

Application of monoclonal antibodies against chicken infectious bursal disease virus (IBDV)

Qiao Zhong(乔 忠)¹, Zhan Lie(詹丽娥)¹, Shi Zhenxin(史振心)¹,
Ma Conglin(马丛林)², Liu Zi(刘 玟)², Ning Guanbao(宁官宝)³

1 Animal Husbandry and Vet Institute, Shanxi Academy of Agricultural Sciences, Taiyuan 030032

2 Military Vet Institute, PLA Vet College, Changchun 130000

3 Shangxi Agricultural University, Taigu 030801

Received April 5, 1993

Summary Preliminary study has been made to test whether three strains McAb(1B₁, 5D₆, 6D₈) are against the same antigen determinant. Through ELISA additive and competition experiments, it proved that these three strains are against different antigen determinant. The result of positive serum antigen component analysis with 2 strains IBDV McAb showed that sample IBD positive serum had obvious inhibition against combination of 5D₆ McAb with corresponding antigen. The results of substitution of corresponding component in ELISA inhibition experiment and comparison of non-IBD serum (SPF chicken serum, ND, MD, IA positive serum) proved that IBD anti-serum was the only one showing inhibition against 5D₆ McAb. Comparison with AGP and electromicroscope observation showed that ELISA inhibition experiment was characterised by high-specificity, rapidity and sensitiveness. 799 serum samples were tested with ELISA inhibition and double immunodiffusion (AGP) experiments. ELISA had gotten 486 positive, with positive rate of 60.83%; and AGP 334, positive rate of 41.8%, indicating that the former was much higher than the later ($P < 0.01$). ELISA inhibition test is suitable not only for individual diagnosis, but also for a great amount of samples, so it is practical for chicken IBD census. Sedimentation feature of IBDV-McAb has been utilized in development of agar double-diffusion and convective immune electrophoresis (CIE) tests to detect virus antibody in bursal disease fowl. In application of rapid IBD virus test for disease fowl, with convective immune electrophoresis test, diagnosis can be rapidly done in 2 hours, while with agar double diffusion, in 24-48 hours.

Key words infectious bursal disease, virus, monoclonal, chicken

Materials

Antigens

- 1 IBDV - CJ801 virus strain, supplied by the National Control Institute of Veterinary Bioproducts and Pharmaceuticals (NCI).
- 2 Agar diffusion antigen, prepared by ourselves.
- 3 ELISA antigen, purified through gradient centrifugation.
- 4 Chicken virus polyarthrititis antigen, presented by the Military Vet. Institute of PLA Vet. College; Chicken Newcastle disease virus antigen (Lasota weak toxic), supplied by NCI; and chicken Marek's disease virus antigen, supplied by Henan Agricultural University.

Serum

- 1 IBD standard negative (S^-) and positive (S^+) serum, supplied by Military Vet. Inst., PLA Vet. College.
- 2 Chicken IBD positive serum, prepared by ourselves.
- 3 SPF chicken serum, supplied by Shandong Poultry Institute.

4 Positive serum of chicken virus polyarthritits, Marek's disease and Newcastle disease, all supplied by NCI.

5 Serum samples from 15 chicken farms in Taiyuan, Yuci, Changzhi and Jincheng. Samples are randomly collected from flock based on age groups.

Bursicle

84 bursae were collected from anatomized ill chickens in Shanxi 661 Chicken Farm, Wenshui Chicken Farm, and Liujiabao Chicken Farm, and stored at -25°C .

Preparation of agar plate

Prepared as routine method, concentration of 1%.

Convectional immune electrophoresis

1 Electrophoresis fluid, barbitol buffer solution pH 8.2, and ion strength 0.05 mol.

2 Electrophoresis agar plate, a $25\text{mm} \times 75\text{mm}$ thin plate casted with ion agar prepared with above mentioned buffer solution.

McAb against chicken IBDV

1 Hybrid tumor cell strain culture supernatant fluid for 3 strains of 1B_1 , 5D_6 , 6D_8 and concentrated McAb, stored at -25°C .

2 Ascitic fluid collected after injection of BALB/C house mouse, and purified McAb, stored at -25°C .

Rabbit anti-mouse IgG enzyme mark antibody

It was supplied by Military Vet. inst., PLA Vet. College.

Methods

To test whether 3 strains McAb combine the same antigen determinant

1. Based on Snyder method (1988), that is, modification of competitive ELISA, agar plate was coated with purified IBDV at 37°C for 2.5 hr. After washing out, ascitic fluid McAb of different extension rates was mixed with equal amount of 1B_1 McAb marked with HRP. Then this mixture liquid 0.1 ml was placed into each hole, reacting at 37°C for 1 hr. The following steps are the same with that in indirect ELISA method.

2. Indirect ELISA additive method The method described by Ailsa (1984), that is, indirect ELISA additive method is used, in which steps are the same with that in indirect ELISA method. But the modification is adding McAb 2 times, each reaction is made for 1 hour at 37°C .

Component assay of serum IBDV antibody

1. The indirect ELISA inhibition experiment is made. The method is as follows:

(1) Coating antigen with purified IBDV antigen, placing 0.1 ml in each hole, at 37°C for 2.5 hr, and washing out.

(2) In each hole placing serum sample 0.01 ml, at 37°C for 1 hr and washing out.

(3) Placing 0.1 ml diluted ascitic fluid in each hole, at 37°C for 1 hr and washing.

(4) Placing rabbit anti-mouse antibody IgG enzyme mark antibody 0.1 ml in each hole, at 37°C for 1 hr and washing.

The following steps are the same with that in ELISA method. 3 control groups are designed in experiment: blank control-place without McAb (substituted with PBS), negative control-place without serum (substituted with PBS) and positive control-place chicken

IBDS⁺.

$$\text{Inhibition ratio} = \left(1 - \frac{\text{OD}_{\text{hole}} - \text{OD}_{\text{blank control}}}{\text{OD}_{\text{negative}} - \text{OD}_{\text{blank control}}}\right) \times 100\%.$$

When the inhibition ratio of sample serum is under 50%, it will be classed as positive inhibitive effect.

2. Using competition ELISA method as described above in experiment.

Use of sedimentation feature of IBDV—McAb in agar diffusion (AGP) and convective immune electrophoresis experiment (CIE)

1. In agar double-diffusion experiment, making plumblossom-like holes in plate; the centre holes and around diameters and interspaces 4mm, 3mm and 3mm respectively.

Placing 1B₁, 5D₆, 6D₈ McAb into the centre hole and bursal cream sample into the around holes; designing positive antigen control and negative control at the same time.

2. Convective immune electrophoresis 3mm thick electrophoresis agar is coated on glass slide of 25mm × 75mm. Three rows of holes are made symmetrically. The hole diameters are 3mm and 4mm and hole interspace is 3.5mm.

1B₁, 5D₆ or 6D₈ McAb and sample bursal cream are placed in symmetrical holes. The negative and positive controls are made at the same time. Measuring of result is carried out in 1 hour after electrophoresis in routine method.

Application of ELISA inhibition experiment in IBD census

By use of ELISA experiment, 1360 samples of chicken serum from 15 chicken farms in Taiyuan, Yuci, Changzhi and Jincheng have been detected, and 779 samples of chicken serum were tested in comparison with agar double-diffusion experiment.

Results

Results of two experiments to detect strains antigen determinant

1. In competition ELISA experiment, 1B₁ McAb 5D₆ McAb and 6D₈ McAb marked with HRP and polyclonal serum were used. The result showed that 5D₆ McAb, 6D₈ McAb and chicken antiserum have no competition with 1B₁ McAb, while 1B₁ McAb showed competition with enzyme mark 1B₁ McAb.

2. In experiment conducted with the indirect ELISA additive method, 3 strains showed additive effect with each other.

Results of specificity experiment

1. In substitution experiment, NS-1 culture supernatant and PBS were used for substituting corresponding component in ELISA inhibition experiment. The results of this experiment showed no inhibitive effect.

2. Reaction with non-IBD chicken serum ELISA inhibition experiment was made with positive serum of MD, ND, IA and SPE chicken serum. The result was compared with IBDS. It showed that, except for IBDS⁺ which had obvious inhibitive effect, the other three sera showed no inhibitive effect.

3. 10 samples of IBD standard negative and positive serum were tested, and the results showed that inhibitive rate for 5D₆ of negative serum was obviously lower than 50%; while positive serum was higher than 50%. So 50% was made as criterion for positive and negative serum.

4. Test for 20 samples of chicken serum were made together with AGP. For 3 samples of chicken bursa (ELISA positive but AGP negative), standard and ultra-thin sections were made and observed with optical and electro microscopes (Hong Tao et al. 1984). The re-

sults showed that 3 samples bursa had notable histological lesion and IBD virus-like granule.

Result of serum antibody component detection.

1. Inhibition tests of chicken IBDS⁺ against 5D₆ McAb and 1B₁ McAb were made in ELISA inhibition experiment and the results showed that IBDS⁺ had obvious inhibitive effect against 5D₆ McAb, but no inhibitive effect against 1B₁ McAb.
2. Result of competition test showed that serum didn't compete for IBDV with enzyme mark 1B₁.
3. Sedimentation feature of IBDV-McAb were used in agar diffusion and convective immunoelectrophoresis experiments. Test of virus-antigen in bursa of disease chickens from 661 farms was made. At the same time, from health young cocks (35—40 days) inoculated with IBDV virus and bursal cream of disease chicken from 661 farms, bursa were collected and prepared into cream and then tested with AGP and CIE experiments. Bursal cream from health chickens was used as control. The results list in Table 1.

Table 1. Result of AGP and CIE experiments to test bursal virus antigen with McAb

| | Disease chicken | Dead chicken | Tested chicken | Bursa collected | Sample bursa | AGP(24—48hr) | | CIE(2hr) | |
|---------------------|-----------------|---------------|----------------|-----------------|--------------|--------------|-------------------|----------|-------------------|
| | | | | | | Positive | Positive ratio(%) | Positive | Positive ratio(%) |
| 661 Farms | 10000 | 2500 | 73 | 73 | 29 | 29 | 100 | 29 | 100 |
| Wenshui farm | 2400 | 450 | 7 | 7 | 3 | 3 | 100 | 3 | 100 |
| Liujiabao farm | 7000 | 1000 | 4 | 4 | 2 | 2 | 100 | 2 | 100 |
| Artificial infected | 23 | Killed in 72h | 23 | 23 | 5 | 5 | 100 | 5 | 100 |
| control | 8 | — | 8 | 8 | 4 | 0 | 0 | 0 | 0 |

Application of ELISA inhibition test in IBD census

ELISA inhibition experiment was made to test 1360 serum samples from 15 chicken farms. 799 samples of them were tested with AGP experiment at the same time. The results were inspected with χ^2 and showed significant difference ($P < 0.01$) between the two methods. The detectability for ELISA and AGP were 60.83% and 41.80%, respectively.

Ages of chicken supplying sample serum range from 8 days to 2 years old, so 4 age groups (0—3, 3—8, 8—20, >20 weeks) were divided to make comparison. It was found that antibody positive rate was different between the various age groups. Ratio of 3—8 weeks group was the highest and ratio of 0—3 weeks group was the lowest.

Discussion

Two experiments to detect 3 strains McAb against the same antigen were conducted. 1B₁ McAb marked with HRP, 5D₆ McAb, 6D₈ McAb and polyclonal serum were used in competition ELISA experiments. The results indicated that 1B₁ competed with enzyme marked 1B₁, but chicken antiserum, 5D₆ McAb and 6D₈ McAb showed no competition with enzyme marked 1B₁. The results suggested that they combined with different determinants. The result of ELISA additive experiment suggested that obvious additive effect occurred in the three strains McAb. These studies lead the authors to believe that 3 strains are against different antigen determinants.

ELISA inhibition experiment is a kind of certified IBD serum diagnosis method with

rapidity, sensitiveness and good specificity. Compared with AGP, ELISA inhibition experiment can be completed in one day and be used for a good deal of samples.

In establishment of ELISA inhibition experiment, it has been found that there are many factors influencing result of ELISA test. For example, in placing test samples in holes on untreated react-plate, result obtained wasn't desirable. When changing the plate into an acid-treated and water-washed plate, we obtained very satisfied result. Thus it is recommended to use enzyme mark react-plate manufactured at the same time in the same experiment. In addition, antigen concentration and time of coating also influence test result. The recommended concentration is $20\mu\text{g/ml}$, which saves antigen as well; recommended time for best effect is 2.5–3 hours at 37°C .

Non-specificity reaction of ELISA experiment background colour is a common problem in ELISA method. Many experiments were conducted and various measures were taken in our study to avoid this problem. For example, cane sugar gradient purified McAb and antigen were used and non-specificity reactive effect was reduced. In addition, designed negative, positive and blank controls for each plate eliminated difference between plates and increased specificity. The result of substitutive corresponding components test indicated that dilution used in experiment and NS-1 supernatant fluid had no influence on result. The result of comparison experiment of MD, ND, IA and IBD positive serum in ELISA inhibition showed that IBD⁺ was the only one to inhibit $5D_6$, while the other 3 had no similar effect.

To prove the specificity of ELISA inhibition experiment, 20 serum samples were tested simultaneously with ELISA inhibition experiment and AGP. For 3 samples of ELISA positive and AGP negative sera, their bursae prepared as standard and ultra-thin sections were observed with optical and electromicroscopes. It was found that in bursae there was obvious tissue lesion and presence of IBD virus granule.

20 samples of IBD standard negative and positive sera were tested, and the results showed that inhibitive rate for $5D_6$ of negative serum was lower than 50%, while positive serum was higher than 50%, therefore 50% was made as criterion for positive and negative serum.

By use of sedimentation feature of IBDV McAb, AGP and CIE tests of bursal virus antigen for IBD disease diagnosis in fowl flock were developed. It's hard to test sedimentation antibody in serum for artificially and sudden naturally infected chicken in 6 days of disease development (Li Shusheng et al. 1984). Using McAb in AGP and CIE test, we can detect virus antigen of bursa in 1–6 days which remedied the defect of failure to test sedimentation antibody. The diagnosis can be done in 1–2 days with AGP of McAb and in 2 hours with McAb CIE.

References

- Hong Tao et al. (1980) Ultrastructure in Biomedicine and Electro Microscope Technology. Beijing: Scientific Press, pp. 394–397 (in Chinese)
- Li Shusheng et al. (1984) Comparison of neutralizing and sedimenting antibodies in chicken infectious bursal disease serum. *J Chinese Vet*, 10(7):13–153 (in Chinese)
- Snyer et al. (1988) Group and strain-specific neutralization sits of IBDV defined with monoclonal antibodies. *Avian Dis*, 32:527–534
- Wang Rongfu et al. (1985) Study on monoclonal antibodies against tuberculoze bacteria. *Bulliten of Monoclonal Antibodies*, (4):24 (in Chinese)