Protective effects of monoclonal antibodies against lethal canine distemper virus infection in mice

Norio Hirayama,^{1*} Megumi Senda,¹ Naohiko Nakashima,¹ Masami Takagi,¹ Makoto Sugiyama,² Yasuhiro Yoshikawa³ and Kazuya Yamanouchi⁴

¹National Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries, 1-15-1 Tokura, Kokubunji, Tokyo 185, ²Department of Veterinary Public Health, Faculty of Agriculture, Gifu University, Yanagido, Gifu 501-11, ³Tsukuba Primate Center, National Institute of Health, Tsukuba, Ibaraki 305 and ⁴Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan

Monoclonal antibodies (MAbs) against the haemagglutinin (H), fusion protein (F) and nucleoprotein of canine distemper virus (CDV) were examined for their ability to protect mice against lethal CDV infection. One MAbs against H and two of six MAbs against F protected mice, the protective effect of the anti-H MAbs being stronger than that of the anti-F MAbs. The anti-H MAbs showed virus neutralizing activity, but the two anti-F MAbs, which recognized the same epitope, did

not. Protection by the anti-F MAbs correlated with cell fusion inhibition, but not with complement-dependent neutralization, complement-dependent cytolysis or antibody-dependent cell-mediated cytotoxicity. These results suggest that neutralization by antibody against H and cell fusion inhibition by antibody against F play important roles in the protective mechanism against CDV infection.

Canine distemper virus (CDV) is a member of the genus morbillivirus in the family *Paramyxoviridae* (Kingsbury *et al.*, 1978). The virus envelope consists of two glycoproteins, haemagglutinin (H) and the fusion protein (F), which have an important role in infection and the subsequent expression of pathogenicity. In general, antibodies against H or haemagglutinin–neuraminidase of paramyxoviruses have neutralizing activity and inhibit virus attachment, and antibodies against F prevent the spread of infection. The protective effects of anti-F antibodies are particularly important in some paramyxoviruses (Löve *et al.*, 1986; Meulemans *et al.*, 1986; Örvell & Grandien, 1982; Rydbeck *et al.*, 1988). In dogs, immunity to F restricts the spread of CDV infection more efficiently than immunity to H (Norrby *et al.*, 1986). In this study, we examined monoclonal antibodies (MAbs) against these two glycoproteins for their ability to protect BALB/c mice against lethal CDV challenge to evaluate the role of each antibody.

The FXNO strain of CDV (Hirayama *et al.*, 1985) was used unless otherwise indicated. In some experiments the YSA-TC strain of CDV (Hirayama *et al.*, 1986), the LA strain of rinderpest virus (RPV) and the Edmonston strain of measles virus (MV) (Sugiyama *et al.*, 1989) were also used.

For the production of MAbs, BALB/c mice were injected intraperitoneally (i.p.) with 10⁸ p.f.u. of virus

and boosted four or five times at 3 week intervals with the same antigen. Their spleens were removed 3 days after the last injection and fused with P3-X63-Ag8-U1 myeloma cells by adding polyethylene glycol 4000 (Merck). Culture supernatants from the hybridoma cells were screened for antibody production by the indirect immunoperoxidase technique (Minamoto *et al.*, 1981) using virus-infected Vero cells as the antigen. Hybridoma cell clones were expanded and injected into the peritoneal cavity of pristane-treated BALB/c mice, and the ascites collected and used as the source of MAbs.

For the virus neutralization test, murine neuroblastoma (MN) cells in 96-well microplates were used. In some experiments, fresh BALB/c mouse serum (1:5) was added to the virus–serum mixture as a source of complement.

Complement-dependent cytolysis (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) tests were carried out as described by Rector *et al.* (1982), virus-infected MN cells being used as the target. In the ADCC test, the effector cells were the peritoneal exudate of BALB/c mice induced by proteose peptone; an effector:target cell ratio of 10:1 was used. In both tests, the extent of cytolysis or cytotoxicity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay (Mosmann, 1983). Briefly 0.5% MTT was used and formazan was extracted with

Table 1. *Immunoreactivity of anti-CDV MAbs with various strains of morbillivirus*

MAb	Isotype	Antigenic site	IFA titre (log ₁₀) against			
			CDV			
			FXNO	YSA-TC	RPV	MV
d-7	IgG2a	H	6	5	<2	<2
k-1	IgG2a	FI	5	5	4	4
g-1	IgG2a	FI	6	5	4	4
f-7	IgG2a	FII	6	5	4	5
b-8	IgG2a	FII	6	5	4	4
a-3	IgG2a	FIII	6	4	3	3
a-8	IgG2a	FIV	5	2	<2	<2
f-5	IgG2a	NPI	6	5	3	<2
e-6	IgG2b	NPII	6	6	<2	<2
c-5	IgG2a	NPIII	5	<2	<2	<2

dimethyl sulphoxide (Takamatsu & Murata, 1986). The highest dilution of MAb that produced 20% cytolysis or cytotoxicity was taken as the antibody titre in the CDC or ADCC tests.

A cell fusion inhibition (FI) test was carried out on MN cells infected with virus at a multiplicity of 0.1. After adsorption for 1.5 h at 37 °C, cells were treated with glycine-hydrochloride-buffered saline pH 3.0 for 5 min on ice to inactivate virus which had not penetrated the cell (Hirayama *et al.*, 1985). The dispersed cells were resuspended in a small volume of PBS and Vero cells grown in 6 cm plastic dishes were inoculated with 0.1 ml of the infected MN cell suspension. After adsorption, dishes were overlaid with agar medium (Hirayama *et al.*, 1978) containing MAb and the number of plaques was counted 6 days later. The highest dilution of an MAb producing 50% plaque formation inhibition was taken as the FI titre.

A total of 54 hybridoma cell lines were established from several independent fusions. Viral polypeptide specificity was determined by immunoprecipitation (Hirayama *et al.*, 1985) and Western blotting (Bunschoten *et al.*, 1989). Of the 54 MAbs, one recognized H, 29 F₁ and four the nucleoprotein (NP); the specificity of the remaining 20 MAbs could not be determined.

Ten MAbs were examined for their antigenic specificity by an indirect fluorescent antibody (IFA) test (Table 1). Antigenic sites on F and NP were identified by their reactivity with MAbs against morbillivirus strains, and a competitive binding assay (data not shown). Four antigenic sites (FI to FIV) were distinguished using anti-F MAbs, three (NPI to NPIII) using anti-NP MAbs.

Using these 10 MAbs we attempted to protect mice against lethal CDV infection. Each group of 10 4- to 5-day-old BALB/c mice was injected i.p. with 0.1 ml of MAb. Immediately thereafter, mice were challenged

Fig. 1. Protective effect of MAbs against lethal CDV infection in suckling mice. Groups of 10 mice were given 0.1 ml of MAb and challenged i.c. with 100 LD₅₀ of CDV. Clinical signs were monitored for 14 days. MAbs were d-7 (○), k-1 (●), g-1 (△), f-7 (▲), b-8 (□), a-3 (■), e-6 (▽), and a-8, f-5, c-5 and control (▼).

intracerebrally (i.c.) with 100 LD₅₀ of virus. As shown in Fig. 1, anti-H MAb d-7 prevented mice developing clinical signs, whereas anti-F MAbs k-1 and g-1 were partially protective, some mice developing clinical signs 8 days post-challenge; no protective effect was observed with the other anti-F (f-7, b-8, a-3 and a-8) and anti-NP (f-5, e-6 and c-5) MAbs. Clinical signs such as wandering, jumping and circling appeared 4 or 5 days post-challenge, a marked decrease in body weight and paralysis were observed, and all mice died between 5 and 7 days after the appearance of symptoms. Pathological lesions were localized in the central nervous system, with a pattern similar to that described by Sawada (1967).

The protective effects of these MAbs were confirmed by the recovery of virus from the brains of challenged mice. Virus was detected from 2 days post-challenge (10^{2.8} TCID₅₀/0.25 g), and high titres (10^{4.5} TCID₅₀/0.25 g) were present until death in the brains of mice which received non-effective MAbs or control ascitic fluid. No virus was detectable in those mice which were clinically protected by MAbs.

The 50% effective dose (ED₅₀) of an anti-H (d-7) and an anti-F (k-1) MAb was the reciprocal of the MAb dilution that protected 50% of mice against challenge with 100 LD₅₀ virus; the protection index was the decrease (log₁₀) in LD₅₀ produced by the administration of an MAb. At the same Ig concentration, the ED₅₀ of the anti-H and anti-F MAbs was 11.3 and 1.1 respectively; the protection indices were 4.0 and 2.7 respectively. To determine whether passive transfer of MAbs could alter the course of an established infection, MAbs were given to mice 1 to 4 days after i.c. challenge with virus; anti-H MAb was effective up to 3 days post-challenge, and anti-F MAb at 1 day. These results

Table 2. Immunological activity of MAb*s* in vitro

MAb	Antigenic site	Neutralization titre		Titre			Protection rate (%)
		Without complement	With complement	CDC	ADCC	FI	
d-7	H	1280	10240	NT*	NT	NT	100
k-1	FI	<10	160	20480	5120	100	50
g-1	FI	<10	320	5120	2560	50	40
f-7	FII	<10	320	5120	5120	<25	0
b-8	FII	<10	320	10240	2560	<25	0
a-3	FIII	<10	320	10240	2560	<25	0
a-8	FIV	<10	<10	20	<20	<25	0
f-5	NPI	<10	<10	NT	NT	NT	0
e-6	NPII	<10	<10	NT	NT	NT	0
c-5	NPIII	<10	<10	<20	<20	<25	0

* NT, Not tested.

indicated that the protective efficacy of the anti-H MAb was higher than that of the anti-F MAb.

To determine the mechanism of protection, the immunological activity of MAb*s* *in vitro* was examined (Table 2). Only anti-H MAb d-7 was found to have neutralizing activity, but five of the six anti-F MAb*s* had neutralizing activity in the presence of mouse complement. Complement-dependent neutralizing activity titres of MAb*s* were almost the same regardless of whether they were protective *in vivo*.

CDC, ADCC and FI tests were carried out to determine the mechanism of protection by anti-F MAb*s*. As shown in Table 2, all anti-F MAb*s* except MAb a-8 had high CDC and ADCC activities. Plaque formation was inhibited under overlay medium containing MAb*s* k-1 or g-1. FI titres of MAb*s* k-1 and g-1 were 100 and 50, respectively, and those of the other anti-F MAb*s* were less than 25, indicating that the FI activity of the anti-F MAb*s* correlated with protection *in vivo*.

The present study has shown that MAb*s* against H and F of CDV protect mice against lethal CDV challenge. The protective effect of the anti-H MAb was stronger than that of anti-F MAb*s*, which do not have neutralizing activity. This result is compatible with a previous report that showed a correlation between the titre of a neutralizing antibody and protection in dogs (Gillespie *et al.*, 1958). Since only one anti-H MAb was available, the protective effect of anti-H MAb*s* recognizing the other epitopes remains to be determined.

This study has revealed that one epitope of F participates in the protective effect. Two anti-F MAb*s* recognizing the same epitope protect mice, indicating that this epitope plays an important role in CDV infection.

In previous studies, neutralizing and non-neutralizing activities of anti-F antibodies have been identified. For

example, the protective effect of MAb*s* against the F of Newcastle disease virus (Meulemans *et al.*, 1986), Sendai virus (Örvell & Grandien, 1982) and parainfluenza virus type 3 (Rydbeck *et al.*, 1988) has been shown to be produced by neutralizing activity whereas the protective activity of non-neutralizing MAb*s* against the F of mumps virus was considered to be relevant to CDC or ADCC (Löve *et al.*, 1986). In the present study, only MAb*s* which protected mice had FI activity, whereas complement-dependent neutralizing activity, ADCC and CDC were found for all MAb*s* regardless of protective capacity. These results suggest that the inhibition of cell-to-cell spread of virus in the brain may be responsible for the protective effect of anti-F MAb*s*.

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