

Pathogenesis of Highly Virulent Infectious Bursal Disease Virus Infection in Intact and Bursectomized Chickens

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ABSTRACT. The pathogenesis of highly virulent infectious bursal disease (IBD) virus (IBDV) infection was studied using 6-week-old intact and 5-week-old bursectomized chickens inoculated with highly virulent strain 90-11 or reference strain I. Chickens inoculated with $10^{0.7}$ EID₅₀ of strain 90-11 showed neither clinical signs nor lesions during the 4-day observation period. In contrast, birds inoculated with $10^{2.7}$ or $10^{4.7}$ EID₅₀ developed severe clinical IBD, as well as gross and histologic lesions, typical of IBD, and produced IBDV antigen demonstrable by immunostaining in the bursa of Fabricius (BF), thymus, spleen and bone marrow from day 2 post-inoculation (PI) onwards. The antigen was also detected by the agar-gel precipitation and latex microsphere agglutination tests in a bursal suspension of these birds from day 2 or day 3 PI on. Birds inoculated with $10^{6.1}$ EID₅₀ of strain I developed only slight clinical signs at day 4 PI. Their lesion- and antigen-scores in the BF were almost the same as those in virulent strain-infected chickens, but lesion- and antigen-scores in the other organs were negligible. Bursectomized chickens inoculated with strain 90-11 did not develop clinical IBD despite the presence of infection that was evidenced by histologic lesions in the thymus and spleen as well as IBDV antigen demonstrable by immunostaining in these organs.—**KEY WORDS:** chicken, highly virulent infectious bursal disease virus, immunohistology, surgical bursectomy.

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Outbreaks of infectious bursal disease (IBD) caused by highly pathogenic virus have been occurring in European countries since 1987 [1, 17, 18]. In Japan, similar acute IBD with high mortality was found in broiler chicken flocks in 1990. Three highly virulent strains of IBD virus (IBDV), designated 90-11, 90-12 and 90-14, were isolated from field cases during the outbreaks of acute IBD [11]. Lin *et al.* [7], in a study on the nucleotide sequence of highly virulent strains of IBDV including the above-mentioned three isolates, revealed that Japanese highly virulent IBDV is more closely related to European virulent strain 52/70 than to Japanese conventional strains.

In the present study, we attempted to elucidate the pathogenesis of highly virulent IBDV infection. Sequential histopathologic and immunohistochemical observations were made on intact and surgically bursectomized specific-pathogen-free (SPF) chickens inoculated with strain 90-11. The chickens were also examined by blood biochemistry and quantitative determination of IBDV antigen in the bursa of Fabricius (BF) by the agar-gel precipitation (AGP) [4] and latex microsphere agglutination (LA) [10] tests as well as antigen-capture ELISA (AC-ELISA) [16]. This paper describes the results obtained by these observations.

MATERIALS AND METHODS

Chickens: A total of 79 chickens were used. They were 5- and 6-week-old SPF White Leghorns of the line M, known to be free of major chicken pathogens including IBDV as previously described [11], and had been bred at the Laboratory Animal Research Station, Nippon Institute for Biological Science (Yamanashi, Japan). All chickens were housed in separate isolation units in a room

under positive air pressure and ventilated with filtered fresh air.

Of these chickens, 12 were bursectomized (BX) and 12 were sham-operated (SO) at the age of 33 days according to the method described previously [14].

Virus: Two strains of IBDV were used. The strain I was isolated in the authors' laboratory from a laying chicken with clinical IBD in 1974 and passaged twice in SPF chickens [11]. This strain was less pathogenic, killing less than 1% of inoculated susceptible birds. The strain 90-11 was isolated from a field case in a 1990 outbreak of IBD and passaged three times in SPF chickens. The strain was proved to be highly pathogenic and induced a mortality rate of 62% in SPF chickens [11].

Blood biochemistry: Heparinized blood samples were collected from chickens at necropsy. Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase, lactate dehydrogenase, creatine phosphokinase (CPK), blood urea nitrogen (BUN), creatine, total cholesterol (T-CHO), phospholipid, total bilirubin (T-Bil), total protein, albumin (Alb), albumin/globulin (A/G) ratio, and calcium (Ca) were analyzed by an autoanalyzer (Clinalyzer SIM-6, JEOL, Tokyo, Japan) except A/G ratio that was calculated by the equation. The hematocrit value was determined by the microhematocrit method [19].

Histopathology: Tissue samples were collected from major organs. They were fixed in a phosphate-buffered 4% paraformaldehyde solution and processed by the conventional paraffin-embedding procedure for hematoxylin and eosin (H & E) staining. The histologic lesions in various organs were scored according to the severity of damage on a scale of zero to 4 [11].

Immunohistochemistry: The streptavidin-biotin-per-

oxidase complex (SABC) method was applied to paraffin-embedded tissues from the BF, thymus, spleen and bone marrow. Deparaffinized and rehydrated sections were treated with 0.25% trypsin-0.02% calcium chloride in 0.1 M phosphate-buffered saline (PBS) for 1 hr at 37°C and then with 10% H₂O₂ in methanol for 30 min at a room temperature to block endogenous peroxidase. The sections were incubated overnight at 4°C with the primary antibody, rabbit hyperimmune serum against strain IQ of IBDV [11]. The sections were processed with a Histofine SAB-PO kit for rabbit primary antibody (affinity-isolated biotinylated goat IgG, Nichirei, Inc., Japan). Quantities of viral antigen in the BF, thymus, spleen and bone marrow detectable by SABC were scored as follows: 0 = none, 1 = a few viral antigen in 2–4 microscopic fields, 2 = a few viral antigen in a microscopic field, 3 = scattered discrete viral antigens in a microscopic field, 4 = diffuse viral antigens in a microscopic field.

Quantitative determination of IBDV antigen by AGP, LA and AC-ELISA: The BFs were collected from all birds at necropsy. A 50% BF suspension in PBS was used as antigen for AGP and LA tests. The plasma samples that had been collected for blood biochemistry in experiment 2 were used as antigen for AC-ELISA.

The AGP test was performed as described previously [4], using IBDV VP2 specific monoclonal antibody (mAb) 811 [10]. The BF suspension was diluted in a twofold series with PBS. The antigen titers were expressed as the reciprocal of the highest dilution of the sample that formed a clearly visible precipitation line in agar gel. The LA test was conducted by the method described by Nakamura *et al.* [10]. The AC-ELISA was carried out using 96-well ELISA plates and mAb 811 essentially as

described by Synder *et al.* [16].

Experimental design: In experiment 1, 55 6-week-old SPF chickens were allocated to groups A to D. Group A chickens were inoculated orally with 10^{0.7} mean embryo infective dose (EID₅₀), group B chickens with 10^{2.7} EID₅₀, and group C chickens with 10^{4.7} EID₅₀ of strain 90–11. Group D chickens were inoculated with 10^{6.1} EID₅₀ of strain I. Each group was housed in a separate isolation unit. Three to 5 chickens from each group were bled and euthanatized daily from day 1 to day 4 postinoculation (PI) and examined for gross lesions.

In experiment 2, 12 BX chickens and 12 SO chickens were inoculated orally with 10^{4.7} EID₅₀ of strain 90–11 2 days after operation. Chickens of both groups were housed together. Three chickens from each group were bled and euthanatized daily from days 1 to 4 PI and examined for gross lesions.

RESULTS

Experiment 1: Clinical signs such as diarrhea, ruffled feathers, anemia, anorexia, depression and coma began to appear at day 2 PI in group C chickens and day 3 PI in group B chickens, and one of group C chickens died at day 3 PI. No overt signs of IBD were observed in group A chickens during the 4-day observation period. In group D chickens, only slight diarrhea and ruffled feathers were observed at day 4 PI. In chickens with clinical signs in the groups B and C, there were the turgid BF, atrophic thymus, fatty bone marrow and discoloration of the liver. Group D chickens had no lesions in any of their organs except the turgid BF.

Chickens having one or more of the following criteria

Table 1. Histologic lesion scores in various organs of chickens inoculated with strain 90–11 or I (Experiment 1)

Organs and lesions	Scores in chickens inoculated with strain (dose given) at postinoculation days:											
	Group B (90–11 10 ^{2.7})				Group C (90–11 10 ^{4.7})				Group D (I 10 ^{6.1})			
	1	2	3	4	1	2	3	4	1	2	3	4
BF ^{a)}												
Lymphoid necrosis	0	0.3 ^{b)}	4.0	4.0	0	2.7	4.0	4.0	0	2.3	3.7	3.8
Inflammatory reaction	0	0.3	2.3	3.5	0	2.3	2.0	2.0	0	2.7	3.3	2.3
Thymus												
Lymphoid necrosis	0	0.3	3.3	1.5	0	1.0	4.0	3.7	0	0.3	1.0	0.8
Spleen												
Lymphoid necrosis	0	0.3	2.8	1.5	0	2.0	3.2	3.0	0	0.7	0.3	1.5
Inflammatory reaction	0	0.3	2.8	1.5	0	2.3	3.6	3.0	0	0.3	0	1.0
Bone marrow												
Myeloid depletion	0	0.3	3.3	1.5	0	2.0	3.2	3.0	0	0	1.0	0.8
Inflammatory reaction	0	2.0	1.5	1.0	0	2.4	3.0	3.0	0	0	0.8	0.8
Liver												
Fatty change	0	0.3	1.9	2.0	0	2.2	2.0	1.9	0	0.3	0.3	0
Inflammatory reaction	0	0.3	1.0	0.5	0	1.0	1.8	1.8	0	0	0	0
Lung												
Inflammatory reaction	0	0.3	2.3	1.5	0	1.3	3.6	3.3	0	0.7	0	0

a) The bursa of Fabricius.

b) Each score represents the mean of three to five birds. See Materials and Methods for the scoring.

were diagnosed as being affected with IBD: necrotic lesion in the BF, positive findings by SABC and positive for viral antigen by AGP test. None of group A chickens inoculated with $10^{0.7}$ EID₅₀ of strain 90-11 developed clinical IBD. All of group B chickens inoculated with $10^{2.7}$ EID₅₀ were affected at day 3 PI and all of group C chickens inoculated with $10^{4.7}$ EID₅₀ at days 2, 3 and 4 PI. On the other hand, all group D chickens inoculated with $10^{6.1}$ EID₅₀ of strain I were affected at days 2, 3 and 4 PI.

In blood biochemical analysis, plasma levels of GOT, GPT, and T-Bil increased markedly at day 3 or 4 PI in groups B and C chickens. In these chickens, an increase in CPK and decreases in T-CHO, Alb, A/G ratio and Ca were also observed at day 3 or 4 PI. In chickens with tubulonephritis, the plasma level of BUN increased at day 3 or 4 PI. No noticeable changes were seen in chickens infected with strain I. In groups B and C chickens, hematocrit values were low at day 1 PI, while the values increased at day 3 or 4 PI.

As shown in Table 1, principal histologic lesions were observed in the lymphoid organs, bone marrows, livers, and lungs of chickens inoculated with strain 90-11. These lesions began to appear at day 2 PI, and the lesion scores at this time of infection were greater in group C chickens than in group B chickens. From day 2 PI onwards, the BF had severe cortical lymphocyte necrosis and depletion of medullary lymphocytes with interstitial inflammation and hyperplasia of epithelial reticular cells. At day 4 PI, bursal follicles of group C chickens occasionally contained amorphous, proteinaceous substance in the empty cortex with remnants of necrotic lymphocytes (Fig. 1). In the thymus, necrotic foci were observed at day 2 PI and cortical lymphocytes disappeared with epithelial reticular cell proliferation at day 4 PI (Fig. 2). Lymphocyte necrosis was severe in the medulla, resulting in marked thymic atrophy. In the bone marrow, hematopoietic cells decreased in the number at day 2 PI and were replaced by adipose tissue at day 3 PI. Sinusoids contained many

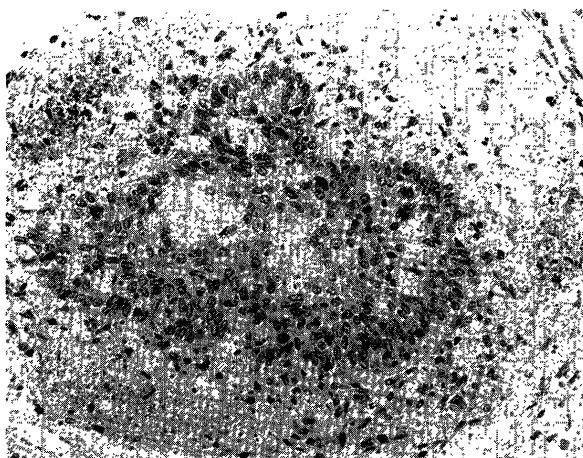


Fig. 1. A bursal follicle of a chicken 4 days after inoculation with $10^{4.7}$ EID₅₀ of strain 90-11. Proteinaceous substance is deposited in the empty cortex with remnants of necrotic lymphocytes. H & E. $\times 260$,

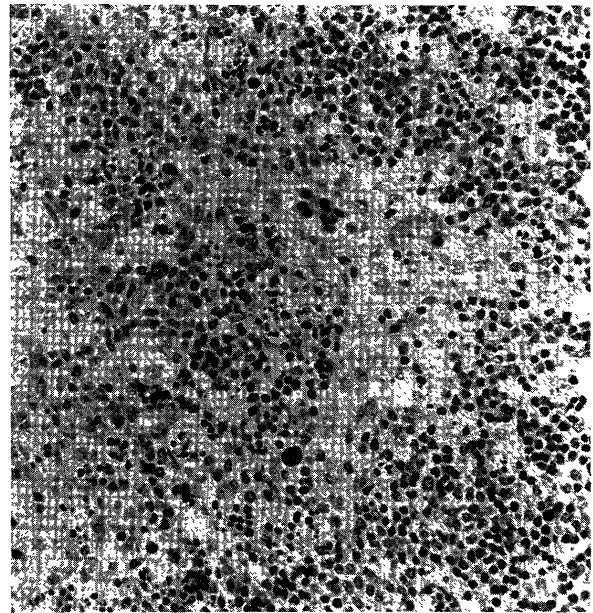


Fig. 2. The thymus of a chicken 2 days after inoculation with $10^{4.7}$ EID₅₀ of strain 90-11. Lymphocyte necrosis is seen in the cortex and medulla. H & E. $\times 480$.

heterophils and macrophages phagocytizing cell debris. The spleen was devoid of lymphocytes at day 3 PI, and infiltrated by heterophils and macrophages. The air capillary walls in the lungs were thickened by infiltration of swollen macrophages ingesting hemosiderin, causing the reduction in blood- and air-capillary spaces. In the liver, there were single cell necrosis and fatty change of hepatocytes, an increase of heterophils in sinusoids and Kupffer cell activation at day 2 PI. In the kidney, degeneration of scattered tubular epithelial cells with heterophil infiltration was observed in about 50% of infected chickens. In group D chickens, small necrotic foci in the bursal follicular cortex appeared at day 2 PI, and follicular lymphocytes completely disappeared at day 3 PI. At day 4 PI, immature lymphoid cells began to appear in the cortex, indicating follicular regeneration (Fig. 3). In the bone marrow and spleen, there were a slight decrease in the number of hematopoietic cells and slight heterophil infiltration and macrophage activation, respectively. The thymus, liver and lung had slight lesions ranging in score from 0.3 to 1.0.

The mean score of IBDV antigen demonstrable by SABC in the BF, thymus, spleen and bone marrow of chickens inoculated with strain 90-11 or I are shown in Table 2. A large amount of granular antigens was found in necrotic foci and macrophages in the BF (Fig. 4). The antigen decreased in amount with progression of necrosis. The antigen in the thymus was located in necrotic foci (Fig. 5). The antigen score in the thymus of group C chickens peaked one day earlier than that in group D chickens. A small amount of antigens was detected in the cytoplasm of promyelocytes and macrophages in the bone marrow and macrophages in the splenic red pulp. No

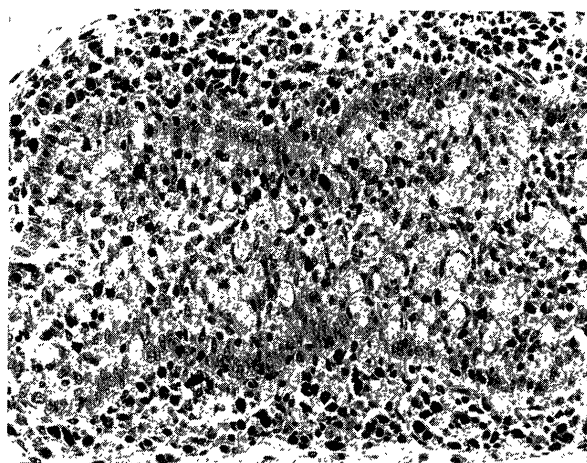


Fig. 3. A bursal follicle of a chicken 4 days after inoculation with $10^{6.1}$ EID₅₀ of strain I. Immature lymphoid cells infiltrate into the cortex, showing follicular regeneration. H & E. $\times 260$.

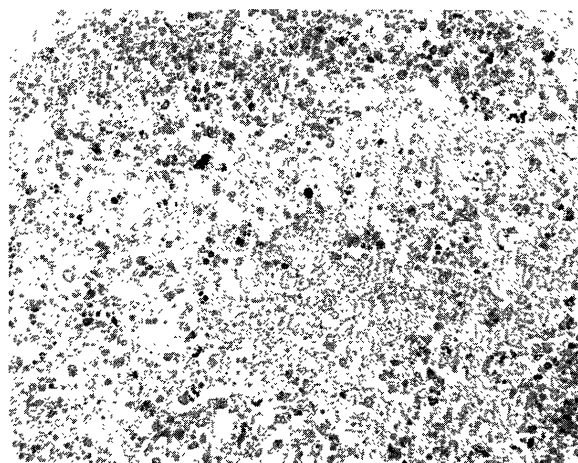


Fig. 4. Immunohistochemical staining of the bursa from a chicken 2 days after inoculation with $10^{4.7}$ EID₅₀ of strain 90-11. A large amount of viral antigens is seen in the follicle. Counterstained with hematoxylin. $\times 260$.

antigen was detected in the liver, lung, and kidney of chickens inoculated with strain 90-11 or I. Table 2 also shows the geometric mean titers of antigen in the BF demonstrated by the AGP and LA tests. The antigen was detected by both tests from day 3 PI onwards in group B chickens, whereas in group C chickens the antigen was detected from day 2 PI on. In chickens inoculated with strain I, almost the same amount of antigen as that in chickens inoculated with strain 90-11 was detected in the BF, thymus, spleen and bone marrow.

Experiment 2: SO chickens inoculated with $10^{4.7}$ EID₅₀ of strain 90-11 had clinical signs, gross lesions, and blood biochemical findings similar to those in groups B and C chickens of experiment 1. As shown in Table 3, the histologic lesion scores as well as scores and distribution of

IBDV antigen demonstrable by SABC in SO chickens were also similar to those of group B or C chickens. In contrast, BX chickens inoculated in the identical manner did not show any clinical signs nor any gross lesions. In these chickens, mild lymphocyte necrosis in the thymic cortex (Fig. 6), and a decrease in the number of lymphocytes in the spleen and a slight heterophil increase in hepatic sinusoid and activation of the mononuclear phagocyte system in the liver and lung were observed histologically from day 2 PI on. A slight myeloid decrease in the bone marrow and mild inflammatory reaction in the lung were also observed from day 3 PI onwards. A small amount of IBDV antigen was detected by SABC from day 1 PI onwards in the thymus (Fig. 7) and spleen and from day 2 PI in the bone marrow. In titration of IBDV antigen

Table 2. Quantity of infectious bursal disease virus antigen in the bursa of Fabricius (BF), thymus, spleen, and bone marrow of chickens inoculated with the strain 90-11 or I as determined by the aga-gel precipitation (AGP) and latex microsphere agglutination (LA) tests and by immunohistological staining (SABC) (Experiment 1)

Methods and organs	Quantity of antigen in chickens inoculated with strain (dose given) at postinoculation day:											
	Group B (90-11 $10^{2.7}$)				Group C (90-11 $10^{4.7}$)				Group D (I $10^{6.1}$)			
	1	2	3	4	1	2	3	4	1	2	3	4
AGP												
BF	0	0	13 ^{a)}	2.8	0	6.3	5.3	4.0	0	4.0	6.3	3.2
LA												
BF	0	0	134	80	0	101	106	56	0	63	80	47
SABC												
BF	0	0.7 ^{b)}	2.3	0.4	0	3.7	1.4	1.0	0	3.0	2.3	1.3
Thymus	0	0.7	1.5	0.4	0	1.7	1.0	1.0	0	0.7	1.3	0.5
Spleen	0	0	0.8	0.3	0	0.3	0.6	0.3	0	0	0.3	0
Bone marrow	0	0.3	1.0	1.0	0	1.0	0.8	0.3	0	0.3	0.6	0

a) Geometric mean titer of positive birds.

b) Mean score of three to five birds.

See Materials and Methods for the scoring system of SABC.

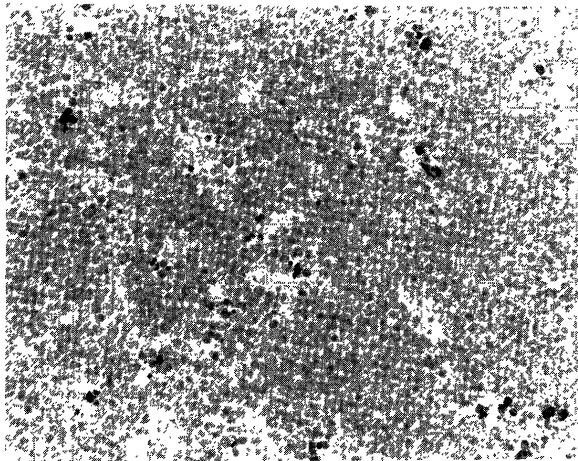


Fig. 5. Immunohistochemical staining of the thymus of a chicken 2 days after inoculation with $10^{4.7}$ EID₅₀ of strain 90-11. Viral antigens are present in necrotic foci. Counterstained with hematoxylin. $\times 480$.

Table 3. Histologic lesion scores and distribution of IBDV antigen detectable by immunohistochemical staining (SABC) in bursectomized (BX) and sham-operated (SO) chickens receiving $10^{4.7}$ EID₅₀ of the strain 90-11 (Experiment 2)

Organs and lesions	Scores in BX and SO chickens at postinoculation days:							
	BX				SO			
	1	2	3	4	1	2	3	4
BF ^{a)}								
Lymphoid necrosis	NA ^{b)}	NA	NA	NA	0	2.3 ^{c)}	4.0	4.0
Inflammatory reaction	NA	NA	NA	NA	0	2.3	2.3	2.3
Thymus								
Lymphoid necrosis	0	0.7	1.0	1.0	0	1.0	3.7	3.7
Spleen								
Lymphoid necrosis	0	0.7	1.7	2.0	0	2.0	3.2	3.0
Inflammatory reaction	0	0.3	0.3	1.0	0	2.3	3.7	3.0
Bone marrow								
Myeloid depletion	0	0	1.0	0.7	0	2.0	3.3	3.0
Inflammatory reaction	0	0	1.0	0.7	0	2.3	3.0	3.0
Liver								
Fatty change	0	0	0	0	0	2.3	2.0	2.0
Inflammatory reaction	0	1.0	1.7	1.3	0	1.7	2.3	1.7
Lung								
Inflammatory reaction	0	0	0.7	0.7	0	1.0	2.3	3.7
SABC								
BF	NA	NA	NA	NA	1.0	2.6	2.0	1.6
Thymus	1.0	1.0	1.0	1.0	1.0	1.3	2.3	1.3
Spleen	0.3	0.6	0.6	0.3	0.3	1.0	1.0	1.0
Bone marrow	0	0.3	0.3	0	0	0.3	1.0	1.0

a) The bursa of Fabricius.

b) Not applicable.

c) Each score represents the mean of 3 chickens. See Materials and Methods for the scoring system.

in plasma samples by the AC-ELISA, 2 of 3 BX chickens at day 2 PI reacted positively at dilutions of 1:4 and 1:1 and one bird was negative. The titers of 3 SO chickens were 1:4, 1:1 and 1:1 at day 2 PI and 1:64, 1:16 and 1:4 at day 3 PI, respectively. No IBDV antigen was detected in

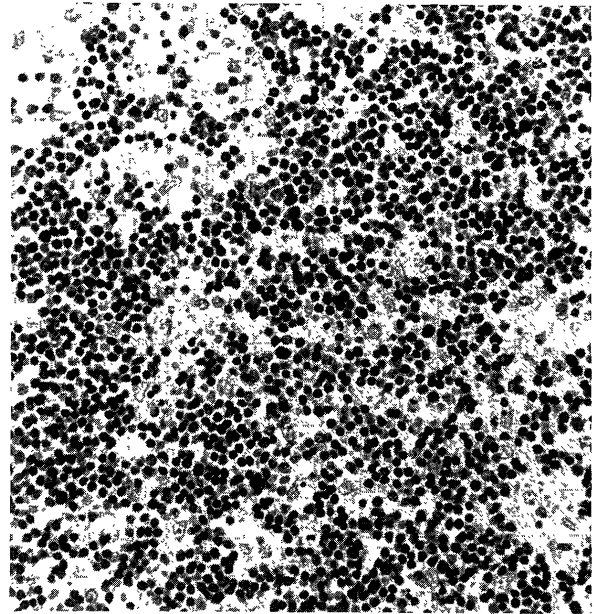


Fig. 6. The thymus of a bursectomized chicken 2 days after inoculation with $10^{4.7}$ EID₅₀ of strain 90-11. Mild lymphocyte necrosis in the cortex is shown. H & E. $\times 480$.

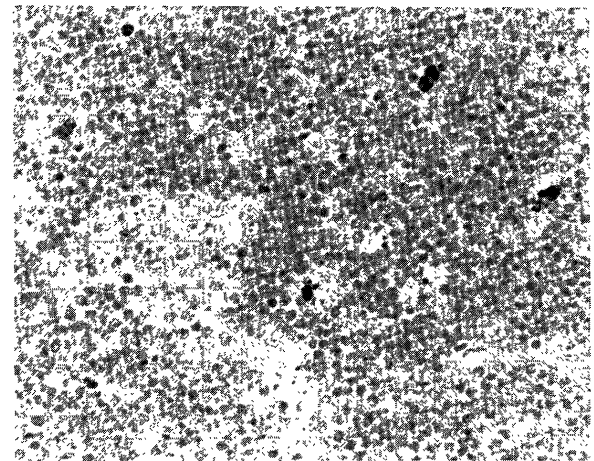


Fig. 7. Immunohistochemical staining of the thymus from a bursectomized chicken 2 days after inoculation with $10^{4.7}$ EID₅₀ of strain 90-11. Viral antigens are scattered in necrotic foci. Counterstained with hematoxylin. $\times 480$.

BX chickens at days 1, 3 and 4 PI and in SO chickens at days 1 and 4 PI.

DISCUSSION

In the experiment 1, the morbidity rate in 6-week-old chickens, which were inoculated orally with the highly pathogenic strain 90-11 of IBDV at the doses ranging from $10^{0.7}$ to $10^{4.7}$ EID₅₀, appeared to be roughly dose-dependent. Of 42 chickens inoculated, however, only one group C chicken died during the 4-day observation period. This is because all other chickens were sacrificed at a moribund stage to make sequential pathologic observa-

tions. In a previous experimental study using chickens of the same age and the same strain of IBDV, the mortality rate of 41% has been reported [11]. Histologic lesion scores in the BF, thymus, spleen, bone marrow, liver and lung were almost the same extent between groups B and C chickens, coinciding with the scores and distribution of viral antigen demonstrable by SABC. There were no significant differences in antigen titers determined by AGP and LA and in histologic lesion scores and SABC scores in the BF between chickens inoculated with strains 90-11 and I. The histologic lesion scores in the organs other than the BF, however, were far less in chickens inoculated with strain I than in birds inoculated with strain 90-11. The liver, bone marrow and lung of chickens inoculated with strain 90-11 had a variety of pathologic lesions, seemingly leading to dysfunction of these organs. In particular, systemic proliferation of activated macrophages was conspicuous. The pathogenic IBDV has been shown to induce interferon in various organs of chickens, while attenuated IBDV induced interferon only in the BF [3], and interferon proved to have macrophage-activating capacity [2]. These observations suggest that a generalized interferon response may have occurred in these birds. In contrast, no comparable lesions in the aforementioned organs were observed in any of chickens infected with strain I. These findings indicate that the highly virulent IBDV affects not merely the BF, which is the target organ of IBDV, but organs other than the BF.

The blood biochemical analysis revealed the elevation of plasma GOT, GPT and T-Bil levels in groups B and C chickens at day 3 or 4 PI. These changes may have resulted from liver damage. Similar results have been reported by Ley *et al.* [6] in 5-week-old chickens inoculated with IBDV. In infected chickens in the groups B and C, hematocrit values appeared to fluctuate according to the degree of dehydration.

Müller *et al* [9] have reported that first IBDV replication occurred in gut-associated macrophages and lymphoid cells 4 hr after peroral inoculation, causing the primary and transient viremia. Subsequently, the BF became infected and massive virus replication occurred in this target organ earlier than 16 hr after inoculation leading to the secondary and pronounced viremia and causing lesions in the other organs. In our studies, the low antigen titer was demonstrated by AC-ELISA in plasma samples from BX chickens at day 2 PI. This positive reaction seems to be caused by secondary virus replication in the BF and other lymphoid organs. Because 2 AC-ELISA positive birds of 3 BX chickens at day 2 PI had a small number of BF follicles recognizable by light microscopy in bursal remnants, and showed positive reactions in these follicles and other lymphoid organs by SABC (data not shown). We presumed that the high antigen titer in plasma samples from SO chickens at day 3 PI may represent IBDV replicated in many normal BF follicles. The difference in antigen titer between plasma samples from BX and SO chickens at day 3 PI may have been related to the number of target cells. The mean score of antigen detectable by

SABC in the thymus of chickens inoculated with $10^{4.7}$ EID₅₀ of strain 90-11 peaked earlier than in chickens inoculated with strain I, and thymic lesions were more severe in the former chickens. This suggests that strain 90-11 may have some more different types of target cells in the thymus than does strain I.

Infection of BX chickens with strain 90-11 was evidenced by the histologic lesion scores in the thymus, spleen, bone marrow, liver, and lung as well as the presence of viral antigen demonstrable by SABC in the thymus, spleen, and bone marrow. That was also supported by the results of AC-ELISA. Nevertheless, none of infected, BX chickens developed any clinical signs, suggestive of IBDV infection. The effects of surgical or chemical bursectomy on IBDV infection and development of clinical IBD are still debatable. It has been reported that IBDV infection with clinical IBD was induced in chemically [8] and embryonally BX chickens [15]. On the contrary, it has been mentioned that chemically [12] and surgically BX chickens [5] did not develop clinical IBD following IBDV infection. Okoye and Uzoukwu [13], in a study on the pathogenesis of IBD in embryonally BX chickens, concluded that the BF is not essential for the establishment of IBDV infection but is required for clinical infection. Our results reported here seem to support their hypothesis. In BX chickens, it is likely that IBDV replication was restricted due to the absence of a sufficient number of susceptible cells, and that vital organs such as the liver, lung and bone marrow escaped from serious damage because of the low virus titer. As a result of these conditions, clinical IBD may not have developed in these birds.

Judging from the appearance and distribution of histologic lesions and viral antigen, primary changes caused by the highly pathogenic strain 90-11 seemed to develop in the BF, thymus and spleen, whereas lesions in the lung and liver appeared to occur secondarily. The amount of viral antigen in the bone marrow was rather small for serious damage to the myeloid tissue, and no antigen was detected in the kidney despite the presence of noticeable lesions. Thus pathogenesis of these lesions remains to be solved.

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