家禽誌 19(1) : 13 ~ 16(1992)　　K.J. Poul. Sci. 19(1) : 13 ~ 16(1992)

Production of Monoclonal Antibody to Avian Infectious Bronchitis Virus

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(Received November 15, 1991)

닭 傳染性 氣管支炎 바이러스에 대한 단클론 抗體 生産

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(1991. 11. 15 접수)

緒 論

마사츄셋형 전염성 기관지염 바이러스(IBV)를 SPF 발육란의 뇨막강내에서 증식시켜 Sucrose 밀도구배 초원심분리에 의해 정제한 다음 BALB/c 마우스에 면역시켰다. 면역 마우스에서 채취한 비장세포와 마우스 골수암세포와 여러 차례 융합시험을 실시하였다. 많은 융합세포 중에서 IBV에 특이적으로 작용하는 단클론 항체(monoclonal antibody : MCA)를 산생하는 hybridoma 클론은 2주밖에 얻지 못했다. 2주의 MCA는 모두 IgG형이었고 IBV중화능이나 혈구응집 억제능이 인정되지 않았다. 간접형광항체법으로 작성된 MCA를 이용하여 인공접종한 닭의 기관도말표본에서 10일간의 시험기간중 계속 IBV를 검출할 수 있었다.

Ⅰ. Interoduction

Avian infectious bronchitis is a highly contagious acute disease caused by IBV belonging to Coronaviridae. The disease was first reported in 1931 in USA and now occurs world wide, causing considerable economic losses to the poultry industry.

In Korea clinical manifestation of the disease was not recognized until 1985 when IBV was first isolated although the presence of IBV had serologically been demonstrated from the early 1960s(Rhee, 1986).

The virus causes respiratory signs in young chicks including coughing, sneezing, rales, ocular and nasal discharge. The virus also induces a marked drop of egg production and quality in laying birds(Hofstad, 1984). Nephrogenic strains causing nephritis and gout in young chicks result in high mortality.

IBVs are positive stranded enveloped RNA viruses and do not retain hemagglutinating ability. However, when treated with enzyme such as trypsin or phospholipase the virus shows hemagglutinating acitivity. IBVs are classified 20 or more serotypes by neutralization or hemagglutination inhibition test. The strains epidemic in Korea are thought to have close antigenic relationship with Massachusetts type. Therefore vaccine made with Massachusetts type are now used in both broiler and layer

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本 硏究는 敎育部 學術硏究費의 지원을 받아 이루어졌음.

farms. The disease often occurs in mixed form with other viral and bacterial diseases, making more difficult to take proper control measures.

In these studies attempt was made to raise monoclonal antibody(MCA) to IBV so that the MCA can be utilized in early detection of infected birds.

Ⅱ. Material and Methods

1. Viruses

IBVs used were vaccine strains of Massachusetts type, Connecticut type and 86-44 Korean isolates. All were obtained from the Institute of Veterinary Research(IVR), Anyang. Other avian and mammalian viruses were also used to determine the specificity of MCA.

2. Cells

Myeloma cells used for hybridoma production was p-3-x63 which obtained from IVR. The cells were proved to produce no immunoglobulins. The cells were cultured as described by Choi et al(1988). Chicken embryo kidney cells were prepared from SPF or commercial eggs for avian virus infection(Choi, 1980).

3. Propagation and purification of the virus

IBV was propagated in 10-day-old SPF eggs obtained from IVR by injecting through allantoic cavity. The virus was passaged 2 or 3 times 2 days interval before final bulk production was made. Allantoic fluid harvested 30 hours postinoculation was centrifuged for 30 minutes at 3000 rpm to remove cellular materials. Supernatant collected was again centrifuged for 3 hours at 27,000 rpm with Beckman type 30 rotor. The pellet was saved 1:100 of original volume. The virus suspension was layered on 30 to 50% sucrose density gradient and centrifuged for 4 hours at 290,000 G. Band formed in interface was collected and dialyzed against PBS and used as an antigen.

4. Immunization of mice

Eight 7-week old BALB/c mice immunized with the IBV antigen by the method described. After final immunization, sera collected were tested for the presence of hemagglutination inhibition(HI) antibody using Massachusetts type antigen.

5. Hybridization and selection

Fusions between immunized mouse spleen cells and mouse myeloma cell, p-3-x63 were performed according to the method described by Kohler and Milstein(1975). Antibody activity against IBV in the medium from cultures of hybridomas, successfully producing specific antibody, was cloned by limiting dilution method. The cloned hybridoma cells were inoculated into BALB/c mice which had already been given pristane. Ascitic fluid collected 3 weeks postinoculation was used for determining MCA properties.

6. Hemagglutination inhibition(HI) test

HI test was performed using antigens obtained commercially by the microtitration method as described by Alexander et al(1983) with some modification in which 0.5% chicken blood cell suspension instead of 1% was used.

Experimental infection with IBV 86-44 via intranasal route at the rate of 10^4 EID_{50} per bird at the age of 3 weeks when maternally derived antibody had waned. The antibody level was examined by HI method. Tracheal smears were made from 3 birds each at 2 day intervals and tested by IFA for the presence of IBV antigen until 10 days postinoculation.

Ⅲ. Results and discussion

A total of 4 fusions was tried between immunized mouse spleen cells and myeloma cells. Out of more than 400 hybridomas established only 2 hybridomas produced MCA specific against IBV of Massachusetts type by IFA test using FITC conjugated antimouse immunoglobulin A+G+M.

The two MCAs produced were reacted against all IBVs tested and did not cross react with other avian and mammalian viruses examined(Table 1).

The two MCAs were typed to belonging to IgG isotype and have no neutralizing and hemagglutinating activity(Table 2). The reacted against 3 strains of IBV at the similar level indicating they are raised against common antigen of IBV. MCA titer in ascitic fluid was in between 10^4 and 10^5 by IFA assay. Bright fluorescence was seen only in the cytoplasm and cell membrane.

IBV antigen was detected in tracheal smears made from chickens infected with IBV during the experimental period of 10 days by IFA using the MCA. Antigen was not detected from samples taken from control noninoculated chickens.

Ⅳ. Summary

Avian infectious bronchitis virus(IBV) was propagated in SPF eggs and purified by sucrose density gradient centrifugation in order to prepare the antigen. Several fusions were made between mouse myeloma cells and spleen cells from BALB/c mouse immunized with IBV antigen and two hybridoma clones producing specific monoclonal antibody(MCA) against the IBV were established.

The MCAs were classified as IgG type and

Table 1. Specificity of monoclonal antibodies to IBV by indirect fluorescent antibody test

Viruses	Cells	Clone No.	
		IB-19	IB-82
−	CEK	−	−
IBV(mass)	CEK	+	+
IBV(Con.)	CEK	+	+
IBV(86-44)	CEK	+	+
TGEV(Pyungtack)	ST	−	−
NDV	CEF	−	−
ILT	CEK	−	−
HVT	CEF	−	−

The two MCAs produced were reacted against all IBVs tested and did not cross react with other avian and mammalian viruses examined(Table 1).

Table 2. Characterization of monoclonal antibodies to IBV

Clone	IFA titer	Isotype	Neutralizing activity	HI activity
IB-19	10^4	IgG	−	−
IB-82	10^5	IgG	−	−

revealed no neutralizing and hemagglutination inhibition activity. Using the MCA, IBV antigen was detected by IFA method in tracheal smears made from chickens infected with IBV during the experimental period of 10 days.

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