IBV) B42株を免疫したマウス脾細胞からN蛋白を認識する単クローン抗体 IF を得た。本抗体は他の IBV の11株に対しても強い反応性を示した。そこで本抗体を用いて酵素抗体法を行ったところ、感染発育鶏卵及び実験感染鶏にウイルス抗原を検出することができた。本成績から、IBV 診断における単クローン抗体による酵素抗体法の有用性が示された。