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Studies on Avian Infectious Bronchitis Virus (IBV)

III. Interferon Induction by and Sensitivity to Interferon of IBV

By

K. Otsuki, J. Maeda, H. Yamamoto, and M. Tsubokura

Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori, Japan

With 2 Figures

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Summary

The induction of interferon by avian infectious bronchitis virus (IBV) and the sensitivity of IBV to interferon were studied. The results of experiments with ten IBV strains are summarized as follows. 1. All the IBV strains tested induced interferon in chick embryo (CE) cells, chicken kidney (CK) cells and embryonated eggs. The Iowa-609 strain induced about 1000 units of interferon in CE cells while the Beaudette-42 strain induced about 200 units of interferon in embryonated eggs; the interferon titers induced by other strains usually ranged from 5 to 60 units. No IBV strain induced interferon in HeLa or BHK-21 cells. 2. IBV particles inactivated by ultraviolet irradiation or by heating lost their ability to induce interferon. 3. The properties of the interferon produced in the present study are similar to those of other interferons produced in chicken cells. 4. HeLa or BHK-21 cells did not acquire resistance to virus infection, after incubation with interferon produced in CE cells. On the other hand, CK cells acquired the same degree of resistance to virus infection as CE cells after incubation with interferon produced in CE cells. 5. All the IBV strains tested were sensitive to interferon in CK cells. The sensitivities of Massachusetts-41 and Holte strains to interferon were similar to that of vesicular stomatitis virus.

Introduction

In 1949, Groupé (5) reported the production of an interfering material in the allantoic fluid of avian infectious bronchitis virus (IBV)infected embryonated eggs. Later, Groupé and Pugh (6) observed that this material interfered with the growth of both influenza virus and IBV in eggs. Then, Lombardi (9) described a similar interference in embryonated eggs produced by the Massachusetts and Connecticut strains of IBV. Furthermore, it has been reported that IBV interferes with Newcastle disease virus (NDV) infection in *in vivo* (7), *in ovo* (14), and *in*

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vitro (1) systems. It has been shown that these interfering phenomena were not due to the action of interferon.

On the other hand, interferon induced by IBV and other coronaviruses has been poorly characterized. Only three reports of interferon induction by IBV have been published. Yurov (18) observed that the IBV-41 strain induced interferon *in ovo*, while Lomniczi (11) reported that only the Beaudette-222 strain could induce interferon in chickens, embryonated eggs and chick embryo (CE) cells. However, Gomez and Raggi (4) failed to induce interferon *in vitro* or in tracheal organ cultures with the Massachusetts strain. The sensitivities of IBV and other coronaviruses to interferon has not been studied.

In this report, ten IBV strains (12, 13) were examined for their ability to induce interferon and for their sensitivities to interferon.

Materials and Methods

Viruses

IBV

Beaudette-42 (Be-42), Massachusetts-41 (IB-41), Connecticut A-5968 (A-5968), Connaught, Holte, Iowa-609, KH, Nerima, Ishida and Shiga strains (12, 13) were studied.

Vesicular Stomatitis Virus (VSV)

The VSV New Jersey serotype was supplied by the National Institute of Animal Health, Tokyo (Director Dr. S. Shibata). This virus had previously been passaged at least three times in embryonated eggs. Before use, the virus was subcultured twice in CE cells.

Cultured Cells

Chicken kidney (CK), CE, HeLa and BHK-21 cells were used (12, 13).

Interferon Induction

Each strain of IBV, at a titer of $10^{6.5}$—$10^{7.5}$ $TCID_{50}$, was inoculated onto monolayers of CE, CK, HeLa or BHK-21 cells. After adsorption of virus for 90 minutes at 37° C, the cells were washed three times with maintenance medium and then cultured at 37° C. Culture media were usually collected 24 or 48 hours after inoculation. The media were heated for 15 minutes at 60° C to inactivate the virus particles and then centrifuged at 30,000 rpm for 60 minutes to pellet the virus. The supernatant fractions were titrated for interferon. Interferon induction in embryonated eggs was examined as follows. Each strain of IBV at titers of $10^{6.0}$—$10^{7.0}$ $TCID_{50}$ was inoculated into the allantoic cavity of 10-day-old embryonated eggs. Allantoic fluids were collected 48 to 120 hours after inoculation from the infected eggs and analysed as described for the cultured cells.

Interferon Titration

In most experiments, the interferon titer was determined by placing 3 ml of serial twofold dilutions of interferon onto CE-cell monolayers seeded in small bottles. When an interferon titration was done in CK, HeLa or BHK-21 cells, the homologous cells were used to titrate the interferon. The cell monolayers were treated with interferon for 16 hours at 37° C, washed three times with Hanks' balanced salt solution, and then infected with VSV at a titer of 50—150 plaque-forming units (PFU). After virus adsorption for 60 minutes at 37° C, 5 ml of Eagle's minimum essential medium containing 1 per cent Bacto agar (DIFCO), 0.1 per cent tryptose phosphate broth (DIFCO) and 0.004 per cent neutral red was added, and cultivation was continued for 36 hours at 37° C. The interferon titer was calculated as the highest dilution producing a 50 per cent reduction in the number of VSV plaques (PR_{50} units).

Ultraviolet (UV) Irradiation of IBV

1.5 ml aliquots of culture medium in petri dishes of 6.5 cm diameter were irradiated with a UV lamp (15 W, 0.5 A) at a distance of 7.5 cm.

Results

Interferon Induction by IBV

Interferon Induction in Cultured Cells

The IBV strains were examined for interferon induction in cultured cells. As shown in Table 1, all the strains except Connaught induced interferon in CE and CK cells. An especially high interferon titer was produced by Iowa-609 strain in CE cells. The other strains induced up to 90 units of interferon in both cell types. All the IBV strains failed to induce interferon in HeLa and BHK-21 cells.

Table 1. *Induction of interferon in cultured cells*

Strain	CE cells		CK cells	
	24[a]	48[a]	24[a]	48[a]
Be-42	20	50	15 (20[b])	25 (25)
IB-41	20	20	5 (5)	45 (50)
Connaught	<5	<5	<5 (<5)	<5 (<5)
A-5968	5	10	<5 (<5)	20 (40)
Iowa-609	1000	1000	<5 (<5)	180 (150)
Holte	20	10	5 (15)	90 (60)
KH	10	20	5 (5)	5 (5)
Nerima	5	5	ND[c]	15 (20)
Ishida	40	60	ND	35 (60)
Shiga	10	20	<5 (<5)	<5 (5)

[a] Period in hours after IBV inoculation
[b] Interferon titers measured on CE cells
[c] Not done

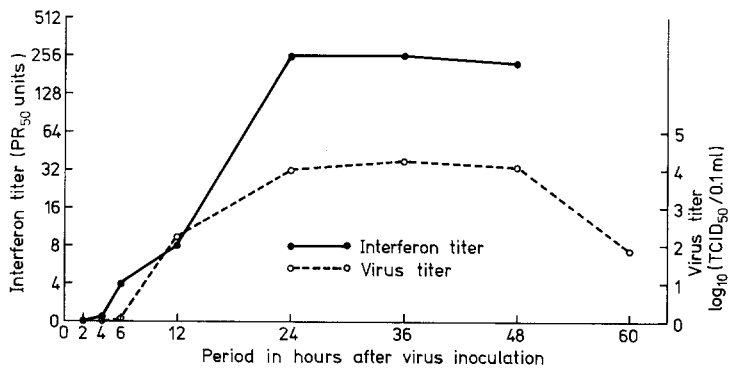

Fig. 1. Production of interferon and virus multiplication in CE cells infected with Iowa-609

The production of interferon in CE cells inoculated with Iowa-609 was examined. Interferon was first observed 6 hours after inoculation (Fig. 1). The amount of interferon increased gradually and reached a maximum level after 24 hours. Progeny viruses appeared after 6 to 12 hours and the peak virus titer was observed after 36 hours.

Interferon Induction in Embryonated Eggs

The results obtained are shown in Table 2. The IB-41, Nerima and Ishida strains failed to induce interferon. However, the other seven strains induced up to 190 units of interferon in embryonated eggs. The Connaught strain, which did not induce interferon in CE or CK cells, induced it in eggs.

Table 2. *Induction of interferon in embryonated eggs*

Strain	Interferon titer				Fate of the infected embryos
	48[a]	72[a]	96[a]	120[a]	
Be-42	150				Death
IB-41		<5	<5		
Connaught		10	5	35	Partial death
A-5968		20	160		
Iowa-609	30	20			Death
Holte		5	20		
KH		20		190	Partial death
Nerima		<5	<5		
Ishida		<5	<5		Partial death
Shiga		10	5		
Allantoic fluid		<5	<5		

[a] Hours after IBV inoculation
Interferon titers measured on CE cells

Interferon Induction With Inactivated IBV

Experiments were done to see whether IBV, inactivated by UV irradiation for 20 seconds or heating for 15 minutes at 60° C, induced interferon. $10^{6.0}$ to $10^{7.0}$ $TCID_{50}$ of virus was used. All the strains completely lost their infectivities on irradiation or heating. Furthermore, none of the inactivated strains, inoculated onto CE cells or into the allantoic cavities of 10-day-old eggs, induced interferon within 96 hours of inoculation.

Some Properties of IBV Induced Interferon

Resistance of Interferon to Treatment With Chemical or Physical Agents

Two interferon preparations were used. One was produced in CE cells inoculated with Iowa-609 and had a titer of 1000 units. The other was produced in embryonated eggs inoculated with Be-42 and had a titer of 150 units. These interferons did not sediment on centrifugation at $100{,}000 \times g$ for 3 hours, were sensitive to treatment with 0.1 per cent trypsin for 1 hour at 37° C, and their activities were reduced by 90 per cent by heating for 10 minutes at 100° C. Treatment of the interferons at pH 2.0 or pH 10.0 for 24 hours at 4° C did not affect their titers. Furthermore, their titers were not reduced by freeze-thawing 10 times; or UV irradiation for 60 seconds.

Antiviral Activities of Interferon Produced in CE Cells

The interferon tested was prepared from CE cells infected with Iowa-609. 250 units of interferon were incubated with HeLa, BHK-21 and CK cells for 16 hours at 37° C, and then about 120 PFU of VSV were inoculated onto the cells. Control cells were not treated with interferon, but were inoculated with 120 PFU of VSV. As shown in Table 3, in interferon-treated HeLa and BHK-21 cells plaque formation by VSV was inhibited slightly, compared with the control, but VSV plaque formation in CK cells was completely inhibited.

Table 3. *Antiviral activities of interferon, induced in CE cells, on VSV infection in HeLa, BHK-21 and CK cells*

	Number of plaques in different cell types			
	HeLa	BHK-21	CK	CE
Interferon (250 units)	26	100	0[a]	0
VSV control	48	98	124	135

[a] Interferon titer was 245 units on CK cells

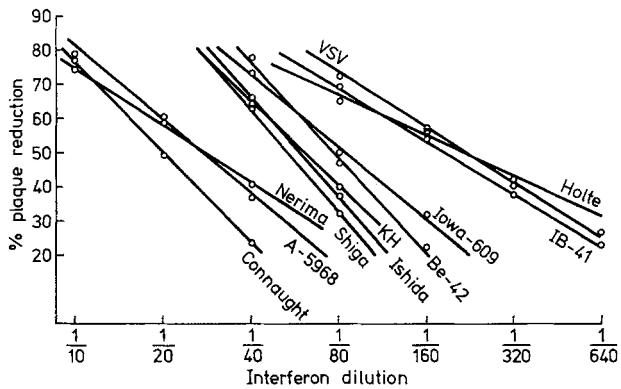

Fig. 2. Sensitivities of IBV strains to interferon produced in embryonated eggs infected with the Be-42 strain. The interferon was assayed in CK cells

Sensitivity of IBV to Interferon

Interferon produced in embryonated eggs infected with the Be-42 strain was used in this study. The sensitivity of the IBV strains to interferon was assayed in CK cells. Approximately 100 PFU of the IBV strains and VSV were added to interferon-treated and control cultures. Plaque reduction between 20 and 80 per cent was plotted, and the PR_{50} units of interferon against each virus was determined. The results obtained are shown in Figure 2. The IB-41 and Holte strains were as sensitive to interferon as VSV, while the sensitivities of the Be-42, Iowa-609, KH, Ishida and Shiga strains were 30—40 per cent that of VSV. The A-5968, Connaught and Nerima strains were only about one-tenth as sensitive as VSV.

Discussion

As all the IBV strains tested induced interferon in CE and CK cells, and in embryonated eggs we suggest that IBV, like many other RNA viruses (8), can induce interferon. However, the ability of IBV to induce interferon appears rather weak, as the interferon titer with most strains was not high and a few investigators (4, 10) have failed to induce interferon with the Massachusetts strain. Furthermore, a large inoculum was required for interferon induction. No interferon was induced in HeLa or BHK-21 cells by any IBV strain, even though the Be-42 and Holte strains grew in BHK-21 cells (13). Thus, we suggest that IBV can only induce interferon in chicken cells or organs. However, other mammalian cells must be tested to confirm this suggestion.

All the IBV strains tested, when inactivated by UV irradiation or heating, lost their ability to induce inteferon. These results agree with those reported previously by Lomniczi (11) and suggest that only infectious IBV particles can induce interferon. It is not known why inactivated IBV particles cannot induce interferon.

Similar amounts of interferon were produced in CK and CE cells on infection with IBV, and furthermore CK cells acquired the same degree of resistance to virus infection as CE cells, when they were incubated with interferon produced in CE cells or embryonated eggs. Thus CK cells can probably be used to test the sensitivity of IBV to interferon.

It is clear that IBV, at least in CK cells, is sensitive to interferon. However, unlike some viruses (2, 3, 10, 15, 16), there is no close relationship between the ability of any strain to induce interferon and its sensitivity to interferon. Similarly, the resistance of the IBV strains to various chemical and physical treatments (12) or their growth in various cells (13) probably bears no relationship to their ability to induce interferon or their sensitivity to interferon. We conclude that IBV is an interferon sensitive virus rather than an interferon inducing virus. However, the interferon sensitivity of the IBV strains should be examined in *in vivo* systems, as the sensitivity of viruses to interferon is not always the same in different systems (17).

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Authors' address: Dr. K. OTSUKI, Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori City, Japan.

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