STRUCTURAL PROTEINS OF AVIAN INFECTIOUS BRONCHITIS VIRUS: ROLE IN IMMUNITY AND PROTECTION

Jagoda Ignjatovic and Lisa Galli

CSIRO Division of Animal Health
Private Bag No 1
Parkville 3052
Australia

ABSTRACT

The antigenicity of the S1, M and N proteins of avian infectious bronchitis virus was compared following immunization of chickens with live and inactivated virus. The N protein was immunodominant antigen inducing cross-reactive antibodies in high titres whereas the S1 glycoprotein induced serotype-specific and cross-reactive antibodies. The M glycoprotein elicited antibodies in low titres and of limited cross-reactivity. Immunization of chickens with the purified N and M proteins did not induce protection against virulent challenge whereas immunization with the S1 glycoprotein prevented replication of nephropathogenic IBV in kidneys but not in tracheas of immunized chickens.

INTRODUCTION

Infectious bronchitis virus (IBV) contains three structural proteins: the peplomer (S) glycoprotein, located at the surface of virion and consisting of two subunits S1 and S2; the membrane (M) glycoprotein partially exposed at the surface of the virion and the nucleocapsid (N) protein which is internally located (1, 2). The antigenicity and role of the S1, S2, N and M proteins in immunity and protection against IBV infection is unclear.

Following infection with IBV, strain specific neutralizing and haemagglutinating antibodies are produced and are directed to the S1 glycoprotein (3-5), however there is no relationship between these antibodies and protection (4,6,7). In one study, the S1 glycoprotein was found to be more antigenic than either the N or the M protein (3) whereas in another study (8) similar titres of the S1 and M antibodies were detected early after infection suggesting that the M protein might have a role in immunity to IBV. The S2 glycoprotein was found to be more antigenic than the S1 glycoprotein, giving rise to cross-reactive antibodies (9). Recent studies have provided evidence that the N protein posses T-cell epitopes (10) and that it has a role in immunity to IBV (11).

The S1 glycoprotein is considered the most likely inducer of protective immunity. Inactivated IBV lacking the S1 glycoprotein did not induce protection in trachea of

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immunized chickens whereas whole inactivated IBV protected chickens against virulent challenge (4). However chickens immunized with the purified S glycoprotein were not protected against virulent challenge (3). With another coronavirus (mouse hepatitis virus), the S glycoprotein (12, 13) and two synthetic peptides which were homologous to two conserved regions in the S2 glycoprotein (14, 15), protected mice against lethal challenge.

The work reported here aimed to compare the antibody response to the S1, M and N antigens of IBV following immunization with live and inactivated virus and to assess if vaccination with the purified S1, N and M antigens, protects chickens against virulent IBV challenge.

METHODS

Nephropathogenic Australian strain of IBV, N1/62, grown in the allantoic cavity of embryonated chicken eggs, was used for preparation of partially purified virus and immunization of chickens. The S1, M and N antigens were obtained from partially purified N1/62 by immunoaffinity purification using CNBr-activated Sepharose 4B to which monoclonal antibodies directed against the S1, M and N proteins (16) were coupled. The method used for immunoaffinity purification was essentially as described by Mocket (8). At two weeks of age specific pathogen-free chickens, were immunized intra-ocularly with live and intra-muscularly with inactivated N1/62 virus, three times, at four-weekly intervals. Antibody responses were followed by ELISA, western blotting (WB), virus neutralization (VN) and haemagglutination inhibition (HI) tests (16). Cell-mediated responses to the S1, M and N antigens were measured by delayed-type hypersensitivity (DTH) reactions (17) in chickens immunized twice with live N1/62.

RESULTS AND DISCUSSION

To determine the profile of the antibody response to IBV antigens, chickens were immunized with live and inactivated N1/62 and appearance of antibodies assessed by VN, ELISA, WB and HI tests. Results obtained in ELISA are shown in Table 1. Immunization with live IBV induces antibodies to the S1, M and N antigens. The purified S2 glycoprotein was not available for this study. The N antigen was immunodominant whereas the M antigen was the least antigenic. Antibodies to the N protein appeared first and were initially detected at 10 days post-infection, at the time when no other antibodies were present. From 2 weeks onwards antibodies against the N protein had the highest titres. Antibodies to the S1 glycoprotein were first detected at 2 weeks post-infection by ELISA and VN, but not by WB. Titre of VN and ELISA antibodies to the S1 glycoprotein rose in parallel, ELISA titres reaching those detected for the N protein after three immunizations. Antibodies to the M protein were first detected by ELISA four weeks after infection, titres being considerably lower than those detected for the S1 and N antigens (Table 1).

Immunization with inactivated N1/62 virus was performed to assess the efficiency of such immunization and profile of antibodies produced (Table 1). Two immunizations with inactivated virus were required to induce antibody response to the S1, M and N antigens, the antibody titres being appreciably lower than those produced following live

Table 1. Titres of antibodies to the S1, N and M proteins in sera of chickens immunized at 4-weekly intervals with (A) live and (B) inactivated N1/62 virus.

	Titre of antibodies[a]			
Antigen[b]	2w[c]	4w	8w	12w
A				
N1/62	500	1600	4500	13500
S1	50	1320	4480	7040
N	400	2720	7040	8860
M	-[d]	80	800	920
B				
N1/62	-	120	500	1600
S1	-	-	300	500
N	-	60	440	1600
M	-	25	240	760

[a]Reciprocal of the last dilution of sera giving positive reactions in ELISA. Titres are mean of five sera tested individually.

[b]The S1, M and N antigens were obtained by immunoaffinity purification from N1/62 virus and used for coating of microtitre plates in ELISA.

[c]Weeks following primary immunization with the N1/62 strain of IBV.

[d]- Antibodies not detected.

immunization. Antibody titres to the M glycoprotein were similar to those obtained by immunization with live virus. The route of immunization (intramuscular or intraperitoneal) with inactivated virus did not alter the antibody response, apart from VN antibodies which appeared earlier, after two intraperitoneal immunizations. Results obtained in ELISA were confirmed by WB (results not shown). Methods used in this study did not allow measurement of antibodies to the S2 glycoprotein.

Epitopes involved in protection are expected to be cross-reactive and unrelated to virus serotype. For that reason it was assessed if the S1, M or N antigens elicit cross-reactive antibodies. Sera raised against IBV strains of different serotypes were titrated in ELISA using the S1, M and N antigens obtained from N1/62 strain of serotype C (Table 2).

The S1 and N antigens both induced cross-reactive antibodies. These cross-reactive antibodies were also detected in WB. The antibodies to the M protein were serotype specific. Only sera against three IBV strains of serotype C reacted in ELISA with the M antigen obtained from N1/62 virus of serotype C.

Table 2. Cross-reactive antibodies in sera obtained against Australian IBV strains of different serotype.

Antisera to[b]	Serotype	A_{450} on antigen[a]				
		N1/62	N	S1	M	NDV
Vac-1	A	0.71	1.10	0.37	-	-
Vic S	B	0.85	0.84	0.64	-	-
N1/62	C	1.56	1.80	1.45	0.44	0.16
N2/62	C	1.30	1.48	1.36	0.37	0.12
N8/78	C	1.10	1.12	1.34	0.40	0.09
N9/74	D	1.15	1.05	0.36	0.11	0.11
Q1/73	E	1.10	1.15	1.25	0.15	0.09
V2/71	F	1.15	1.40	1.10	0.18	0.16
V1/71	G	1.15	1.70	1.10	0.15	0.11

[a]Microtitre plates were coated with partially purified N1/62, the N, S1 and M antigens obtained from N1/62 strain or Newcastle disease virus (NDV).

[b]Sera obtained after two immunizations.

Table 3. Delayed-type hypersensitivity reaction elicited by purified S1, M and N antigens in chickens immunized with N1/62 strain of IBV.

Antigen inoculated[a]	No positive/ No tested	Wattle thickness after inoculation of antigen (increase)[a]			
		0 h	24 h	48 h	72 h
PBS	0/5	1.31	1.35 (**0.04**)	1.35 (**0.04**)	1.48 (**0.17**)
N1/62	4/5	1.23	1.69 (**0.46**)	2.13 (**0.90**)	2.00 (**0.77**)
PBS	0/5	1.62	1.73 (**0.11**)	1.81 (**0.19**)	1.87 (**0.25**)
S1	5/5	1.63	2.86 (**1.23**)	2.71 (**1.08**)	2.25 (**0.62**)
PBS	0/5	1.51	1.62 (**0.11**)	1.75 (**0.24**)	1.84 (**0.33**)
M	4/5	1.49	1.75 (**0.26**)	2.04 (**0.56**)	1.84 (**0.35**)
PBS	0/5	1.73	1.85 (**0.12**)	1.97 (**0.24**)	1.97 (**0.24**)
N	3/5	1.63	2.23 (**0.60**)	2.31 (**0.68**)	2.42 (**0.79**)

[a]Wattle thickness in mm. Increase in wattle thickness of >0.40 mm was considered positive.

[b] PBS and antigens in 50 μl were inoculated into left and right wattle, respectively. The S1, M and N proteins were purified from N1/62 virus. N1/62 was inactivated virus.

Since it is suspected that immune responses other than antibodies are involved in the mechanism of protection to IBV, we attempted to determine if the S1, M and N antigens also induce cell-mediated responses. This was assessed by measuring DTH responses in immune chickens following inoculation of the purified S1, M and N antigens into the wattle. All three antigens induced DTH responses in majority of chickens (Table 3).

To identify the protective antigen(s) of IBV, chickens were immunized with the purified S1, M and N antigens and inactivated N1/62 virus, and challenged with nephropathogenic N1/62 strain. Two intramuscular immunizations with inactivated N1/62, S1 and N antigens induced ELISA but not VN antibodies. None of the chickens challenged with the nephropathogenic N1/62 strain of IBV were protected. After three immunizations with inactivated N1/62 and the S1, M and N antigens, VN antibodies were detected in some chickens vaccinated with inactivated N1/62 virus and the S1 glycoprotein. The majority of chickens which were immunized with the inactivated N1/62 virus were protected and virus could not be isolated either from the trachea or kidneys. The majority of chickens immunized with the S1 glycoprotein were protected when isolation of virus from the kidneys was used as the criteria of protection. However these chickens were not protected when isolation of virus from trachea was used as a criteria of protection. Protection did not correlate with the presence of VN and HI antibodies.

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