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The Pathogenesis of Virulent and Avirulent Avian Infectious Bronchitis Virus

By

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With 1 Figure

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Summary

This study compared the infections of chickens with virulent and avirulent strains of avian infectious bronchitis virus (IBV). Sixty 3-week-old chickens were infected by aerosol with a virulent strain of IBV and 60 with an avirulent strain. Immunofluorescent staining of impression smears and cryostat sections of the trachea revealed that the virulent IBV infected and destroyed the ciliated epithelial cells lining the trachea. The virus then became localized in subepithelial cells and was detectable for 21 days. The avirulent IBV infected only a few ciliated epithelial cells and there was no significant desquamation of the epithelium. The avirulent IBV, like the virulent, became localized in subepithelial cells and was detectable for 24 days. The immunologic response to virulent and avirulent IBV, as measured by virus neutralization titers, was similar at 19 days post infection. After this time, there was a divergence of the responses, with the virulent virus inducing ascending antibody titers while the antibody titers descended with the avirulent virus.

1. Introduction

A number of reports have been made on the histopathologic changes of the trachea that occur in IBV infections (5, 6, 8, 11, and 12). None of these reports, however, correlated the changes with immunofluorescent studies to determine the relationship of the changes to the site of replication of the virus. There are also no reports as to the effect, if any, of avirulent strains of IBV on the trachea.

One of the biologic properties of IBV is that it becomes avirulent and less immunogenic with successive passage of the virus in embryos (4). The Beaudette strain of IBV is one such high embryo passage virus that is not considered to be immunogenic. The purpose of this study was to determine if, indeed, the avirulent virus replicates in chickens and, if so, to compare it to the replication of a virulent strain of IBV.

2. Materials and Methods

2.1. Viruses

The Massachusetts strain of IBV was used as the virulent virus and was in its eighth serial passage in chicken embryos. The titer of the virus was $10^{7.5}$ EID_{50}/ml. The avirulent virus was the Beaudette strain and has been through hundreds of serial passages in chicken embryos. The titer was $10^{8.2}$ EID_{50}/ml.

2.2. Chickens

The chickens used for this study were Athens-Canadian randombred chickens that were free of neutralizing antibodies against IBV. At one day of age, they were placed in positive pressure isolation units and reared to three weeks of age.

2.3. Experimental Design

Six isolation units containing 20 3-week-old chickens each were used for this experiment. Sixty chickens in 3 units were infected by aerosol with virulent IBV and the 60 chickens in the other 3 units with avirulent IBV. Each stock virus was diluted 1:5 in Hanks' balanced salt solution and 0.025% lactalbumin hydrolysate. A quantity of 2.0 ml of virus was administered to each unit with a nebulizer[1] under a constant pressure of 15 lb/in^2.

The observation period for this experiment was 35 days. Chickens from each group were sacrificed and the tracheas removed aseptically. Each trachea was divided into 4 sections and used: 1. to make impression smears for immunofluorescent staining, 2. to make cryostat sections for immunofluorescent staining, 3. to prepare tracheal organ cultures for observation of ciliary activity and virus isolation, and 4. to fix in formalin for histopathologic studies after staining with hematoxylin and eosin. The number of birds sacrificed from each group and the days postinfection were as follows: three chickens on PI days 1—8, 10, 12, 15, 17, 19, and 21 and two chickens on PI days 24, 26, 32, and 35. All chickens were observed daily for clinical signs of disease.

The immunofluorescent staining of tracheal impression smears and cryostat sections was done with a fluorescein isothiocyanate labeled anti-IBV globulin prepared as previously described (10). Cryostat sections were counterstained with 1:300 rhodamine.

Tracheal organ cultures were prepared as previously described (2). If there was no ciliary movement or after ciliary movement ceased in tracheal organ cultures, the fluids were harvested and up to 3 serial passages at 24-hour intervals made in embryonating hens' eggs for virus reisolation.

Serum samples were collected from each chicken at the time of sacrifice and virus neutralization (VN) tests done on the pools of serum collected on days 1, 19, 24, and 35. The VN tests were conducted using a plaque assay in chicken embryo kidney cells as previously described (9).

3. Results

3.1. Clinical Observations

Only the chickens infected with the virulent IBV developed clinical signs of respiratory distress, nasal discharge, tracheal rales, sneezing and malaise. These signs were not detected after the eighth day PI. None of the chickens infected with the avirulent virus became visibly ill.

3.2. Immunofluorescent Staining

Cells infected with IBV were found in impression smears of tracheas infected with the virulent IBV for 6 days, and many positive cells were seen in the smears.

[1] Devilbiss nebulizer 40, Devilbiss Co., Sumerset, PA 15501, U.S.A.

Only a few infected cells were seen in impression smears from tracheas infected with the avirulent virus and smears were negative after the third day (Table 1). The predominant cell types seen in the smear were erythrocytes, and epithelial cells with a few white blood cells also present. Only the epithelial cells were infected with IBV.

The staining of cryostat sections revealed that tracheas infected with virulent IBV were positive from the first to the twenty-first day PI and those from tracheas infected with avirulent IBV were positive from the third day to the twenty-fourth day PI (Table 1). The difference between the virulent and avirulent infected tracheas was in the cell types that were infected. With the virulent virus the epithelial cells were infected up to the sixth day PI and subepithelial cells were infected from the first to the twenty-first day. The epithelial cells were never observed to be infected in cryostat sections with avirulent IBV but the subepithelial cells were infected by the third day and were positive up to the twenty-fourth day.

3.3. Tracheal Organ Cultures

The ciliary movement of epithelium in organ cultures derived from tracheas infected with virulent IBV was not evident during the first 7 days PI but movement appeared on the eighth day and was observed through the thirty-fifth day (Table 1).

Table 1. *The Immunofluorescence, Ciliary Activity and Virus Isolation of Avian Infectious Bronchitis Virus Infected Chickens*

Virus Strain	Virus Indicator	Days post infection										
		1	2	3	4	5	6	7	8	10—21	24	26—35
	FA Staining											
Virulent	1. Smears	+	+	+	+	+	+	−	−	−	−	−
	2. Cryostat section	+	+	+	+	+	+	+	+	+	−	−
	Ciliary movement	−	−	−	−	−	−	−	+	+	+	+
	Virus isolation	+	+	+	+	+	+	+	+	+	−	−
	FA Staining											
Avirulent	1. Smears	+	+	+	−	−	−	−	−	−	−	−
	2. Cryostat section	−	−	+	+	+	+	+	+	+	+	−
	Ciliary movement	+	+	+	+	+	+	+	+	+	+	+
	Virus isolation	+	+	+	+	+	+	+	+	+	+	−

The tracheal organ cultures derived from the avirulent IBV infected chickens exhibited ciliary movement throughout the experiment (Table 1). The average number of days that ciliary movement lasted in tracheal organ cultures derived from each bird is summarized in Figure 1. The cessation of ciliary movement and death of the epithelium within 5 to 7 days was attributed to IBV infection during the first 21 to 24 days of the experiment. This was demonstrated by immunofluorescent staining of the organ cultures and isolation of virus from them. Tracheal organ cultures prepared from birds not infected with IBV exhibited ciliary movement for an average of 13 days (1).

3.4. Virus Isolation

Virus was isolated from the tracheal organ cultures derived from the virulent IBV infected chickens for 21 days and for 24 days from the avirulent IBV infected chickens (Table 1). The avirulent IBV isolated during the first 12 days PI caused death of embryos but after that the virus caused stunting of embryos. The virulent IBV caused only stunting of embryos when isolated from the tracheal organ cultures.

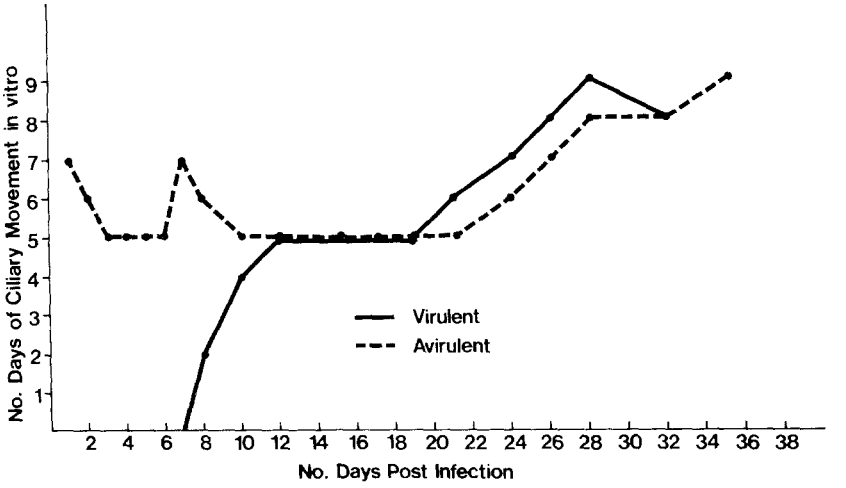

Fig. 1. The average number of days of ciliary movement in tracheal organ cultures derived from chickens infected with virulent and avirulent strains of avian infectious bronchitis virus

3.5. Histopathology

The histopathology of the tracheas from birds exposed to the virulent and avirulent IBV was markedly different. The virulent virus resulted in the loss of the ciliated surface epithelium and goblet cells during the first 5 days PI and there was hyperplasia of the remaining epithelial layer, dilation of blood vessels and definite mononuclear cell infiltration. During days 6, 7 and 8 PI there was a diminishing of the inflammatory response and a return of the normal architecture of the epithelial lining, with ciliated epithelial cells evident on the eighth day. The hyperplasia of the epithelial cells was evident until the fifteenth day PI.

The avirulent virus caused no detectable damage to the ciliated surface epithelium. Beginning on day 2 there was a definite hyperplasia of the epithelial layer of cells with the dilation of blood vessels and infiltration of this area with mononuclear cells. The hyperplasia was present until the ninth day PI at which time the tracheas appeared to have returned to normal.

3.6. Virus Neutralization

The neutralizing antibody response to the virulent and avirulent viruses is summarized in Table 2. The neutralizing antibody activity was about the same on the nineteenth day PI but the chickens infected with the virulent virus main-

tained their neutralizing antibody titer throughout the experiment. The antibody titers fell significantly in the 24 and 35 day serum pools from chickens infected with avirulent virus.

Table 2. *The Virus Neutralizing Antibody Response of Chickens to Virulent and Avirulent Avian Infectious Bronchitis Virus (IBV)*

	Days post infection			
Virus Strain	0	19	24	35
Virulent	<1.0[a]	2.4	2.8	2.7
Avirulent	<1.0	2.1	1.4	1.3

[a] Log_{10} reduction in titer of Beaudette cell culture adapted IBV.

4. Discussion

The findings of this study indicate that both virulent and avirulent IBV will replicate in the chicken. The main difference appears to be their ability to infect and replicate in the ciliated epithelial lining of the trachea. It appears that a few of the epithelial cells are infected as evidenced by the fact that some infected cells were detected by immunofluorescent staining of impression smears, where a large portion of the cells are epithelial. It was evidently a matter of probability that no infected epithelial cells were detected in cryostat sections of trachea during the first three days.

Both the virulent and avirulent viruses persisted in the chickens for essentially the same period of time and there was perfect correlation between the detection of viral infection by immunofluorescence and the ability to reisolate the virus. A recent report by PURCELL and MCFERRAN (12) indicated that they could isolate virus from the trachea up to the twelfth day PI. COOK (3) was able to isolate IBV for up to 49 days PI. In this study, the virus was reisolated by making organ cultures from the tracheas. When the ciliated epithelium was destroyed the isolation of virus was carried out in the embryonating hen's egg. The virus evidently resurfaces from the subepithelial cells and infects the surface epithelium. JOHNSON *et al.* (7) found that ciliated epithelial cells from tracheas of immune chickens are just as susceptible to IBV as those from non-immune chickens.

It would appear that the differences in pathogenic and immunogenic properties of virulent and avirulent IBV lies in their ability to destroy the surface epithelium lining the trachea. The avirulent virus infected only a few epithelial cells before establishing an infection of subepithelial cells. The antibody response invoked by the avirulent virus was almost comparable to that of the virulent virus of 19 days PI. However, it did not maintain a high level and in fact was descending from the nineteenth to the thirty-fifth day PI in contrast to the continued rise in antibody titer with the virulent IBV infection.

The histopathologic changes of IBV infections have been described by a number of investigators (5, 6, 8, 11, 12) with only minor discrepancies; they are all quite similar and agree with the histologic changes observed in this study.

One puzzling point that this study revealed is the apparent lack of correlation between the susceptibility of the ciliated epithelium to IBV *in vivo* and *in vitro*. The ciliated cells of the trachea appear to resist infection in the non-immune chickens, but the ciliated cells of tracheas maintained *in vitro* are quite susceptible to avirulent IBV and are rapidly destroyed (2). This would indicate that the tracheal organ cultures are not the ultimate model for *in vivo* conditions in a defined artificial environment.

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