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INTERFERON INDUCTION BY SEVERAL STRAINS OF AVIAN INFECTIOUS BRONCHITIS VIRUS, A CORONAVIRUS, IN CHICKENS

K. OTSUKI, T. NAKAMURA, Y. KAWAOKA, M. TSUBOKURA

Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori 680, Japan

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Summary — Eight U.S. and Japanese strains of avian infectious bronchitis virus (IBV) were tested for their interferon-inducing ability in chickens. All strains induced interferon (IFN) in several organs of six-week-old SPF chickens. However, the extent of IFN formation in these chickens was not necessarily the same from one strain to another.

Key words: avian infectious bronchitis virus; coronavirus; interferon

Introduction

Interferon (IFN) has been thought one of the important factors in defending a virus infection. There is limited information about IFN of avian infectious bronchitis virus (IBV). Only a few reports have been published by some investigators. They pointed out that IBV appears to be a poor IFN inducer (Gomez and Raggi, 1974; Holmes and Darbyshire, 1978; Lomniczi, 1974; Yurov, 1967). Holmes and Darbyshire (1978) reported that none of IBV strains investigated was susceptible to the inhibitory effects of chick IFN. We also reported that 10 U.S. and Japanese strains of IBV induced IFN in chick embryo (CE) cells, chick kidney (CK) cells and embryonated hen's eggs but their IFN-inducing capacity was moderate and that they were generally sensitive to IFN at least in the CK-cell system (Otsuki *et al.*, 1979*c*).

INF induction by IBV *in vivo* has been poorly characterized. Only two reports regarding this subject have been published. Lomniczi (1974) and Otsuki *et al.* (1982) demonstrated that the Beaudette strain of IBV induced IFN in chickens. In this investigation, eight U.S. and Japanese strains of IBV were examined for their ability to induce IFN in chickens.

Materials and Methods

Viruses. The strains of IBV tested were Massachusetts-41 (IB-41), Connaught, Holte, Connecticut A-5968 (A-5968), KH, Nerima, Shiga and Ishida (Otsuki *et al.*, 1979*a*). Latter four strains were isolated in Japan. Each strain had been passaged twice in embryonated hen's eggs

and seven times in CK cells before they were analysed in this laboratory. The vesicular stomatitis virus, New Jersey serotype, was used to determine the IFN titre (Otsuki *et al.*, 1979c). Before use, this virus was subcultured twice in CE cells.

Chickens and embryonated hen's eggs. Six-week-old SPF chickens and 10-day-old SPF embryonated hen's eggs, kindly supplied by Dr. Y. Iritani, Aburahi Laboratories, Shionogi Co., Koka-cho, Koga-gun, Shiga Prefecture, Japan were used in this investigation.

Cell cultures. CK and CE cells were used (Otsuki *et al.*, 1979*a*, 1979*b*).

Virus titration. Virus titration was performed by inoculating the sample to embryonated hen's eggs via the allantoic cavity and the infectivity was expressed as 50% egg infectious doses (EID_{50}).

Experimental infection with IBV. Ten SPF chickens were inoculated with IBV of $10^{5.0}$ EID_{50} intratracheally for each strain. Two chickens of a group were killed daily for five days. Their laryngeal swab and plasma were taken and trachea, lungs, liver, spleen and kidneys were removed aseptically and examined for virus recovery and interferon. These organs were homogenized with sterile sand and suspended to approximately 10% in Eagle's MEM. After clasification by centrifugation for 30 minutes at 3000 rev/min, portions of the supernatants were frozen at −80 °C until virus infectivity and IFN were examined.

IFN assay. To avoid the interference with infective IBV (Otsuki *et al.*, 1983) samples were acidified with 1 mol/l HCl to pH 2 and kept at 4 °C for overnight and then pH was readjusted to 7 with 1 mol/l NaOH. These suspensions were heated for 15 min at 60 °C and then ultracentrifuged at 30 000 rev/min for 90 min to pellet the virus and cell debris. The supernatant fractions were titrated for IFN by the method described previously (Otsuki *et al.*, 1979c).

Results

First, four U.S. strains of IBV were tested for their IFN-inducing ability in chickens. Results obtained are shown in Fig. 1. A considerable titre of IFN was found in chickens inoculated with IB-41 or Holte strain. A high titre of IFN was demonstrated in respiratory organs of chickens inoculated

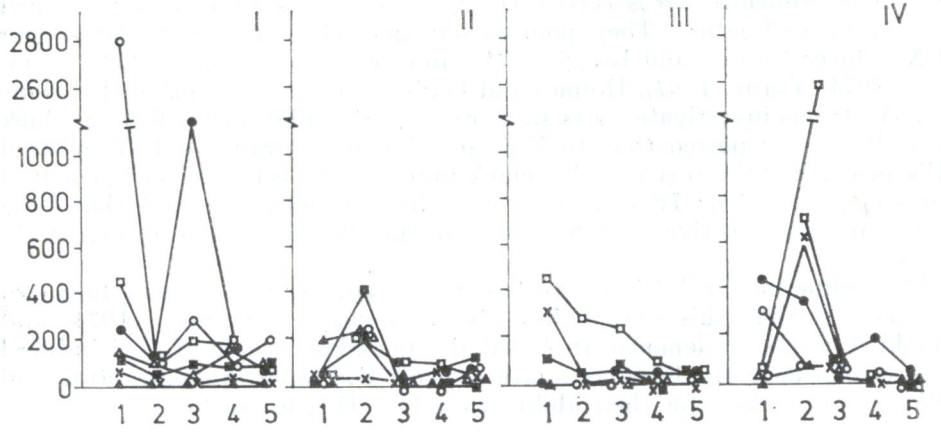

Fig. 1

IFN production in chickens inoculated with the U.S. strains of IBV

(I) IB-41 strain. (II) Connaught strain. (III) A-5968 strain. (IV) Holte strain.
Abscissa: days p.i.; ordinate: IFN titre.

○: trachea, △: laryngeal swab, ●: lungs, ▲: liver, □: spleen, ×: kidneys, ■: plasma

with strain IB-41 (Fig. 1-I); in the trachea 2800 units of IFN were demonstrated on day 1 post-inoculation (p.i.) and 280 and 200 units on days 3 and 5, respectively. In the lungs 250 units were found on day 1 and 1150 and 150 units on days 3 and 4, respectively. A fair titre of IFN was also detected in the spleen but its activity was weak in kidneys. The chickens inoculated with the Holte strain had considerable titres of IFN in spleen, plasma and kidneys on day 2, but in respiratory organs the titre was not so high (Fig. 1-IV). The Connaught and A-5968 strains induced only low titres of IFN. Virus recovery from the organs of inoculated chickens was also performed. All strains of IBV were recovered from trachea, lungs, liver, spleen, kidneys and plasma for five days p.i. The chickens inoculated with IB-41 or Holte strains showed mild respiratory signs from days 2 to 5 p.i.

Next, four Japanese strains were tested for their IFN-inducing ability. As shown in Fig. 2, all IBV strains tested induced IFN in the chickens inoculated. Their IFN-inducing capacity was not necessarily the same from one strain to another and was different from that of the U.S. strains. Shiga and Ishida strains induced high titre of IFN; the chickens inoculated with Shiga strain produced 6400 and 1700 units in lungs and trachea respectively, and 640, 690 and 400 units in plasma, kidneys and spleen, respectively on day 5 (Fig. 2-I). Alternatively, 2000, 440, 260 and 240 units of IFN were demonstrated in spleen, trachea, lungs and kidneys, respectively, on day 4. The IFN disappeared by the next days from these organs when induced by the Ishida strain (Fig. 2-III). The IFN-inducing capacity of KH and Nerima strains was low (Figs. 2-III and 2-IV). All Japanese IBV strains were suc-

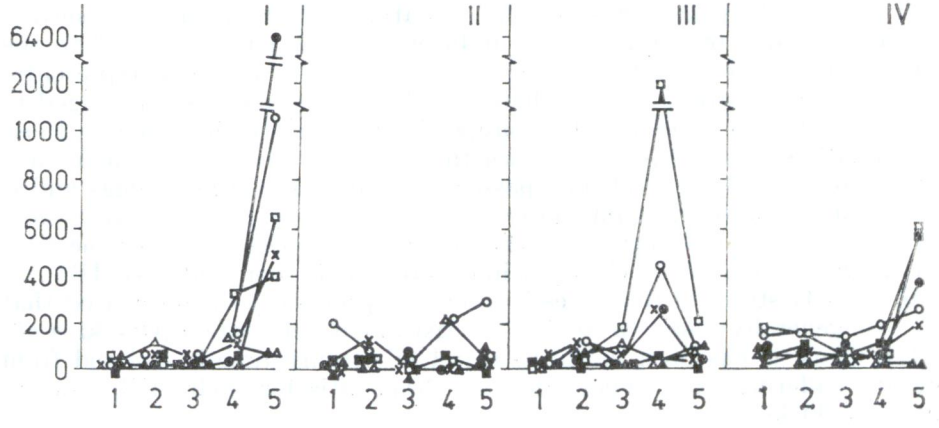

Fig. 2

IFN production in chickens inoculated with Japanese strains of IBV

(I) Shiga strain. (II) Nerima strain. (III) Ishida strain. (IV) KH strain.

○: trachea, △: laryngeal swab, ●: lungs, ▲: liver, □: spleen, ×: kidneys, ■: plasma

cessively recovered from any organs of inoculated chickens for five days. None of the birds inoculated with these strains showed any clinical signs during the investigation.

Discussion

As stated above, IFN induction by IBV *in vivo* has been poorly characterized. Lomniczi (1974) reported that six-week-old chickens injected with strain Massachusetts 41 or with two Hungarian strains of IBV failed to produce IFN but they produced IFN after administration of the Beaudette strain. Otsuki *et al.* (1982) also reported that the Beaudette-42 strain induced 100 to 600 IFN units in the chicken plasma or trachea.

The present investigation revealed that some representative U.S. and Japanese strains of IBV induced considerably high titre of IFN in chickens. Formation of IFN in these was not necessarily the same from one strain to another. There was an obvious difference among the eight strains of the IBV tested for the amounts of IFN produced, the interval of its peak titre and the organs in which the highest IFN titre had been demonstrated, although virus replication was rather similar with any strain tested (data not shown). While IB-41 and Holte strains caused mild respiratory signs in SPF chickens, they induced a fair IFN titre, Ishida and Shiga strains induced high titre of IFN but did not cause any clinical signs of disease. We are not certain why the IFN-inducing potency of IBV varied from strain to strain. As stated in *in vivo* examinations (Holmes and Darbyshire, 1978; Otsuki *et al.*, 1979*c*), no simple relationship between IFN-inducing potency and virulence of IBV was found.

Holmes and Darbyshire (1978) suggested that IFN plays a limited protective role in the pathogenesis of IBV in natural infections in fowls because most IBV strains tested were poor inducers of it and they resisted to the anti-viral effects of it in tracheal organ culture. However most representative and vaccine strains of IBV had already been numerously passaged in *in vivo* or *in vitro* systems. Their properties are hardly the same as those of field IBV strains; it is well known that the latter easily loss their virulence following only several-time passage in embryonated hen's eggs and it is difficult to cause experimentally severe disease to chickens with most representative IBV strains. Nevertheless, we notice that considerable titre of IFN was produced in chickens inoculated with representative IBV including IB-41 strain in this investigation. We previously demonstrated that IBV was generally sensitive to IFN at least in CK-cell system (Otsuki *et al.*, 1979*c*). Thus, it has been useful to test IBV strains directly isolated from chickens suffering from avian infectious bronchitis for their IFN-inducing ability in chickens.

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