USE OF MONOCLONAL ANTIBODIES TO ASSESS ANTIGENIC RELATIONSHIPS OF AVIAN INFECTIOUS BRONCHITIS VIRUS SEROTYPES IN THE UNITED STATES

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Avian infectious bronchitis virus (IBV), the etiology of a highly infectious and contagious respiratory disease of young chickens, has been under scrutiny for about 50 years. Since the early reports on the manifestations of the disease, a large number of apparently distinct serotypes of the virus have been identified in the United States. One classification scheme was derived in our laboratory by reciprocal virus neutralization tests carried out in tracheal organ culture[3]. However, there is some controversy as to the absolute number of IBV serotypes in existence and the groups into which they are assigned. Hopkins[2] obtained seven serotypes as determined by neutralization assays with cloned virus in cell culture.

All but a few designated serotypes, and new isolates appear to have a distribution peculiar to a given area. Control of the disease is attempted with modified-live virus vaccine administered to chicken flocks on the basis of prevalence of the disease causing serotype in that area. In the Delaware-Maryland-Virginia (Delmarva) area four serotypes are currently employed as vaccines. They are the Mass-41, Conn, JMK and ARK-99 serotypes.

Infectious bronchitis outbreaks in flocks which have been vaccinated for IBV do occur and there are several apparent causes for this. First, vaccines themselves may cause disease in stressed birds; second, non-indigenous viruses may be imported from other geographic areas of the U.S.; and third, the appearance of antigenic variants in the environment may also cause these breaks.

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The poultry industry is interested in preventing disease, whereas virologists wish to know whether new serotypes are evolving. Therefore, both the poultry industry and virologists would like to define the antigenic repertoire of IBV. However, a rapid serotypic identification of IBV is not possible when using currently available isolation and identification procedures. Further, *in vitro* serotypic differences may have little practical significance in relation to what *in vivo* protection a serotype may or may not elicit. It is this parameter which is ultimately important, but one that has only in recent years begun to be defined. Results of *in vivo* cross-challenge studies by us and others, employing IBV serotypes defined as distinct by serum neutralization tests, both support and contradict *in vitro* results.

We have recently confirmed and extended the use of a tracheal ciliary activity test[5] first reported by Darbyshire[1], for use in the assessment of *in vivo* protection to various serotypes of IBV. Briefly, two-week-old specific pathogen free chickens are vaccinated with the serotypes of interest. The chickens are allowed to develop an immunity for three weeks and are then challenged with homotypic and heterotypic viruses. After three days, the chickens are sacrificed and their tracheas are removed and tracheal rings are examined for ciliary activity. Tracheal rings from protected birds show strong ciliary movement while those from unprotected birds show cessation of ciliary activity. This criterion may also be used to correlate *in vivo* resistance with enzyme-linked immunosorbent assay (ELISA) antibody titer to IBV[6].

Experimental results thus far from *in vivo* cross-challenge studies indicate that putative, distinct antigenic serotypes *in vitro* may actually be very closely related from a protection standpoint. Further, certain serotypes elicit protection against themselves and other serotypes, but others elicit only one-way protection. Thus, one-way crosses of protection may be obtained, which suggests further variation in antigenic complexity as recognized *in vivo*. Table 1 shows results of *in vivo* reciprocal JMK-Conn challenges which exemplify one-way cross protection. This and similar work indicate the need for caution when extrapolating *in vitro* results for use *in vivo*, particularly when one is selecting potential vaccine candidates.

To facilitate rapid diagnostic methods, and in order to map the antigenic relationships of IBV at the epitope level, we have prepared batteries of monoclonal antibodies (mcabs) to three of four serotypes prevalent on the Delmarva Peninsula. They are the Mass-41, Conn and JMK serotypes. Two monoclonal antibodies, which are specific for the Mass-41 serotype and one monoclonal antibody apparently specific for the JMK serotype have been obtained.

Table 1. Results of Cross-Protection Tests in Chickens Immunized with the JMK and Conn Serotypes of Infectious Bronchitis Virus.

Immunizing Virus	Challenge Virus	Three days post challenge: Ciliostasis	Three days post challenge: Virus Recovery
None	Conn	4/4[a]	4/4
Conn	Conn	0/6	0/6
Conn	JMK	4/4	6/6
None	JMK	4/4	4/4
JMK	JMK	0/6	0/6
JMK	Conn	0/6	0/6

[a] Number positive of number tested.

Specificities of the antibodies were determined by indirect ELISA and fluorescent antibody tests. Monoclonal antibodies, specific for the Mass-41 serotype, were observed by specific fluorescence to be reacting in the cytoplasm of Mass-41 virus infected chorio-allantoic membrane cells, but not in heterotypically infected cells, or those from normal control membranes. Forty-four other mcabs have been derived, eight of these have unique ELISA reactivity patterns against the four serotypes and are under further study. All eight antibodies have been tested for specificity against the four IBV serotypes (Mass-41, Conn, JMK, ARK-99), purified Newcastle disease virus, normal allantoic fluid preparations and actin. Actin was included in these studies because of previous reports[4], which we have confirmed, that it will co-purify with egg propagated IBV. The ELISA reactivity patterns of these mcabs are reported in Table 2.

We conclude that mcabs to IBV will be useful in rapid diagnostics and epidemiological studies as serotype specific antibodies become available for serotypes present in a given geographic area. They will also be useful as probes for dissecting the virion structure and elucidating functional relationships.

At this time we are characterizing derived mcabs to these three serotypes of IBV in order to map their polypeptide specificities and to study the functions of the viral proteins.

Table 2. ELISA Reactivity Patterns of Monoclonal Antibodies Against Selected Antigen Preparations.

Monoclonal Antibody	Solid phase antigen						
	Mass-41	Conn	JMK	ARK-99	NDV[a]	NAF[b]	Actin
LAS I	++++[c]	-[d]	-	-	-	-	-
LAS II	+/-	-	++++	-	-	-	-
8.1	++	++	++	++	-	-	-
H	++++	+	++	ND[e]	-	-	-
C	++++	++++	+	ND	-	-	-
2	++	+	++++	ND	-	-	-
H.7	++++	++++	++++	ND	-	+/-	-
8b	++++	++++	++++	ND	++++	++++	-

[a] NDV is Newcastle disease virus.

[b] NAF is normal allantoic fluid.

[c] Normalized reaction strength, + is weak, ++++ is strong.

[d] Negative reaction.

[e] Not done.

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