

Infectious bursal disease virus in poultry: current status and future prospects

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Abstract: Infectious bursal disease virus (IBDV) affects immature B lymphocytes of the bursa of Fabricius and may cause significant immunosuppression. It continues to be a leading cause of economic losses in the poultry industry. IBDV, having a segmented double-stranded RNA genome, is prone to genetic variation. Therefore, IBDV isolates with different genotypic and phenotypic diversity exist. Understanding these features of the virus and the mechanisms of protective immunity elicited thereof is necessary for developing vaccines with improved efficacy. In this review, we highlighted the pattern of virus evolution and new developments in prophylactic strategies, mainly the development of new generation vaccines, which will continue to be of interest for research as well as field application in the future.

Keywords: epidemiology, IBDV, immunity, poultry, vaccine

Introduction

In all poultry producing regions of the world, infectious bursal disease virus (IBDV) continues to be a major constraint for poultry farmers. The consequences of immunosuppression associated with IBDV are vaccination failure and susceptibility of chickens to opportunistic pathogens. It was also shown that IBDV-infected birds may become a good propagator for other viral pathogens. For example, low pathogenic duck adapted avian influenza virus becomes more virulent when serially passaged in IBDV-infected chickens.¹ Moreover, highly virulent IBDV can cause high mortality in unprotected flocks.

IBDV is a double-stranded RNA (dsRNA) virus,² which targets immature B lymphocytes of the bursa of Fabricius (BF), a primary lymphoid organ in avian species, and subsequently causes B-cell depletion in bursal follicles. Macrophages may be susceptible to IBDV (reviewed by Khatri and Sharma³) and recently IBDV-positive T-cell populations were detected in the BF.⁴ Efficient horizontal virus transmission between flocks through ingestion of feed and water contaminated with infectious feces is remarkable, once a rearing site has been contaminated. However, there are no indications of vertical transmission.

The virus may use one or more putative host cell receptors/structures such as N-glycosylated polypeptide(s),⁵ heat shock proteins (HSPs),⁶ $\alpha 4\beta 1$ integrin,⁷ or lipid raft endocytic pathways⁸ to enter into permissive cells. Mononuclear phagocytic cells and lymphoid cells of the gut mucosa may serve as targets for initial IBDV infection and replication following oral exposure. Infected macrophages transport the virus to the BF, where extensive replication takes place.⁹ Virus spread from the bursa to other lymphoid organs may occur depending on the virulence of the infecting IBDV strain.

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Since the first discovery of classical IBDV strains in the USA over 50 years ago,¹⁰ the virus has spread throughout the world and complex evolution of the virus has taken place. Our understanding of IBDV epidemiology has been much more comprehensive by today. All major poultry producing regions report the coexistence of two or more strains of variable pathogenicity.¹¹ A recent study addressing the global molecular epidemiology of IBDV from four continents revealed that 60%–76% of IBDV isolates are very virulent (vv) IBDV strains. However, other strains including variants are also gaining grounds and are the most prevailing in the USA.

Besides biosecurity, vaccination is the most important measure to control IBDV in the field. Classical and variant strains have been used for many years, but extensive usage of live vaccines in the field is thought to favor the emergence of new strains. The emerging viruses evade vaccine-induced immunity, and may in addition contribute their viral genome to reassortment and recombination events. In addition, some IBDV live vaccines are expected to maintain quasispecies nature, which in the face of selection pressure may favor the outgrowth of more virulent antigenic variants or mutants in the viral population.¹² Understanding the current epidemiology of IBDV and the implementation of new prophylactic measures are vital to control IBDV. This review article bridges the current understanding of IBDV from epidemiological and immunological perspectives, to possible control measures and new developments in prophylactic strategies against IBDV.

IBDV genome

IBDV has a bisegmented dsRNA genome.² Segment A contains two partially overlapping open reading frames (ORFs). The larger ORF encodes for a polyprotein (PP), which is autocatalytically cleaved into the two structural proteins, virus protein (VP)2 and VP3, and a serine (S) protease, VP4.¹³ The PP is considered as the main mediator of IBDV-induced immunosuppression and pathogenicity.¹⁴ VP2 contains the major antigenic sites responsible for induction of neutralizing antibodies (Abs).¹⁵ VP5, which is encoded by the small ORF, is involved in the dissemination of the virus from infected cells.¹⁶

The crystal structure of VP2 indicates that most amino acid (aa) changes are localized in the exposed projection domain that contains the hypervariable region (hVP2).¹⁷ hVP2 has two major hydrophilic domains namely major hydrophilic peak A (aa 212–224) and peak B (aa 314–325)¹⁸ that form loop P_{BC} (aa 219–224) and P_{HI} (aa 316–324),^{19,20}

respectively. The minor hydrophilic peak 1 (aa 248–254) and peak 2 (aa 279–290) of hVP2 form loop P_{DE} (aa 249–254) and P_{FG} (aa 279–284), respectively.¹⁹

Segment B-encoded polymerase (VP1) mediates viral RNA replication.²¹ The VP1 crystal structure shows three domains: the N-terminal (aa 1–167), central polymerase (aa 168–658), and C-terminal (aa 659–878) regions.²²

IBDV is constantly evolving in the field with varying virulence

Historically, IBDV evolution has three major events: the first description of a classical IBDV outbreak in 1957 and the occurrence of antigenic variants in 1980s, both in the USA as well as the detection of vvIBDV in late 1980s in Europe.^{10,23} While classical and variant strains dominated field outbreaks for nearly 5 decades in the USA, the first IBDV outbreak related to vvIBDV in the USA was reported in 2008.²⁴ IBDV-neutralizing Ab escape mutants that have been reported in most recurrent outbreaks in vaccinated flocks were the result of mutations at major hydrophilic peak A and B domains (loop P_{BC} and P_{HI}) of hVP2.^{25,26} Moreover, the emergence of reassortant field strains plays a significant role in current IBDV evolution.

In recent years, IBDV field strains from different continents showed aa exchanges at minor hydrophilic peak domains (loop P_{DE} and P_{FG}) of hVP2.^{27–29} Single or combined mutations at these regions affect the virulence of IBDV field isolates. Experimentally, the substitution of aa at positions 253 (Q253H), 279 (D279N), and 284 (A284T) in the VP2 of vvIBDV isolates resulted in loss of virulence.³⁰ However, a single aa mutation at position 253 (H253Q/N) or 249 (R249Q) in VP2 markedly increased the virulence of an attenuated IBDV strain.³¹ In addition, mutation at position 212 (D212N) is common in most recent vvIBDV isolates and may influence the structure of VP2 and consequently the antigenicity of the virus.³² Moreover, a S residue instead of glycine (G) at position 254 (loop P_{DE}) was reported from vvIBDV field isolates detected from chickens vaccinated with classical IBDV vaccines,³³ suggesting a role of this aa mutation in vaccination failure.²⁸ Chickens vaccinated with a live Delaware (Del-E) variant and challenged with a neutralizing Ab escape Del-E mutant that contains S at position 254 developed severe bursal lesions.²⁸

In the USA, one-third out of 300 IBDV field isolates failed to react with any of the known monoclonal Abs raised against VP2, which were used to identify IBDV strains for the last 2 decades and hence demonstrate enormous virus evolution.²⁷ IBDV field isolates bearing VP2-epitopes chimeric of at least

three strains are reported, which contribute to an enormous variation in antigenicity and virulence diversity.³⁴ Frequent mutations in VP2 indicate that certain codons are under constant purifying selection pressure and point mutations (antigenic drift) may favor emerging neutralizing Ab escape mutants.³⁵

IBDV ecological diversity and phylogenetic clustering

Based on geographic origin, phylogenetic analysis using either partial or complete VP2 nucleotide sequences clustered vvIBDV strains in one well-defined major monophyletic lineage.^{36,37} In contrast to these reports, recent vvIBDV isolates from major chicken producing regions of People's Republic of China showed rather divergent VP2 phylogenetic clusters. When compared with earlier Chinese vvIBDVs, the present Chinese vvIBDVs have unique mutations in both segments and are more virulent compared with the typical European vvIBDV, despite similarity in antigenic features.³⁸ In support of a direct correlation between geographic origin of IBDV and phylogenetic clusters, Jackwood and Stoute³⁹ described spatially restricted IBDVs in northeast Ohio that contain mutations unique to this geographic region in both VP2 and VP1 gene. On the contrary, a large number of sequences obtained from VP2 of IBDV field isolates from the USA were phylogenetically different from any known IBDV VP2 sequences.²⁷ The VP1 gene of most IBDV strains, however, shows multiple phylogenetic lineages providing evidence of VP1 contribution in IBDV epidemiology.^{37,40} All these reports highlight the continuing evolution of both segments of the virus and the circulation of genetically diverse IBDVs.

In addition, the existence of a worldwide-spread genetic lineage of IBDVs designated as distinct IBDVs has been described, which cause only immunosuppression.⁴¹ A discriminant analysis of principal components, a novel specific multivariate method that identifies clustering patterns of IBDVs, grouped distinct IBDVs as a distinct cluster of genetically related viruses separated from the other typical IBDV strains. Genetic distance estimation predicts that these viruses are one of the most genetically divergent IBDV and showed the highest between-group variance and genetic distance from vvIBDVs.

Recombination events in IBDV

Sequence diversity among field IBDVs may also be due to homologous recombination between VP2 genes. IBDV isolates were identified which gained aa sequences from classic IBDV within the P_{BC} and P_{HI} loops and sequences

from Del-E variant strains within the minor P_{DE} and P_{FG} loops.³⁴ Other isolates were described to have undergone intrasegment recombination in their segment B between two vvIBDV donors.⁴² If the exposed capsid residues (253H and 284T), which are involved in particle-particle interaction are affected by recombination events, the orientation of the capsid domains may be modified and vaccine failure may be observed.⁴³

Genetic reassortments play a significant role in IBDV epidemiology

Basically, reassortment contributes to the first emergence of vvIBDV in the late 1980s in Europe.^{23,44} Prediction of the most recent common ancestor of vvIBDV describes an approximate most recent common ancestor of vvVP2 around 1960 and of vvVP1 from an unidentified avian reservoir around 1980 indicating that these two proteins of vvIBDV have evolved at different time points.^{44,45} Recent genetic analysis showed that most reassortant IBDVs reported from around the world have segment A from vvIBDV that maintain key virulence marker aa in their VP2 (222A, 256I, 294I, and 299S,^{46,47} attenuated strains have 253H and 284T, and 253Q and 284A are often found in variant strains³³) and segment B from attenuated vaccine strains.^{45,48} Reassortants with segments from serotype 1 and serotype 2 IBDV or segment A from an attenuated and segment B from vvIBDVs have also been reported.⁴⁹ Different genotypes of IBDV are sometimes isolated from the same bursa implying coinfection being common in the field.⁵⁰

The frequent isolation of reassortant IBDV field isolates indicates that phylogenetic markers need to include VP1 in the molecular epidemiology of IBDV, especially if considering the contribution of VP1 to IBDV virulence.⁵¹⁻⁵³ A field reassortant IBDV comprising segment A of vvIBDV and segment B of an attenuated strain caused reduced mortality in specific pathogen free (SPF) chickens compared with a typical vvIBDV isolate.⁵⁴ When regions of VP1 of a vvIBDV strain were exchanged with VP1 counterparts of an attenuated IBDV, the resulting recombinant virus showed reduced virulence and bursal lesions in chickens.⁵⁵ Recent studies have identified putative virulence markers in VP1 of IBDV field isolates.⁵⁶ A TDN (threonine/aspartic acid/asparagine) motif was identified at aa residues 145, 146, and 147 of all vvIBDVs tested, which is absent in most non-vvIBDV isolates.⁵⁷ Replacement of the TDN motif of a vvIBDV strain with TEG (threonine/glutamic acid/glycine) or NEG (asparagine/glutamic acid/glycine) resulted in loss of virulence, and the change of NEG to TDN increased the virulence for an attenuated strain, indicating the contribution of these three

aa to the polymerase activity.⁵⁸ Yu et al⁵⁹ further evaluated the pathogenicity of a vvIBDV strain by exchanging a single aa at VP1 position 4 (V4I) which resulted in attenuation of the respective mutated virus.

Host immunity to IBDV: innate and adaptive immune responses

Role of pattern recognition receptors in the immune response to IBDV

The innate immune response is the first-line of defense against pathogens in vertebrate species. This response is mediated by pattern recognition receptors (PRRs), which detect pathogen-associated molecular patterns (PAMPs) for induction of effector molecules.⁶⁰ In chickens, ten different Toll-like receptors (TLRs), a well-characterized member of PRRs, have been identified and their natural or synthetic ligands representing PAMPs have been characterized.⁶¹ Chickens mucosally infected with IBDV showed upregulation of TLR transcripts including TLR3^{62,63} and TLR21⁶² and molecules associated with TLRs such as MD-1 and MD-2⁶⁴ in the BF during the acute phase of virus replication. Other studies have reported the differential regulation of TLR3 and TLR7 after mucosal IBDV infection.^{62,65} The results of these studies suggest that TLRs may be involved in IBDV recognition for initiation of innate immunity. The chicken melanoma differentiation-associated gene (MDA5) has also been suggested as intracellular PRRs for IBDV.⁶⁶ In the course of mucosal IBDV infection, Smith et al⁶² revealed increased expression of MDA5 in the BF during early stages of IBDV infection.

Expression of innate response genes during in vitro and in vivo IBDV infection

When administered per os, IBDV can be detected at 8–12 hours post infection in intestinal mononuclear phagocytic cells, which transport the virus to the BF for extensive replication in B-cells. IBDV infection activates intracellular signaling cascades such as the nuclear factor (NF)- κ B pathway.⁶⁷ Increased macrophage infiltration into the BF may cause higher expression of proinflammatory cytokines (interleukin [IL]-6, IL-1 β , and IL-18) and inducible nitric oxide synthase.^{63,68} Higher expression of interferon (IFN)- γ and chemokines (CXCLi2) was detected in the BF compared with spleen specimens.⁶⁹ Further IBDV infection studies in newly hatched chickens or late-stage chicken embryos (infected at embryonation day 18) resulted in higher expression of other components of innate immunity such as stress response proteins, complement components, and β -defensins in primary lymphoid organs.^{64,70}

Transcriptome analyses may help to identify other candidate host genes involved in the development of clinical disease, bursal lesions, and protection.⁶³

Rauf et al⁷¹ and Sharma and Lee⁷² did not find significant evidence of natural killer (NK)-cell involvement in the early immune response after IBDV infection. Smith et al⁶² demonstrated higher expression of NK lysin to approximately 15-fold at 4 days post infection (dpi) compared with virus-free controls suggesting cytotoxic T-cells and NK cells involvement. Still, the role of NK cells in IBDV pathogenesis or immunity is not fully understood.

Type I IFN responses during IBDV infection

IBDV infection leads to upregulation of antiviral response genes such as type I IFNs in the BF at 3 dpi and the expression of IFN-stimulated genes such as viperin, Mx, and chicken ZAP in BF and spleen tissues.^{63,73,74} This was confirmed in other studies,⁶⁴ where genes with regulatory functions (IFN regulatory factor 7) and janus kinases/signal transducer and activators of transcription (JAK/STAT), as well as other classes of IFN-stimulated genes including interferon induced protein with tetratricopeptide repeats 5, IFN-induced transmembrane protein (IFITM) 1, IFITM3, IFITM5, 2'-5'-oligoadenylate synthase, and dsRNA-dependent protein kinase were induced following IBDV infection.^{62,64} Recent studies indicated that suppression of the IFN response may promote IBDV replication, supporting the role of IFN in IBDV control and pathogenesis.⁷⁵ Understanding innate immune mechanisms will benefit the development of strategies to target the antiviral pathways in the chicken to control IBDV.

Adaptive immune responses and mechanisms to prevent IBDV infection

IBDV evades innate responses by interfering with the IFN pathway⁷⁶ or impairment of dendritic cell function.⁷⁷ For further control of infections, chicken rely on adaptive immunity. Virus neutralizing Abs against the conformation dependent neutralizing epitopes can be detected after few days of infection or vaccination and provide protection against antigenetically related viruses.^{15,78} The roles of T-cell subpopulations in IBDV protection were previously described (reviewed by Mahgoub et al⁴).

The status of IBDV vaccines has been recently reviewed by Müller et al.⁷⁹ Here, we have summarized commercial and alternative vaccine candidates and their mechanisms of protection against IBDV (Table 1). Conventional live attenuated IBDV vaccines categorized as mild, intermediate, or intermediate plus are suitable for mass vaccination and

Table 1 Examples of experimental candidate and commercial new generation IBDV vaccines

Vaccine types	Target antigen or gene	Expression systems or vectors	Parameters evaluated and references
Subunit	Hypervariable region of VP2	<i>Pichia pastoris</i>	70% protection against challenge ¹²²
	N-terminal VP2 (aa 18–139)	<i>Escherichia coli</i>	↑ ELISA titer, 100% protection against mortality ¹²³
	Mimotope	<i>E. coli</i>	↑ ELISA and VN Ab titer, 100% protection against mortality ¹²⁴
Chimeric virus-like particles	VP2	Plants	Seroconverted, 80% protection against mortality ^{125,126}
	Neutralizing epitope from the P _{BC} loop of VP2	Bamboo mosaic virus	↑ ELISA titer, mild-to-moderate bursal lesions after challenge ¹²⁷
Chimeric proteins	VP2 and N-terminus M2 extracellular domain of H9 AIV	Baculovirus vector	↑ ELISA titer and VN Ab titer, improved protection against challenge ¹²⁸
	Mimotope polypeptide	Human hepatitis B virus	↑ ELISA and VN Ab titer, 100% protection against mortality ¹²⁹
Viral vectored	VP2	Fowlpox virus	14% and 33% of the chickens protected against gross and histological lesions, respectively ¹³⁰
		Marek's disease virus	55% protection against bursal lesions, no sterile immunity ¹³¹
		Semliki forest virus	Induction of VN Ab ¹³²
		Vaccinia virus	Induction of VN Ab ¹³³
		Avian adenovirus	↑ VN Ab titer, mortality up to 20% after challenge ¹³⁴
		T4 bacteriophage	↑ ELISA titer, no clinical signs or death ¹³⁵
		Canarypox	Induction of VN Abs ¹³⁶
Bacterial delivery	VP2	<i>E. coli</i>	Seroconversion, 95% protection against mortality ¹⁰⁶
	PP	<i>Salmonella</i> Typhimurium	73% protection against mortality and seroconversion ¹⁰⁷
DNA vaccine	Immunodominant VP2 gene fragment (VP2 ₅₂₋₄₁₇)	<i>E. coli</i>	75% protection against bursal lesions, 90% survival, ↑ IgY and splenocyte proliferation ¹³⁷
	VP2 and HSP70 (fused and expressed in one plasmid), recombinant VP2	<i>Saccharomyces cerevisiae</i> (DNA prime-protein boost)	100% protection against mortality, ↑ ELISA Ab and cell proliferation, ↑ expression of IFN-γ and IL-12, IL-10 ⁹⁹
	PP and chicken IL-18 (cloned in one plasmid)	<i>E. coli</i>	93% protection, ↑ ELISA Ab and cell proliferation and ↑ induction of IFN-γ and IL-4 ⁹³
DNA/protein vaccine	VP2	VP2 DNA (<i>E. coli</i>) and rVP2	100% protection against vvIBDV-induced mortality, ↑ ELISA, and VN Ab, ↑ lymphocyte proliferation, ↑ induction of IL-4 and IFN-γ ¹³⁸

Note: Most of the vaccines evaluated in these studies were administered to chickens of different ages by parenteral vaccination (intramuscular and subcutaneous), with exception of the ones delivered by bacterial vectors or plants.

Abbreviations: aa, amino acid; Ab, antibody; AIV, avian influenza virus; ELISA, enzyme-linked immunosorbent assay; HSP, heat shock protein; IBDV, infectious bursal disease virus; IFN, interferon; IL, interleukin; PP, polyprotein; VN, virus neutralizing; VP, virus protein; vv, very virulent; ↑ upregulation or increased.

when applied in drinking water can induce robust immunity.⁸⁰ The potential for reversion to virulence,⁸¹ residual immunosuppressive effects,⁸² as well as their role as genetic sources for the generation of reassortant new viruses³² are major safety concerns. Killed vaccines for breeder vaccination to transfer maternal Abs to progeny⁸³ and infectious bursal disease (IBD)-immune complex vaccines developed for in ovo (administered at embryonation day 18)⁸⁴ as well as posthatch vaccination of broilers⁸⁵ have been used in the past. IBD-immune complex vaccines are prepared by combining certain quantity of IBDV-specific hyperimmune sera with live intermediate plus IBDV.⁸⁶

As the viral capsid protein, VP2 carries immunodominant epitopes responsible for the induction of a protective humoral immune response.¹⁵ The PP gene as a whole,

the mature VP2, or immunogenic/neutralizing domains of VP2 are targeted to produce new generation candidate vaccines. Viral-vectored vaccines such as the herpesvirus of turkeys-IBD vaccine were licensed for in ovo and posthatch vaccination of broilers and layers in various countries.^{87,88} These vectored vaccines induce strong systemic neutralizing and/or mucosal Abs. Limitations are possible interference with other herpesvirus of turkeys vaccines given at the same time, which may affect vaccine efficacy by reducing their replication in host tissues. IBDV DNA vaccines may elicit cell-mediated immunity including memory T-cell responses,^{89,90} contributing to the protective efficacy of the vaccine. IBDV-VP2 subunit vaccines produced in yeast and *Escherichia coli* expression systems have been licensed for commercial use.^{91,92}

The way forward: improving the quality of vaccine-induced immune responses by molecular adjuvants and vaccine delivery systems

New generation vaccine adjuvants

Due to partial protection offered by vaccination, especially by nonreplicating constructs (subunit and DNA vaccines), the development of new adjuvants and adjuvant formulations may help to improve the immunogenicity and protection provided by these vaccines. Vaccination of chickens with a DNA vaccine coexpressing VP2/4/3 and IL-18 led to protection against challenge compared with the DNA vaccine expressing only VP2/4/3.⁹³ Good indicators of strong cell-mediated immunity were higher T-cell counts and higher antigen-specific T-cell proliferation in the cytokine-adjuvant group. The immune potentiating effect of IL-2 was found to be significant when cloned together with the VP2 gene into a bicistronic eukaryotic expression vector.⁹⁴ Recombinant chicken cytokines such as IL-12 were used to improve vectored IBD vaccines.⁹⁵ But not in all cases tested recombinant cytokines improved the IBDV vaccine response significantly as shown with recombinant IFNs and IL-1 β in combination with an inactivated IBD vaccine.⁹⁶

The administration regime of adjuvants may modify their efficacy as shown by Negash et al,⁹⁷ who demonstrated that adjuvant application at staggered intervals improved vaccine efficacy compared with simultaneous application of vaccine and adjuvant. Other adjuvant candidates have also been experimentally tested, including porcine lactoferrin,⁹⁸ HSPs,⁹⁹ chicken beta-defensin-1,¹⁰⁰ or synthetic PAMP mimics such as CpG oligodeoxynucleotides^{97,101,102} in combination with different vaccine types. A DNA vaccine encoding the C-terminal domain of the HSP70 of *Mycobacterium tuberculosis* genetically fused with the full-length VP2 gene induced higher Ab levels compared with the VP2 DNA vaccine without the HSP. It induced a mixed T helper 1- and T helper 2-like response as well as provided complete protection in a “DNA prime-protein boost” approach.⁹⁹

Mucosal vaccine delivery approaches as a way to enhance immunity

Another approach to improve vaccine efficacy is to optimize the delivery of candidate vaccines by targeting specific immunological compartments, for example, antigen-presenting cells. Microparticle (MP) and nanoparticle delivery systems, particularly those made from biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) and chitosan have been

tested for mucosal delivery of poultry vaccines.⁹⁷ Antigen delivery by this system has several advantages including preventing vaccine degradation by mucosal enzymes and a slow controlled antigen release to avoid frequent boosting.^{103,104} Targeted delivery and increased antigen uptake by antigen-presenting cells are other benefits.¹⁰⁵ Mucosal immunization of chickens with PLGA MPs with an adsorbed IBDV DNA vaccine in conjunction with plasmids encoding chicken IL-2 or CpG oligodeoxynucleotide adsorbed onto different PLGA MPs induced a detectable T-cell response and protection against challenge.⁹⁷ In addition, bacterial vectors including *Salmonella* and *E. coli* were used for the delivery of IBDV DNA vaccines through mucosal surfaces.^{106,107} In this case, the recombinant plasmid pCI-VP2/4/3 was transformed by electroporation into an attenuated *Salmonella* Typhimurium. Oral immunization of chickens with transformed bacteria elicited Ab responses offering approximately 73% protection against virulent IBDV challenge.¹⁰⁶

Reverse genetics and vaccinology

The introduction of vaccines targeting reassortant subsets of circulating IBDVs may be necessary in future IBDV control strategies. Different reverse genetic systems for IBDV have been described, which can be used for better IBDV characterization and for IBDV vaccine development.^{108,109} A modified IBDV that contained an authentic 3' RNA sequence generated by *cis*-acting hepatitis delta virus ribozyme was less pathogenic to the BF compared with a cell line-adapted variant E IBDV strain, but induced higher Ab responses as early as day 7 post infection.¹¹⁰ The risk of reversion to virulence of the genetically modified viruses may exist.¹¹¹

Conclusion and future perspectives

Prevention of IBDV-related losses associated with immunosuppression and secondary infections will continue to be a specific focus in the field.¹¹² Moreover, the identification and characterization of new emerging IBDV strains remains a major reason for the development of new vaccination strategies. In this regard, new sequencing technologies and bioinformatics will be used in the future to understand IBDV epidemiology and possibly predict the distribution of certain strains in the field.

It is suggested that the progression of an infection within a host will determine the ability of a pathogen to transmit to new hosts and to maintain itself in the population,¹¹³ therefore, additional understanding of IBDV pathogenesis and its interactions with the innate immune system is still required to provide the basis for improved prophylactic strategies.¹¹⁴

New technologies including transcriptomics and metabolomics may help to elucidate these aspects further.

Vectored IBD vaccines as well as immune complex vaccines have been licensed in recent years to overcome some of the problems related to classical vaccines. DNA vaccines have not been introduced to the field mainly due to variable or insufficient protection, especially after their mucosal delivery; the method of choice for IBDV-field vaccination. Therefore, efforts will continue to improve alternative vaccination strategies by testing new delivery systems and/or adjuvants to stimulate innate and acquired immunity. In addition to traditional adjuvants, other innovative strategies improving vaccine-induced immunity are under development, including inhibitors of metabolic pathways, modulators of baseline inflammation levels, monoclonal Abs targeting checkpoint inhibitors, and compounds depleting regulatory cells (reviewed by De Gregorio¹¹⁵). Possibly single-cycle replication incompetent and nontransmissible vector vaccines will gain more importance to overcome the risk of reversion to virulence of classical attenuated vaccine strains.¹¹⁶

New alternative prophylactic strategies may be investigated further, including the use of recombinant Abs, recombinant single chain variable fragment Abs, or nanobodies specific for vvIBDV.^{117,118} MicroRNAs targeting specific viral proteins may also provide protection against IBDV challenge.¹¹⁹

Recently, the roles of mechanistic/mammalian target of rapamycin (mTOR), an intracellular regulator of innate and adaptive immunity, for controlling viral infections have been described. By regulating the autophagy machinery, mTOR leads in the generation of significant amounts of peptide epitopes by delivering antigens to the autophagosomes. These major histocompatibility complex class II antigens derived from intracellular sources may be presented by dendritic cells and cross-prime CD8⁺ T-cells (reviewed by Puleston and Simon¹²⁰). In IBDV infection, the interaction of VP2 protein with an avibirnavirus-binding receptor induces autophagy in an AKT-mTOR dependent pathway.¹²¹ Therefore, targeting autophagy may have promising implication in IBDV intervention strategy.

Disclosure

The authors report no conflicts of interest in this work.

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