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Evaluation of the Use of Monoclonal Antibodies to Hemagglutinin and Fusion Glycoproteins of Newcastle Disease Virus for Virus Identification and Strain Differentiation Purposes

By

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Summary

Monoclonal antibodies detect evident antigenic variations in NDV HN and F protein. However, the A/PMV-1 viruses can be identified by HI test using a preparation made of the combination of two different monoclonals.

A primary evaluation of the pathogenicity of the isolated viruses can be made by HI test using monoclonal antibodies but needs always confirmation using conventional pathogenicity tests.

Introduction

In a previous work, we reported on the preparation and characterization of monoclonal antibodies directed against the hemagglutinin (HN) and fusion (F) glycoproteins of Newcastle disease virus (NDV) (10). Eighteen monoclonal antibodies directed against HN were obtained and divided into four groups according to their reactivity toward native HN, non glycosylated HN precursor and heat denaturated HN blotted on nitro-cellulose membranes.

Five monoclonals which neutralized viral infectivity but failed to inhibit hemagglutination were demonstrated by radioimmunoprecipitation tests to be directed against the F protein (10).

In this study, we tested the biological activity of representative anti HN and anti F monoclonals in hemagglutination inhibition and sero-neutralization tests in order to evaluate their usefulness for NDV identification and strain differentiation purposes.

Materials and Methods

Viruses

The viruses used in this study are listed in Table 1.

Representative strains of the different avian paramyxovirus groups were received from D. J. Alexander, International Reference Laboratory, Weybridge, United Kingdom. All viruses were grown in the allantoic fluid of 9 to 11 day-old embryonated SPF eggs (Valo, Lohmann). Harvest of the allantoic fluid was performed 48 hours post inoculation for velogenic strains and 72–96 hours post inoculation for lentogenic isolates.

Table 1. *Characteristics of the different A/PMV-I viruses*

Viruses	Virulence			Origins		
	Type	ICPI[c]	IVPI[d]	Month/year	Country	Host
Herts '33[a]	VV[e]	1.88	2.64	1933	England	Fowl
Italien[b]	VV	1.86	2.81	1945	Italy	Fowl
G.B.Texas[a]	NV[j]	1.75	2.66	1948	U.S.A.	Fowl
Kuwait[a]	V[f]	1.75	2.57	1968	Kuwait	Fowl
Essex '70[a]	VV	1.86	2.53	9/1970	England	Fowl
621/72[b]	VV	1.73	2.37	10/1972	Belgium	Fowl
647/72[b]	VV	1.75	2.52	10/1972	Belgium	Fowl
707/72[b]	VV	1.81	2.62	10/1972	Belgium	Fowl
763/72[b]	VV	1.76	2.39	11/1972	Belgium	Fowl
1176/78[b]	VV	1.86	2.65	5/1978	Belgium	Parrot
Beaudette C[a]	M[g]	1.46	1.23	1946	U.S.A.	Fowl
1/83[b]	M	1.40	0.43	4/1983	Belgium	Pigeon
14/83[b]	M	1.20	0.46	10/1983	Belgium	Pigeon
21/83[b]	M	1.28	0.00	11/1983	Belgium	Pigeon
B 1[a]	L[h]	0.25	0.00	1948	U.S.A.	Fowl
F[a]	L	0.25	0.00	1949	England	Fowl
La Sota[b]	L	0.38	0.00	1952	U.S.A.	Fowl
Ulster 2 C[a]	L	0.00	0.00	1966	N.Ireland	Fowl
MC 110[b]	L	0.00	0.00	1977	France	Duck

[a] PH RUSSELL and ALEXANDER D.J. (15)
[b] MEULEMANS G., unpublished results and reference (11)
[c] ICPI: intracerebral pathogenicity index in day-old chicks
[d] IVPI: intraveinous pathogenicity index in six-week-old chickens
[e] VV: viscerotropic velogenic
[f] V⁻: velogenic of unknown tropism
[g] M: mesogenic
[h] L: lentogenic
[j] NV: neurotropic velogenic

Monoclonal Antibodies

Four monoclonal antibodies directed against HN and two monoclonals directed against F were used (Table 2).

Ascites fluid was obtained by intraperitoneal injection of 1×10^7 hybridoma cells to mice that had been pristane (2, 6, 10, 14–tetramethylpentadecane) primed at least 10 days before injection.

Table 2. *Characteristics of the monoclonal antibodies*

Clone	Isotype	Specificity
8 C 11	IgG 2 A	Anti HN, group I
4 D 6	IgG 2 A	Anti HN, group II
5 A 1	IgG 2 A	Anti HN, group III
7 D 4	IgG 1	Anti HN, group IV
2 C 1	IgG 1	Anti F, La Sota
1 C 3	IgG 1	Anti F, Italien

Hemagglutination Inhibition (HI) Tests

Hemagglutination inhibition tests were done in microplates using twofold dilutions of ascites, 1 per cent chicken red blood cells and 4 hemagglutinating units following the method of ALLAN and GOUGH (3). Monoclonal antibodies were mouse ascitic fluid which had been diluted 1/10 in PBS before testing. Titres were expressed as the reciprocal of the highest dilution which caused inhibition of the hemagglutination.

Virus Neutralization Assays

α Virus Neutralization Procedure

Serial tenfolds dilutions of virus "Italien" were mixed with a standard dilution (1/10) of heat-inactivated (56° C, 30 minutes) ascites fluids. The ascites-virus mixtures were incubated during 2 hours at 37° C and assayed for residual infectious virus by inoculating secondary culture of chicken embryo fibroblasts or primary chick kidney cells prepared in 24 wells titerplates. For each viral dilution, 5 wells were inoculated with 0.2 ml of the virus-ascites mixture. Controls consisting of mixtures of viral dilutions with a negative serum were included in each test. After 1 hour absorption, cultures were added with maintenance medium, incubated at 38.5° C for 96 hours and examined for cytopathic effects. The neutralizing index represents the difference between the virus titers in the control experiment and the virus titer in the mixture virus-ascites. The virus titers were calculated according to REED and MUENCH (14).

β Virus Neutralization Procedure

Ascites fluids were diluted 10 fold with PBS, heat-inactivated at 56° C for 30 minutes, then submitted to serial twofolds dilutions. The different ascites dilutions were mixed with a constant virus dose of 100 $TCID_{50}$. Running of the test was as described above. The neutralization titer is the dilution of the ascites which neutralizes 50 per cent of the virus.

Results

Monoclonals Directed Against HN

Results of the hemagglutination inhibition tests are given in Table 3. Monoclonal 8 C 11 of group I reacted with all strains it was tested with indicating that it was raised against an epitope present on all isolates. However, its reactivity differed from strains to strains indicating antigenic variations between the different NDV strains tested.

Monoclonal 4 D 6 (group II) lacked reactivity with some velogenic, mesogenic and lentogenic strains. However, a mixture of antibodies from

Table 3. *Hemagglutination inhibition tests*

Viruses	Monoclonal antibodies				
	Group I (8 C 11)	Group II (4 D 6)	Group III (5 A 1)	Group IV (7 D 4)	Group I + II (8 C 11 + 4 D 6)
Herts '33	1,280	1,280	< 10	< 10	5,120
Italien	1,280	2,560	< 10	< 10	2,560
G.B.Texas	5,120	5,120	< 10	< 10	10,240
Kuwait	5,120	20,480	< 10	< 10	20,480
Essex '70	10,240	10,240	< 10	< 10	20,480
621/72	40	10,240	< 10	< 10	10,240
647/72	2,560	< 10	< 10	< 10	2,560
707/72	320	2,560	< 10	< 10	2,560
763/72	2,560	5,120	< 10	< 10	5,120
1176/78	2,560	80	< 10	< 10	2,560
Beaudette C	2,560	10,240	640	20	10,240
1/83	10,240	< 10	< 10	< 10	5,120
14/83	2,560	< 10	< 10	< 10	2,560
21/83	10,240	< 10	< 10	< 10	5,120
B 1	5,120	2,560	1,280	20	5,120
F	640	20,480	1,280	5,120	5,120
La Sota	160	2,560	5,120	2,560	10,240
Ulster 2 C	320	2,560	< 10	< 10	10,240
MC 110	320	< 10	< 10	< 10	160

groups I and II (8 C 11 + 4 D 6) inhibited the hemagglutination of all viruses tested at high titre. This monoclonal preparation never reacted with other PMV than A/PMV-1 as HI titres recorded against A/PMV-2, A/PMV-3, A/PMV-4, A/PMV-6 and A/PMV-7 were always less than 10 (Data not shown).

Monoclonal 5 A 1 of group III did not react with velogenic strains; it detected only one mesogenic and three lentogenic viruses including the two commonly used vaccinal Hitchner and La Sota viruses.

Table 4. *Reactivity of monoclonal 7 D 4 with different Hitchner and La Sota vaccinal strains*

Viral strains	HI titre
La Sota A	2,560
La Sota B	2,560
La Sota C	2,560
La Sota Cl 30	1,280
Hitchner A	20
Hitchner B	20
Hitchner C	20
Hitchner D	20

Monoclonal 7 D 4 (group IV) only showed HI activity with two lentogenic viruses: the La Sota and F strains and was further demonstrated to be able to discriminate between La Sota and Hitchner commercial vaccines (Table 4).

Monoclonals Directed Against F

Results of the sero-neutralization tests performed with monoclonal 2 C 1 prepared with strain La Sota and monoclonal 1 C 3 prepared with the velogenic strain Italien are given in Table 5. It is remarkable that the monoclonal 1 C 3 prepared against strain Italien was reactive toward all NDV strains except strain MC 110 whilst 2 C 1 raised against La Sota lacked also significant reactivity toward some velogenic isolates tested.

Table 5. *Seroneutralization tests*

	α Neutralization		β Neutralization	
	Monoclonals			
Viruses	1 C 3	2 C 1	1 C 3	2 C 1
Herts '33	4	2.75	2.45	1.85
Italien	4.34	1.55	2.15	0.00
G.B.Texas	2.12	2.00	1.71	≥ 3.85
Kuwait	1.82	1.69	2.76	1.65
Essex '70	3.49	1.64	1.90	1.20
621/72	3.49	1.00	1.94	0.00
647/72	4.00	1.06	1.78	0.00
707/72	4.45	0.88	2.11	0.00
763/72	3.87	0.54	1.85	0.00
1/83	4.80	N.D.[a]	N.D.	N.D.
14/83	5.00	N.D.	N.D.	N.D.
21/83	5.12	N.D.	N.D.	N.D.
B 1	≥ 5.33	≥ 5.33	3.65	4.38
F	≥ 5.33	≥ 5.33	3.65	4.31
La Sota	3.25	3.87	2.31	3.74
Ulster 2 C	≥ 5.13	≥ 5.13	3.55	3.25
MC 110	0.37	0	N.D.	N.D.

[a] N.D. = not done

Diskussion

It is interesting to note that representative monoclonals of the four groups of anti HN antibodies showed differential reactivities against the NDV strains they were tested with, which indicates that they were probably raised against different epitopes.

Antibody 8 C 11 of group I reacted with representative strains of the different antigenic groups described among A/PMV-1 viruses by RUSSEL

and ALEXANDER (15), including pigeon and duck isolates, and is probably similar to monoclonal 1/29 described by ISHIDA *et al.* (9). This observation indicates that antibody 8 C 11 was raised against an epitope present on all isolates what confirms the findings of NISHIKAWA *et al.* (12) who established that there is a highly conserved region on the HN glycoprotein of different NDV strains. The use of a monoclonal antibody directed against this epitope such as 8 C 11 would therefore be an excellent tool to identify NDV. However, the monoclonal 8 C 11 showed a low reactivity with some strains (e.g. strain 621/72) and for this reason it could be preferable to use a mixture of monoclonals of group I and II (8 C 11 and 4 D 6) for NDV identification purposes (Table 3). This monoclonal preparation could be preferred to the polyclonal antiserum presently used in HI identification tests as it eliminates all cross reactions described between NDV and the other avian paramyxoviruses (1).

Control of the identification of an isolate as NDV could also be performed by sero neutralization test using an anti F monoclonal antibody reacting with all strains as antibody 1 C 3.

Antigenic variations among NDV strains have been reported using polyclonal (4, 5, 6, 7, 8, 13, 16, 17) and monoclonal antisera (12, 15). These variations result from changes in the epitopes exposed on HN among NDV strains (12). Until now, no correlation was however found between antigenic variations and pathogenicity (15). Results obtained in this study with monoclonal 5 A 1 (group III) are therefore highly interesting as this monoclonal reacted preferentially with lentogenic viruses and not at all with velogenic strains in HI tests. However, monoclonal 5 A 1 did not react with Ulster and duck type strains and was not able to discriminate totally between lentogenic and mesogenic strains. Nevertheless, a primary evaluation of the pathogenicity of an isolated NDV could be easily performed using this monoclonal antibody in HI test and could be confirmed by the commonly used ICPI and IVPI tests. Using this methodology, we were able to show an excellent correlation between these two tests as we identified 36 out of 38 lentogenic isolates by HI test using monoclonal 5 A 1 (Meulemans, G., unpublished).

Monoclonal against NDV glycoproteins can be used as strain markers (2, 9). In this study, we obtained a monoclonal (7 D 4, group IV) which reacted specifically in HI test with the La Sota and F strains. This monoclonal could be very helpful in evaluating the circulation of these viruses among vaccinated and non vaccinated flocks and to discriminate between Hitchner and La Sota. In this respect, it would be very interesting to obtain a similar-monoclonal for all commonly used vaccines strains as the Hitchner strain.

Antigenic variations among NDV result not only from changes in the epitopes exposed on HN but also from changes in the epitopes exposed on F as demonstrated by variations in the results of seroneutralization tests per-

formed using anti F monoclonal. This variation in F protein is particularly evident for the duck strain MC 110.

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