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Production of Monoclonal Antibody to Newcastle Disease Virus and its Diagnostic Use

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뉴캣슬병 바이러스에 대한 단크론성 抗體生産 및 診斷에 利用

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摘　　要

뉴캣슬병바이러스(NDV)인 LaSota 주를 SPF 발육난의 요막강내에 증식시켜 순수 정제한 것을 BALB/c 흰쥐에 면역시킨후 추출한 비강세포와 흰쥐 골수암세포와의 융합방법에 의하여 NDV에 특이하게 작용하는 단크론성항체(MCA)를 생산하는 3주의 Hybridoma를 작성하였다. 이 3주의 MCA는 모두 IgG형에 속하였으며 흰쥐 복강내에 접종하여 생산된 복수항체의 항체가는 간접형광항체법으로 10^3-10^6에 달하였고 약독 및 강독 NDV에 모두 동일한 수준으로 작용하였다. 중화능은 인정되지 않았고 3주중 1주만이 혈구응집 억제능을 약하게 나타냈다. 이 MCA를 이용하여 간접형광항체법으로 인공감염시킨 닭에서 NDV항원 검출을 시도한 결과 기관점막을 비롯한 각종 장기의 도말표본에서 접종 3일후부터 뚜렷한 검출이 가능하였다.

I. INTRODUCTION

Newcatle disease caused by NDV belonging to an avian paramyxovirus 1 (Alexander 1982) is one of the most important diseases in poultry industry in Korea. The disease attacks all breeds of chickens regardless of age. Clinical signs and severity of disease varies greatly depending on the virulence of the virus. Mild strains induce only transitory respiratory signs and interruption of egg production. Strains of moderate virulence induce incoordination, paralysis and low to moderate mortality. Highly virulent velogenic viruses produce severe respiratory and digestive signs with mortality reaching nearly 100% (Beard and Hanson 1984). Although NDVs show varying pathogenicity there are no serological differences recognized between strains.

In Korea the disease is a continuous problem

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especially in broiler industry because many broiler farmers disinclined to use live vaccine so as to avoid possible negative effect of the vaccination such as side reaction and vaccination labor. As the disease often occurs plurally with other respiratory diseases, to take proper control measures it requires early accurate diagnosis.

In this studies MCAs specific to NDV were produced by cell fusion methods and utilized in detecting viral antigens from various tissues of chickens.

II. MATERIALS AND METHODS

∘ Viruses : NDVs used were a virulent strain, Kyojungwon and vaccine strains, B_1, Lasota and V_4. Other avian and mammalian viruses were also used to determine the specificity of MCA.

∘ Propagation and purification of the virus : NDV was propagated in 10-day-SPF eggs obtained from Veterinary Reserach Institute by inoculating allantoic cavity. Allantoic fluid harvested was inactivated in 0.2% formalin at 4℃ overnight and centrifuged at 3000rpm for 30 minutes to remove rough material. Supernatnt collected was again centrifuged at 40000G for 4 hours and the pellet saved was suspended in TNE (0.01M Tris-HCL, 0.1NaCl, 0.002M EDTA) buffer, subjected to 30 to 60% sucrose density gradient centrifugation. The fraction containing virus was used as antigen after dialysis.

∘ Immunization of mice : A group of 7-week-old BALB/c mice was immunized with the NDV antigen by the method of An and Kim 1983. After final immunization sera prepared from 2 mice were tested for the presence of hemagglutination inhibition antibody.

∘ Hybridization and selection : Fusions between immunized mouse spleen cells and mouse myeloma cell, SP2/0 were performed according to An and Kim(1983) and Choi et al (1986). Antibody activity against NDV in the medium from clutures of successfully hybridized cells was screened by IFA or ELISA. Hybridomas producing specific antibody were cloned by limiting dilution methods. Ascitic antibody was produced by injecting hybridoma cells into BALB/c mice by intraperitoneal route.

∘ Hemagglutination-Inhibition test : The test was performed by micromethods as described by Allan and Gough (1974) and Anon (1975). Indirect Immunofluorescent assay and ELISA test was conducted as described by Choi et al (1986).

∘ Detection of NDV antigen from experimentally infected chickens : Six-week old SPF chickens were inoculated with NDV Kyojungwon at 10^5 ELD_{50} per bird via intramuscular route. Slide smears with tissues collected from various organs were prepared from 3 to 5 days after infection. Acetone fixed smear preparations were examined for NDV antigen using MCA by IFA.

Commercial chickens reared for 6 weeks without any vaccination were also infected with NDV, LaSota and frozen tissue sections were similarly examined for NDV antigen from 1 to 7 days after infection.

III. RESULTS AND DISCUSSION

∘ Production of MCA : Out of several fusions made between immunized mouse spleen cells and myeloma cells many hybridomas were produced. Among those hybridomas 3 clones were found to produce specific antibody against NDV (Table 1).

Table 1. Characteristics of monoclonal antibody against Newcastle disease virus

Clones	Isotypes	IFA	Neutralizing titers	HAI titers	Specific polypeptides
ND-13	IgG,	10^{3*}	-	-	ND
ND-27	IgG	10^4	-	16	ND
ND-52	IgG	10^6	-		41K

ND : Not detectable by immunobloting

* : Titers in ascitic fluid

They were all typed to belong to IgG type and have no neutralizing and HAI activity except one, clone ND-27 which showed low HAI titer. Antibody from clone ND-52 showed highest IFA titer

when raised in mouse abdominal cavity. Clone ND-52 reacted against polypeptide of 41 kilodalton by immunobloting whereas 2 clones, ND-13 and ND-27 were not detectable against any polypeptides prepared. Considering polypeptide size clone ND-52 appears to react to membrane protein and clone ND-27 might have been raised against glycoprotein HN since it showed mild HAI activity.

◦ Specificity of MCA : Three MCA produced were specific to NDV and did not corss-react with infectious laryngotracheitis virus, infectious bursal disease virus, Japanese encephalitis virus and infectious bovine rhinotracheitis virus (Table 2). These antibodies reacted with all NDV strains tested indicating that they are unable to discriminate the possible antigenic differences among NDV strains.

Table 2. Specificity of monoclonal antibody against Newcastle disease virus(NDV) by IFA

Viruses-Cells	Clone No.		
	ND-13	ND-27	ND-52
Control-CEF	−	−	−
Control-CK	−	−	−
NDV-CEF			
-Kyojungwon	+	+	+
-B_1	+	+	+
-LaSota	+	+	+
-V_4	+	+	+
ILTV-CK	−	−	−
IBDV-CEF	−	−	−
JEV-BGK	−	−	−
IBRV-MODK	−	−	−

IFA : Indirect immunofluoresence assay
CEF : Chick embryo fibroblast cells
CK : Chicken kidney cells
ILTV: Infectious laryngotracheitis virus
JEV : Japanese encephelitis virus
BGK : Black goat kidney cells
IBRV: Infectious bovine rhinotracheitis virus

◦ Detection of NDV antigen from experimentally infected chickens : NDV antigen could be detected from tracheal smears 3 days onward after infection with virulent NDV by IFA using the MCA (Table 3). At 4 dyas after infection, viral antigen was detected from various organs including liver, lung, spleen, air sac membrane, conjunctiva, intestine, kidney, pancreas, ovary. However, in one experiment NDV antigen was not detected well from tissues of chickens infected with lentogenic strain, LaSota during 7 days postinfection.

Table 3. Distribution of viral antigen in tissues of chicken infected experimentally with a virulent Newcastle disease virus, Kyochugwon strain

Tissue	Days after infection			
	Before	3	4	5
Conjunctiva	−	NT	2+	+
Trachea	−	2+	3+	3+
Brain	−	−	+	−
Liver	−	−	2+	±
Lung	−	±	2+	±
Intestine	−	±	+	±
Kidney	−	±	+	−
Spleen	−	±	3+	±
Pancreas	−	−	+	+
Ovary	−	±	+	±
Air sac	−	NT	2+	3+

NT : Not tested
Results were scored as negative(−), suspicious(±), weak(+), moderate(2+) and intense reactions(3+) by indirect immunofluorescence assay.

These results indicated that using the MCA field NDVs might be more readily detectable than mild or vaccine strains. More experiments should be carried out with many other virulent and avirulent strains before any conclusions are made. Recently Srinivasappa et al (1986) produced a MCA designated as AVS-1 having high binding affinity to only lentogenic NDVs. As our clones can react broadly with velogenic and len-

togenic strains, field virulent NDV could easily be differentiated from vaccine or mild strains if we can use two MCAs, one of ours and ASV-1 of theirs.

Ⅳ. SUMMARY

A total of 3 hybridoma clones producting monoclonal antibody (MCA) against Newcastle disease virus(NDV) was raised by cell fusion method. The MCAs did not cross react against other avian or mammalian viruses tested. However, these antibodies reacted with all strains of velogenic and lentogenic NDVs tested indicating that they are unable to discriminate the possible antigenic differences among NDVs. All the MCAs were classified as IgG type and did not show neutralizing and hemagglutination inhibition (HAI) activity except one clone which has low HAI activity. One of these MCA raised in mouse ascites revealed the titer of 10^6 by indirect immunofluorescent antibody (IFA) test. Using the MCA, virulent NDV could easily be detected from tracheal and conjunctival smears made 2 to 3 days after experimental infection.

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