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REVIEW

Pathological Changes and Pathogenic Mechanisms of Infectious Bursal Disease (IBD) in Chickens Infected with IBD Viruses of Different Pathogenicities

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Abstract

Infectious bursal disease (IBD), a highly contagious immunosuppressive disease in young chickens, causes significant losses to the poultry industry worldwide. The pathological changes in IBD are reviewed in this study. Oral inoculation with $10^{5.5}$ mean egg infective dose of a very virulent Ehime/91 IBD virus (IBDV) strain caused 40%-60% mortality in 3- to 4-week-old specific-pathogen-free chickens within 5 days postinoculation. In the acute phase, the virus produced severe necrotic and inflammatory lesions in the bursa of Fabricius (BF) and other lymphatic organs, bone marrow, and vital organs including the lungs. Under similar conditions, the classical virulent J1 strain caused no mortality and produced moderate to severe lesions in the BF and mild lesions in the thymus without damaging other organs. The antigenic variant-E strain caused no mortality and induced rapid BF atrophy without severe inflammatory lesions. IBD pathogenesis is determined by different pathogenicities among IBDV isolates and variable host factors. The pathogenicity of IBDV correlates with the replication efficiency of the virus in immature B cells and cells of the monocyte–macrophage lineage and the degree of systemic acute inflammatory reaction. Moreover, host factors including age, breed, and immune status are known to alter the clinical course of IBDV infection.

Discipline: Animal Science
Additional key words: clinical signs, gross lesions, histopathology

Introduction

Infectious bursal disease (IBD) is an acute viral infection of young chickens caused by the IBD virus (IBDV), classified in the family *Birnaviridae*, genus *Avibirnavirus* (Eterradossi & Saif 2020). The primary target cells for the IBDV infection are actively dividing surface IgM-bearing B cells in the bursa of Fabricius (BF) (Hirai & Calnek 1979, Hirai et al. 1981, Müller 1986). The cells of the monocyte–macrophage lineage can be persistently and productively infected and play a crucial role in the dissemination of the virus (Burkhardt & Müller 1987, Inoue et al. 1992) and the onset of the disease (Kim et al. 1998, Rasoli et al. 2015, Sharma & Lee 1983). Severe acute disease, usually in 3- to 6-week-old birds, is associated with high mortality (Ingrao 2013, van den Berg 2000). In addition, the immunosuppressive effects of the disease lower the resistance of birds to other infections and reduce the responsiveness to commonly used vaccines (Eterradossi & Saif 2020). As IBD outbreaks cause enormous damage to the poultry industry, it is designated as a notifiable disease under the Act on Domestic Animal Infectious Diseases Control in Japan (Ministry of Justice 2004). IBD is also included in the Office International des Epizooties (OIE)-listed diseases (OIE 2020) because the disease is an important hazard in international trade. The isolates of pathogenic serotype 1 IBDV are categorized as classical virulent strains, very virulent strains, or antigenic variant strains on the basis of their antigenicity and pathogenicity (Dey et al. 2019, Eterradossi & Saif 2020, van den Berg 2000, van den Berg et al. 2004). Reviews have been published

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on various aspects of IBD, including the acute form of IBD caused by very virulent IBDV (vvIBDV) (van den Berg 2000), host–pathogen interactions (Ingrao et al. 2013, Li & Zheng 2020, Qin & Zheng 2017), pathogenesis and immunosuppression (Sharma et al. 2000), the current status of vaccines against IBD (Müller et al. 2012), and management strategies (Dey et al. 2019). However, the molecular basis for the pathogenicity of the virus has not been fully determined (Qi et al. 2013, Eterradossi & Saif 2020), and the pathogenesis of IBD remains to be elucidated. Pathological studies are required to investigate the relationship between pathogen localization and histological changes and to clarify the pathogenesis of this infectious disease. This review focuses on the pathological changes caused by the Ehime/91 (E/91) strain of vvIBDV (Tsukamoto et al. 1992) and describes the differences in pathological changes caused by the classical virulent J1 strain (Tanimura et al. 1994, 1995) and the antigenic variant-E (VE) strain (Tanimura & Sharma 1998). These pathological analyses are supplemented with bibliographic considerations.

Clinical signs and gross lesions

In 3-week-old specific-pathogen-free (SPF) chickens orally inoculated with the E/91 strain ($10^{5.5}$ mean egg infective dose [EID_{50}]), we observed clinical signs such as depression, ruffled feathers, trembling, and diarrhea from 2 to 5 days postinoculation (dpi), which led to 40% mortality (Tanimura et al. 1994, 1995), whereas in 4-week-old SPF chickens, 60% mortality was recorded (Tsukamoto et al. 1992) (Table 1). The BF showed edema, necrosis, and hemorrhage from 2 to 4 dpi (Fig. 1A), followed by becoming severely atrophic from 7 dpi (Fig. 1B). The following lesions were also observed during the acute phase: atrophy of the thymus (3-10 dpi, Fig. 1C), yellowish discoloration of the femoral bone marrow (3-4 dpi, Fig. 1D), hemorrhage of the thigh and pectoral muscles (2-5 dpi), hemorrhage on the mucosal surface of the proventriculus (2-5 dpi), hyperemia of the palpebral conjunctiva (2-5 dpi), pulmonary congestion (3-5 dpi), yellowish discoloration of the liver (2-5 dpi) and kidneys (3-5 dpi), and urate accumulation in the ureters (3-5 dpi). Surviving chickens quickly recovered after 5 dpi.

Histopathology

1. Bursa of Fabricius

In the BF of chickens inoculated with the E/91 strain (Tanimura et al. 1994, 1995), viral antigens have been detected in the medullary lymphocytes using immunohistochemistry as early as 6h - 12h postinoculation. Before the infiltration of the heterophils and macrophages, $CD3^+$ T cells infiltrate around the capillary vessels in the bursal follicles and accumulate in the follicles (Tanimura & Sharma 1997). From 1 to 2 dpi, viral antigens spread to numerous B cells both in the cortex and the medulla of bursal follicles, where heterophils begin to accumulate both within and around the capillary vessels. Transmission electron microscopy

Table 1. Mortality and gross pathological changes in chickens inoculated with Ehime/91, J1, or variant-E strains of the infectious bursal disease virus (IBDV)

	Ehime/91 strain (very virulent IBDV)	J1 strain (classical virulent IBDV)	Variant-E strain (antigenic variant IBDV)
Mortality (3- to 4-week-old SPF[a] chickens)	40% - 60%	0%	0%
Bursa of Fabricius: Acute inflammation	+++[c] (2 to 4 dpi[d])	++ (2 to 4 dpi)	–
Necrosis, Hemorrhage	+++ (2 to 4 dpi)	++ (2 to 4 dpi)	+ (2 dpi)
Atrophy	+++ (from 5 dpi onward)	+++ (from 5 dpi onward)	+++ (from 4 dpi onward)
Thymus: Cotrical atrophy	+++ (3 to 10 dpi)	+ (4 dpi)	–
Bone marrow: Yellowish discoloration	+++ (3 to 4 dpi)	–	–
Proventriculus, Muscles[b]: Hemorrhage	+++ (2 to 5 dpi)	–	–
Lung: Congestion	+++ (3 to 5 dpi)	–	–
Liver: Yellowish discoloraton	+++ (2 to 5 dpi)	–	–
Kidney: Yellowish discoloration	+++ (3 to 5 dpi)	–	–

[a] specific-pathogen-free
[b] pectral and thigh muscles
[c] +++: severe, ++: moderate, +: mild, −: no lesions
[d] days postinoculation
Modified with permission from Tanimura (2016) published in *J. Jpn. Soc. Poult. Dis.*

findings have revealed that viral particles are present in the cytoplasm of the lymphocytes and reticular cells. From 2 to 3 dpi, viral antigens are detected in the macrophages infiltrating both within and outside the bursal follicles. At this stage, severe lesions, including necrosis and depletion of lymphocytes, edema, and infiltration of heterophils and macrophages, are evident. Inflammatory edema and cell infiltration extend from bursal follicles to the muscle layer and subserosal tissues. Monocytosis and hyperemia are observed in capillary vessels and small veins, some of which are followed by hyaline thrombosis and hemorrhage. Extensive hemorrhage develops throughout the bursal folds in some chickens. At 4 dpi, the number of viral antigen-positive macrophages gradually decreases, but viral antigens are detected in macrophages at least until 13 dpi. As the acute inflammatory reaction declines, the atrophy of bursal follicles ensues, followed by the hypertrophy and hyperplasia of epithelial cells and interstitial fibrosis.

The very virulent, classical virulent, and antigenic variant strains of IBDV cause significantly different pathological changes in the BF and other organs (Table 1). Although the pathogenetic mechanisms inducing these different pathological changes remain unknown, T cells and macrophages infiltrating the BF may play a crucial role (Ingrao et al. 2013). In acute IBD, T cells infiltrating the BF may modulate IBDV pathogenesis in different ways. (1) $CD4^+$ T cells promote protective antibody production by B cells, whereas $CD8^+$ T cells exert cytotoxicity against virus-infected cells. The humoral and cellular immunity against IBDV limits viral replication in the BF and promotes recovery from infection. In addition, (2) intrabursal T cells produce cytokines, such as IFN-γ, and activate macrophages.

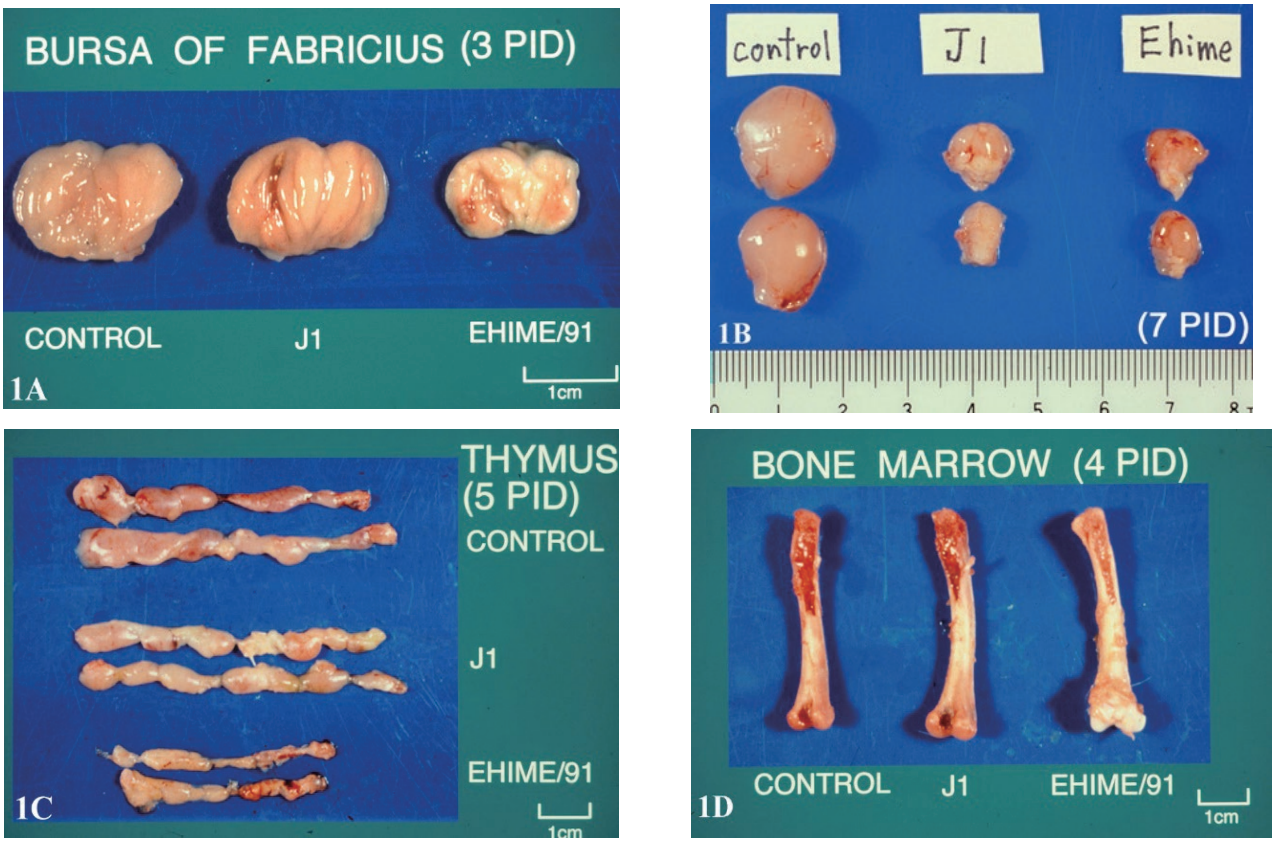

Fig. 1. Bursa of Fabricius (BF) (1A, 1B), thymus (1C), and femoral bone marrow (1D) of normal control, J1 strain-inoculated, or Ehime/91 strain-inoculated chickens

PID: postinoculation days

1A: Necrosis (yellowish-white discoloration), hemorrhage, and edema in the BF of J1 strain- and Ehime/91 (E/91) strain-inoculated chickens. Necrosis and edema were more severe in the BF of E/91 strain-inoculated chickens compared with in those with the J1 strain. 1B: Both the BFs of the J1 strain- and E/91 strain-inoculated chickens were severely atrophic. 1C: The thymus of the E/91 strain-inoculated chickens was severely atrophic. J1 strain inoculation resulted in no significant changes in the thymus. 1D: Bone marrow of E/91 strain-inoculated chickens showed yellowish discoloration. J1 strain inoculation caused no significant change in the bone marrow. Figures 1A and 1B reproduced with permission from Tanimura (2016) published in *J. Jpn. Soc. Poult. Dis.*

Activated macrophages not only inhibit viral replication but promote tissue damage and delay tissue recovery (Kim et al. 2000, Rauf et al. 2012, Rauw et al. 2007, Rautenschlein et al. 2002a, Rautenschlein et al. 2002b). In the bursal tissues of 3-week-old chickens, the very virulent UK661 strain induced IFN-γ mRNA expression to a greater degree than did the classical virulent F52/70 strain, indicating that the UK661 strain exacerbates inflammatory reaction via IFN-γ-activated macrophages (Eldaghayes et al. 2006).

The BF of chickens inoculated with the antigenic VE strain showed a slight whitish-yellow discoloration at 2 dpi, but no edema or hemorrhage was observed. The bursal weight of VE-IBDV-inoculated chickens rapidly decreased from 2 dpi, without appreciable bursal swelling. Histologically, bursal follicles show severe B cell depletion, moderate heterophil infiltration, and slight inflammatory edema (Tanimura & Sharma 1998). In the BF of 3-week-old chickens inoculated with a variant Indiana strain (Rauf et al. 2011), the gene expression of inflammatory chemical mediators (IL-6, IL-8, MIP-α, and iNOS) was shown to be lower than that in the classical virulent standard challenge (STC) strain-inoculated chickens. The Indiana strain downregulated the expression of TLR3 in the BF, whereas the STC strain upregulated its expression. These significant differences in the gene expression of inflammatory chemical mediators and TLR3 may explain the milder inflammatory response in the BF induced by the antigenic variant strain.

2. Spleen

In the spleen of chickens inoculated with the E/91 strain (Tanimura et al. 1994, 1995), viral antigens have been detected in the B cells in the lymph follicles, macrophages, and reticular cells in the ellipsoid and red pulp from 1 to 5 dpi. Lymphocytes in the lymph follicles and periarterial lymphoid sheaths become necrotic and depleted. Heterophils and macrophages infiltrate the ellipsoid and red pulp. Some macrophages engulf erythrocytes and accumulate hemosiderin. Hyaline materials are deposited around ellipsoids.

As with the bursal macrophages, splenic macrophages might play an important role in regulating inflammatory reactions during IBDV infection. In the acute phase of IBD caused by the classical virulent IM strain, splenic macrophages have been reported to exhibit enhanced gene expression of type I IFN, IL-6, and IL-8 (Kim et al. 1998), and the bursal macrophages were observed to exhibit enhanced gene expression of IL-1β, IL-6, IL-18, and iNOS (Khatri et al. 2005). These inflammatory chemical mediators produced by macrophages may lead to an acute inflammatory response and cause damage to the spleen and BF.

3. Thymus

In the thymus of E/91 strain-inoculated chickens (Tanimura et al. 1994, 1995), viral antigens have been detected in the lymphoid cells and macrophages from 1 to 7 dpi. In the thymic cortex, there is an increased number of tingible body macrophages engulfing the fragments of pyknotic lymphocytes, where no viral antigens are detected. The thymic cortex becomes transiently atrophic because of cortical lymphocyte depletion from 3 to 10 dpi.

The pathogenesis of thymic atrophy due to IBDV infection remains unresolved. *In vitro* experiments have shown that the virulent strain 73688 of IBDV replicates in B cells transformed by the avian leukosis virus and in the IgM-bearing B cells derived from the BF, but not in the T cells transformed by Marek's disease virus and in the thymic lymphocytes deprived of B cells (Hirai & Calnek 1979). In contrast, IBDV serotype 1 (strain L) is adsorbed to Marek's disease-derived lymphoid tumor cells (MSB-1 cells), where it can multiply (Lam 1988). In *in vivo* experiments, viral antigens were not detected in the bursal and splenic T cells in chickens inoculated with the classical virulent F52/70 strain (Vervelde & Davison 1997). In contrast, viral antigens were found to be present on the surface of cortical thymocytes in chickens inoculated with the very virulent UK661 strain, suggesting that the strain can adhere to immature T cells (Williams & Davison 2005). However, none of the studies have demonstrated that IBDV replicates within T cells derived from a normal chicken thymus. Transmission electron microscopy findings have revealed that IBDV particles are present within the thymic epithelial reticular cells of chickens inoculated with the classical virulent J1 strain (Tanimura et al. 1995), the B cells and epithelial reticular cells in the thymus of chickens inoculated with the very virulent HPS-2 strain (Inoue et al. 1994), and the necrotic foci in the thymus of chickens inoculated with the very virulent 90-11 strain (Nunoya 1992). Altogether, IBDV can replicate in B cells, epithelial reticular cells, and macrophages in the thymus. In addition, the adherence of viral particles or viral components released from the infected cells to cortical thymocytes may induce apoptosis or necrosis. Moreover, the inflammatory mediators produced both in the thymus and other organs may affect the differentiation of immature thymocytes and induce apoptosis or necrosis (Inoue et al. 1994, Tanimura & Sharma 1998, Williams & Davison 2005).

4. Bone marrow

In the femoral bone marrow of the E/91 strain-inoculated chickens (Tanimura et al. 1994, 1995), viral antigens have been detected in monocytes and macrophages from 1 to 5 dpi. A number of erythrocytic and myelocytic hematopoietic cells were found to transiently decrease from 2 to 7 dpi. There is a greater decrease in the myelocyte population compared with that in the erythrocyte population. An increased number of monocytes and macrophages are observed engulfing the cellular debris. In surviving chickens, these lesions are followed by the hyperplasia of hematopoietic cells.

The femoral bone marrow of chickens infected with the very virulent HPS-2 strain showed severe lysis and depletion of heterophil myelocytes without virus replication (Inoue et al. 1999). The myelocytes of HPS-2-infected chickens showed morphological characteristics of apoptosis. Although the pathogenesis of hematopoietic cell depletion in the bone marrow caused by vvIBDV infection remains unknown, it is possible that the inflammatory mediators released from the activated macrophages and other cells in the bone marrow and other organs may alter the cytokine milieu in the bone marrow, thereby affecting hematopoiesis (Khatri & Sharma 2009).

vvIBDV infection in SPF chickens also causes a deficiency of thrombocytes and coagulation factors, which may result in hemorrhages in the BF, thigh muscle, and mucosal junction between the proventriculus and gizzard (Zeryehun et al. 2012).

5. Other organs

In chickens inoculated with the E/91 strain, the following lesions have been observed during the acute phase.

(1) Harderian glands

From 3 to 5 dpi, plasma cells in the lamina propria are transiently depleted, and the epithelial cells of the secretory gland become hypertrophic. From 7 to 10 dpi, plasma cells repopulate the lamina propria.

(2) Lungs

From 3 to 5 dpi, the lungs show infiltration by heterophils and swollen macrophages around the air capillaries, hyperemia of capillary vessels, and stenosis and occlusion of air capillaries.

(3) Liver

From 3 to 5 dpi, the liver shows fatty degeneration of hepatocytes, swelling of Kupffer cells, and monocytosis in small veins.

(4) Kidney

During the acute phase, the kidneys of some chickens, especially moribund or dead chickens, exhibit the accumulation of small urate spheres and hyaline casts in the renal tubules, necrosis and sloughing of renal tubular epithelial cells, and infiltration of heterophils.

Host–pathogen interaction

The clinical course of IBD is determined by the pathogenicity of IBDV and the age, breed, and immune status of chickens (Eterradossi & Saif 2020). The pathogenicity of IBDV correlates with the replication efficiency of IBDV (Tsukamoto et al. 1995b) (Fig. 2) and the degree of tissue damage (Tanimura et al. 1994, 1995; Tanimura & Sharma 1998) (Table 1) in chickens. Very virulent E/91 strain-infected chickens develop severe necrosis and inflammation in the BF and cecal tonsils due to the destruction of B cell. Concurrently, severe tissue damage is observed not only in the thymus, spleen, and bone marrow but in other vital organs, such as the lungs and liver, accompanied by viral antigen-positive monocytes and macrophages. These pathological changes caused by the E/91 strain were also observed in chickens inoculated with the DV86 strain of vvIBDV isolated in the Netherlands (Tanimura et al. 1994, 1995). The E/91 strain may have a high proliferation potential in the cells of the monocyte–macrophage lineage as well as IgM-bearing B cells. Activated macrophages may release excessive inflammatory chemical mediators, such

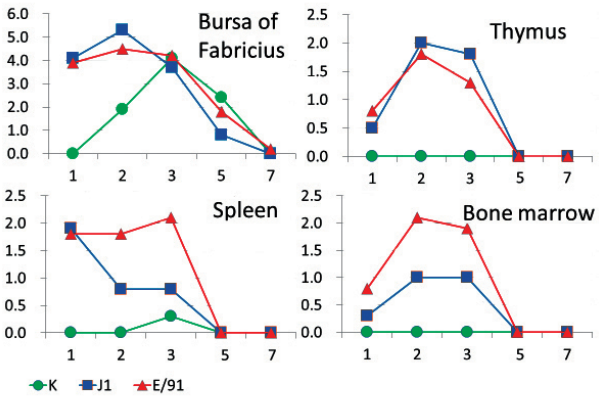

Fig. 2. Infectious bursal disease virus (IBDV) titers in four hematopoietic tissues in chickens inoculated with the K, J1, or Ehime/91 strains of IBDV
Vertical axis: $TCID_{50}$/0.01 mL of each tissue homogenate expressed as $\log_{10}$ N
Horizontal axis: days postinoculation
K: live vaccine K strain, J1: classical virulent J1 strain, E/91: very virulent Ehime/91 strain
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as pro-inflammatory cytokines, thereby exacerbating the systemic inflammatory response and tissue damage in vital organs, leading to the destruction of homeostasis and high mortality rates (Fig. 3).

IBDV has variable pathogenicity among different isolates. For example, "classical virulent" strains include the following: (1) J1 strain: 3-week-old SPF chickens orally inoculated with $10^{5.5}EID_{50}$ virus showed no mortality (Table 1); (2) STC strain: 3-week-old SPF chickens intraocularly inoculated with $10^{4}EID_{50}$ virus exhibited 8% mortality (Rauf et al. 2011); and (3) Faragher 52/70 strain: 6-week-old SPF chickens intranasally inoculated with $10^{5}EID_{50}$ virus exhibited 24% mortality (Le Nouën et al. 2006). The phylogenetic analysis of IBDV (Le Nouën et al. 2006) suggested a general coevolution of the two genome segments, indicating that genetic evolution from the initial vvIBDV clone through reassortment has led to the emergence of new and diversified vvIBDV-related viral strains.

Host factors, such as age, breed, and immune status, also alter the clinical course of IBDV infections. In SPF chickens inoculated with 100 LD_{50} of the 849 VB strain of vvIBDV at 1, 2, 3, and 4-6 weeks of age using an eyedropper showed 1%, 55%, 73%, and up to 100% mortality, respectively (van den Berg et al. 1991). SPF white leghorn egg-laying chickens are more susceptible to the deleterious effects of vvIBDV infection compared with SPF white Plymouth Rock broiler chickens, determined on the basis of clinical signs, mortality, and pathological changes (Sá e Silva et al. 2016). Chickens conferred with maternal anti-IBDV antibodies or active immunity by live vaccine inoculation are resistant to vvIBDV infection (Eterradossi & Saif 2020, Tsukamoto 1995a).

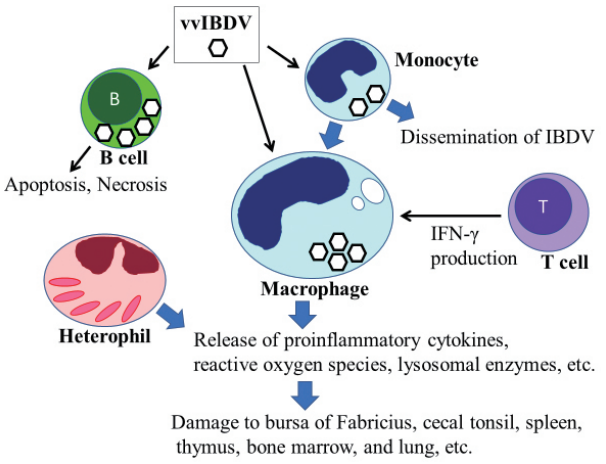

Fig. 3. An outline of the mechanism of tissue injury caused by very virulent infectious bursal disease virus (vvIBDV)

Conclusions

Clinical IBD can be diagnosed via a combination of characteristic signs as well as grossly visible and histological changes in the BF observed postmortem (OIE 2019, Eterradossi & Saif 2020). The diagnosis can be confirmed by detecting viral antigens or viral genomes in tissues. In the case of vvIBDV infection, in addition to the BF, pathological changes are observed in multiple organs, including the thymus, bone marrow, lungs, and liver. Therefore, the pathological examination of all body organs is essential to accurately comprehend IBD pathogenesis. This will help confirm the presence or absence of secondary infections and enable accurate differential diagnosis. As the antigenicity and virulence of IBDV have been altered by mutations in the viral genome, research on host–pathogen interactions should be continued to improve the diagnostic and control measures regarding IBD.

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