伝染性ファブリキウスのう病ウイルスによる補体の活性化とリンパ系細胞でのウイルス増殖との関連性

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Complement Activation by Infectious Bursal Disease Virus and its Relevance to Virus Growth in the Lymphoid Cells

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Infectious bursal disease virus (IBDV) is a non-enveloped virus of the double-stranded RNA type which belongs to the *Birnaviridae* family. Subsequent to the oral inoculation of chickens, this virus replicates in chicken macrophage and lymphoid cells within the gutassociated lymphoid tissues, leading to a marked growth in the bursa of Fabricius [1, 2]. Such predilection of IBDV for the bursal lymphocytes results in the induction of immuno-suppression. In severe cases of infection, both humoral and cellular immunities are markedly suppressed, and a low level of complement activity has also been reported [6].

We reported previously that IBDV is neutralized by fresh normal chicken serum (NCS) in the absence of antibody specific to IBDV and suggested that the antibody-independent activation of complement via an alternative pathway by IBDV is responsible for the virus inactivation [5]. To investigate the significance of such complement activation *in vivo*, we examined in this study the growth capacity of attenuated IBDV treated with complement in spleen cells and demonstrated that complement-treated IBDV could replicate in adherent cells but not in lymphocytes. The relevance of this finding with respect to virus-induced immuno-suppression was also discussed.

Fertilized specific-pathogen-free (SPF) eggs were purchased from SPAFAS Co. Ltd., (Norwich, CT). Primary monolayer cultures of chicken embryo (CE) cells were prepared in plastic plates or dishes (Falcon 3002, 3008, Becton Oxnard, Calif.) from 10 to 11 day-old SPF chicken embryos. The J1 strain of IBDV [7] was passed into CE cells, and cloned three times. The spleen cells were obtained from 3 week-old SPF chickens. The lymphocyte fraction was separated from the spleen cells by using low-speed centrifugation after mixing with Lymphocyte-Separator-Solution (Sigma Chemical Co., St Louis, MO.). The adherent cells were collected after incubation of the spleen cells in plastic dishes at 37°C for 1 hr. The culture medium for the spleen cells, the spleen lymphocytes and the adherent cells consisted of RPMI 1640, supplemented with 5% heat-inactivated fetal bovine serum, and the medium used for the CE cells consisted of Eagle's minimum essential medium, also supplemented with 5% heat-inactivated fetal bovine serum. These cells were suspended in the culture medium at a concentration of 10^6 cells per m*l*. The SPF chickens were raised in isolation and bled by heart puncture at an age of 4 to 7 weeks and their sera were separated at 4°C, pooled and stored at −80°C until use. The above prepared NCS was then tested for the absence of antibodies specific to avian viruses including IBDV by means of the neutralization test. Complement activities of NCS involved in both the calssical and alternative pathways were determined as described previously [4], and their values were at titers of 24 to 32 for the former and 20 to 24 for the latter, respectively. To obtain anti-IBDV serum, about 100 day-old hens were immunized by killed IBDV vaccine and their offsprings were bled during their first day of life. The anti-IBDV antibody titer measured by 50% plaque reduction method amounted to 4,500 units.

Procedures for the decomplementation of fresh NCS were described previously [4]. Briefly, 1 m*l* of fresh NCS was mixed with 2 mg of zymosan A (Sigma Chemicals) and the mixture was incubated at 37°C for 1 hr. Subsequently, the zymosan was removed by low-speed centrifugation at 4°C. The complement activity of the supernatant was decreased to a value lower than 5% of the activity value contained in the fresh

Table 1. Effect of treatment with fresh NCS upon the infectivity titer of IBDV

IBDV treated with	Titer of IBDV (log $TCID_{50}$)
Untreated	6.5
Fresh NCS	5.5
Fresh NCS absorbed by IBDV infected CE cells	6.5
Heated NCS (56°C, 30 min)	6.5
Zymosan-treated NCS	6.5
EGTA-Mg-treated NCS	5.5
Fresh NCS and anti-C3 serum	4.0
Culture medium and anti-C3 serum	6.5

After incubation at 37°C for 1 hr, infectivity titers of IBDV treated with each serum were measured in CE cells.

NCS.

The effect of the fresh NCS on the infectious capacity of the IBDV was examined by incubating the stock of IBDV with an equal volume of fresh NCS for 1 hr at 37°C. As shown in Table 1, the incubation with fresh NCS caused a reduction in the infectivity titer determined in CE cells by 1 log $TCID_{50}$. A similar level of reduction was observed in the presence of EGTA-Mg^{2+}, or by NCS which was absorbed 5 times by IBDV-infected CE cells. Such reduction was not observed by the decomplemented serum or by the control culture medium. Subsequently, the IBDV treated with fresh NCS was further incubated with anti-quail C3 serum which cross-reacts with chicken C3 [3] . As a result, the infectivityy titer was decreased by 2.5 log, suggesting the C3 deposition on IBDV virion. These results suggested that the reduction in the infectivity titer was due to the antibody-independent activation of the complement by IBDV via an alternative pathway.

The neutralization kinetics of the IBDV infectivity induced by fresh NCS was compared with the neutralization induced by anti-IBDV serum diluted to 100 and 200 units. As shown in Fig. 1, the infectivity titer decreased more gradually in the sample neutralized by the fresh NCS than by anti-IBDV serum. These results further confirmed that the reduction of infectivity titer induced by the fresh NCS was due to the activation of the complement and not by the

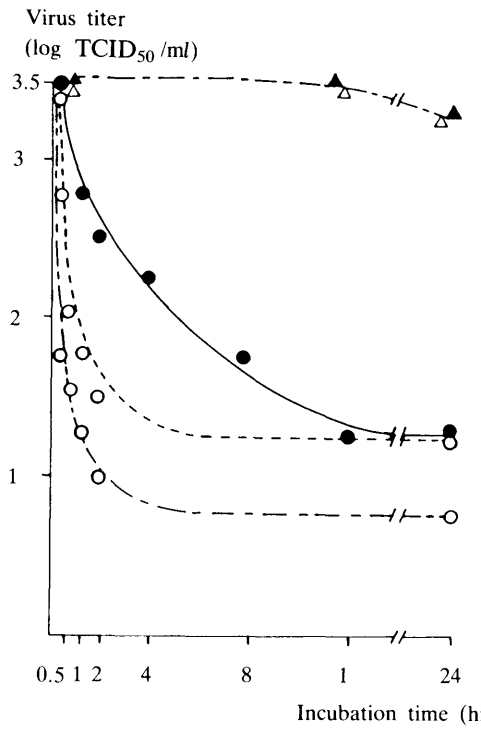

Fig. 1. Kinetics of virus neutralization by means of NCS and specific antibody. Virus was mixed with an equal volume of NCS or antibody, incubated at 37°C, and the infectivity titers were predetermined intervals. Fresh NCS: ●——●, specific antibody with 100 units: ○----○, specific antibody with 200 units: ○-·-·-○, zymosan-treated NCS: ▲----▲, heated NCS: △-·-·-△.

Table 2. Growth in chicken cells of IBDV treated with fresh NCS[a)]

Inoculated into	Virus yield after treatment with	
	fresh NCS	Heated NCS
CE cells	<1.5	6.5
Spleen cells	2.8	5.8
unfractionated lymphocytes	<1.5	4.8
adherent cells	3.3	3.0

a) Mean titers obtained from triplicate experiments are indicated as log $TCID_{50}$/m*l*.

anti-IBDV antibody. A more gradual decrease in the infectious capacity induced by fresh NCS may be due to the effect of the multistep reactions within the complement system.

Since the major target cells *in vivo* of IBDV are the lymphoid cells, the fate of the IBDV treated with fresh NCS was examined by means of the infectivity test involving spleen cells as well as CE cells. The stock virus with titer of $10^{3.0}$ $TCID_{50}$/m*l* was incubated at 37°C for 1 hr with fresh NCS or with inactivated NCS at 56°C for 30 min, and virus inoculum was adjusted to contain 10 $TCID_{50}$/m*l* of preincubation titer. By implementing this minimal dose, the fresh NCS-treated virus was determined to contain no infectious virus. One m*l* of virus solution was then inoculated into each 4 plastic dishes seeded with CE cells, unfractionated spleen cells, spleen lympocytes or adherent spleen cells. After incubation at 37°C for 4 days, the infectivity titers of the culture supernatants were measured in CE cells.

As summarized in Table 2, virus growth was not observed in the CE cell sample. In the unfractionated spleen cell sample, virus was detected at a titer of $10^{2.8}$ $TCID_{50}$/m*l*. In the adherent spleen cell sample, the virus yield was $10^{3.3}$ $TCID_{50}$/m*l* but in the lymphocyte sample virus growth was not detected. The result indicated that the virus neutralized by fresh NCS was apparently still capable of replication in the case of the adherent spleen cells, which consist most probably of macrophages.

Growth of the fresh NCS-treated virus in the adherent spleen cells, in spite of its failure to grow in CE cells, was an unexpected result. The present results may indicate some aspects of biological significance involving the role of the complement in attenuated IBDV infection. The decrease in the infectious capacity induced by the antibody-independent activation of the complement points to its significant role in the mechanism of early non-specific defence involved in several virus infections. The C3b deposit caused by the complement activation may also lead to the masking of virus binding sites to its cellular receptor. This mechanism may partially explain why the complement proves ineffective in retarding the preferential growth of attenuated IBDV occurring within lymphoid tissues.

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要　　約

伝染性ファブリキウスのう病ウイルスによる補体の活性化とリンパ系細胞でのウイルス増殖との関連性（短報）：小峯健一・大田博昭・鎌田信一[1)]・内田和夫[1)]・吉川泰弘[2)]・山内一也[2)]・岡田秀親[3)]（㈱ゲン・コーポレーション，[1)]日本獣医畜産大学獣医衛生学教室，[2)]東京大学医科学研究所実験動物研究施設，[3)]福岡大学医学部微生物学教室）――伝染病ファブリキウスのう病ウイルス（IBDV）に対する特異抗体を特たない新鮮正常ニワトリ血清は，IBDV に対する中和活性を示した．この中和活性は Mg^{2+}依存性，Ca^{2+}非依存性であり，さらに抗ウズラ C_3血清の添加により増強されたことから，抗体非依存性の補体第２経路の活性化によることが示唆された．新鮮正常ニワトリ血清で中和されたIBDV は，ニワトリ胚細胞では増殖できなかったが，ニワトリ脾細胞中のマクロファージと考えられるガラス壁付着細胞では増殖することがみいだされた．