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BRIEF REVIEW

An overview of infectious bursal disease

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Abstract Infectious bursal disease (IBD) is a viral immunosuppressive disease of chickens attacking mainly an important lymphoid organ in birds [the bursa of Fabricius (BF)]. The emergence of new variant strains of the causative agent [infectious bursal disease virus (IBDV)] has made it more urgent to develop new vaccination strategies against IBD. One of these strategies is the use of recombinant vaccines (DNA and viral-vectored vaccines). Several studies have investigated the host immune response towards IBDV. This review will present a detailed background on the disease and its causative agent, accompanied by a summary of the most recent findings regarding the host immune response to IBDV infection and the use of recombinant vaccines against IBD.

IBDV

Infectious bursal disease virus (IBDV) has a selective tropism for bursal B cells. Infectious bursal disease (IBD) involves massive destruction of B cells in lymphoid organs, resulting in lymphopenia (immunosuppression) and secondary infection of the infected birds [52]. Immunosuppression is a state of immune system dysfunction that leads to increased disease susceptibility [73]. Immunosuppression is considered to be one of the major problems threatening the poultry industry. In general, any infectious disease can cause immunosuppression.

There are two distinct types of IBDV, designated as serotypes I and II [52, 61]. While serotype I viruses are pathogenic to chickens, serotype II viruses are isolated from turkeys and are avirulent for chickens [59]. IBDV serotype I isolates have different levels of virulence and different replication efficiency in bursal cells [82]. Beginning in 1990, variant strains of serotype I virus emerged in the United States, Western Europe, and parts of Southeast Asia that were more virulent than classical strains and caused mortality rates of over 50 % [52, 61]. These variant strains were isolated from flocks that had been vaccinated with classical-strain vaccines, and they are antigenically different from the classical strains and resistant to current commercial vaccines. Comparing the disease outcome of one of the classical virulent IBDV (vIBDV) strains (F52/70) to the variant/very virulent IBDV (vvIBDV) strains, there was an increase in the mortality rate, from 50 % with vIBDV to 90 % with vvIBDV, which was accompanied by more-severe immunosuppression across the lymphoid organs.

IBDV is a member of the genus *Avibirnavirus*, family *Birnaviridae* [52, 61]. Its genome consists of two segments of linear double-stranded RNA, designated A and B, 6 kb in length in total. Segment A is 3.2 kb in length and contains two partly overlapping open reading frames (ORF). The largest ORF encodes a polyprotein that is autocatalytically cleaved into two structural proteins, VP2 and VP3, and a serine protease, VP4 [6, 45].

VP2 is considered to be the major host-protective antigen and contains the major antigenic site responsible for eliciting neutralizing antibodies (Abs) [26]. At least two neutralizing epitopes are located on this polypeptide. VP2 induces virus-neutralizing Abs that protect susceptible chickens from vIBDV. It is responsible for antigenic variation, tissue-culture adaptation and viral virulence [12].

VP2 is folded into three main domains (the base, shell and projection domains) [8, 20, 46, 53, 65]. The base and

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shell domains are formed by the conserved N- and C-termini of VP2. The projection domain is formed by the hypervariable region of VP2 [amino acids (AAs) 206 to 350] (Fig. 1) [3]. Within the VP2 region, two hydrophilic regions (A and B) were identified (Fig. 1). Region A spans AAs 212 to 224, and region B spans AAs 314 to 325 [2]. These regions constitute two loops, P_{BC} and P_{HI} (neutralising Ab-binding domains), which represent the outmost part of the projection domain (Fig. 1) [46]. Two additional loops were identified in the projection domain, P_{DE} and P_{FG} (Fig. 1) [20]. Moreover, the putative AAs responsible for virulence and cellular tropism were identified to be glutamine at AA position 253, aspartic acid at AA position 279, and alanine at AA position 284 [12] (Fig. 1).

Segment A also encodes a 17-kD non-structural protein, VP5, from the small ORF [60]. VP5 is a class II membrane protein with a cytoplasmic N-terminus and an extracellular C-terminal domain [50]. It is highly basic, cysteine-rich, and semi-conserved among all serotype I IBDV strains [60], and it has been incriminated in the induced bursal pathology [92]. Moreover, it has a role in virus dissemination from infected cells [50]. VP5 accumulates within the cell membrane, resulting in its disruption and decreasing cellular viability. VP2 and VP5 have been shown to induce apoptosis in *in vitro* culture [70, 92].

Segment B is 2.8 kb in length and encodes VP1, a 97-kD protein with polymerase activity [61]. VP1 exists as a genome-linked protein, circularizing segments A and B. DNA vector-based RNA interference, directed towards VP1, prevents IBDV replication in Vero cells [29].

Receptors

The first report investigating the IBDV receptor was made by Ogawa et al. [62]. They described the protein as an N-glycosylated protein associated with the expression of B cell surface immunoglobulin (Ig) M^{+}. In a later study by Lin et al. [49], chicken heat shock protein 90 was suggested to be part of the IBDV-receptor complex. A more recent report demonstrated the ability of $\alpha 4\beta 1$ integrin to act as an IBDV receptor [22].

Pathogenesis, clinical signs, and pathology of infected bursae

Following oral infection, the virus replicates in gut-associated macrophages and lymphoid cells and enters the portal circulation, leading to primary viraemia [61]. The viral antigen is detectable in the macrophages and lymphoid cells of the caecum as early as 4 h postinfection (hpi) and reaches the liver by 5 hpi, and following primary viraemia, the virus reaches the BF by 11 hpi [52, 61]. Following IBDV replication in the BF, the virus enters the blood stream to cause secondary viraemia, which results in virus spread to other tissues.

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F52/70 1    MTNLQDQTQQ  IVPFIRSLLM  PTTGPASIPQ  DTELKHTLRS  ETSTYNLTVG 50
UK661       MTNLQDQTQQ  IVPFIRSLLM  PTTGPASIPQ  DTELKHTLRS  ETSTYNLTVG

F52/70 51   DTGSGLIVFF  PGFPGSIVGA  HYTLQSNGNY  KFDQMLLTAQ  NLPASYNYCR 100
UK661       DTGSGLIVFF  PGFPGSIVGA  HYTLQSNGNY  KFDQMLLTAQ  NLPASYNYCR

F52/70 101  LVSRSLTVRS  STLPGGVYAL  NGTINAVTFQ  GSLSELTDVS  YNGLMSATAN 150
UK661       LVSRSLTVRS  STLPGGVYAL  NGTINAVTFQ  GSLSELTDVS  YNGLMSATAN

F52/70 151  INDKIGNVLV  GEGVTVLSLP  TSYDLGYVRL  GDPIPAIGLD  PKMVATCDSS 200
UK661       INDKIGNVLV  GEGVTVLSLP  TSYDLGYVRL  GDPIPAIGLD  PKMVATCDSS

F52/70 201  DRPRVYTITA  ADDYQFSSQY  QPGGVTITLF  SANIDAITSL  SIGGELVFQT 250
UK661       DRPRVYTITA  ADDYQFSSQY  QAGGVTITLF  SANIDAITSL  SIGGELVFQT

F52/70 251  SVQGLVLGAT  IYLIGFDGTA  VITRAVAADN  GLTAGTDNLM  PFNLVIPTNE 300
UK661       SVQGLILGAT  IYLIGFDGTA  VITRAVAADN  GLTAGTDNLM  PFNIVIPTSE

F52/70 301  ITQPITSIKL  EIVTSKSGGQ  AGDQMSWSAS  GSLAVTIHGGN  YPGALRPVTL 350
UK661       ITQPITSIKL  EIVTSKSGGQ  AGDQMSWSAS  GSLAVTIHGGN  YPGALRPVTL

F52/70 351  VAYERVATG   SVVTVAGVSN  FELIPNPELA  KNLVTEYGRF  DPGAMNYTKL 400
UK661       VAYERVATG   SVVTVAGVSN  FELIPNPELA  KNLVTEYGRF  DPGAMNYTKL

F52/70 401  ILSERDRLGI  KTVWPTREYT  DFREYFMEVA  DLNSPLKIAG  A     441
UK661       ILSERDRLGI  KTVWPTREYT  DFREYFMEVA  DLNSPLKIAG  A
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Fig. 1 Deduced AA sequence of VP2, from AA positions 1 to 441 of vIBDV strain F52/70 and vvIBDV strain UK661. Sequences were taken from Brown and Skinner [13]. Yellow highlighted residues are AAs that differ the two strains. Green highlighted residues are the AAs responsible for virulence and cellular tropism. The two underlined segments represent hydrophilic regions A and B, respectively. Blue segment, P_{BC} loop; green segment, P_{DE} loop; brown segment, P_{FG} loop; red segment, P_{HI} loop (color figure online)

Apoptosis is an individual and active type of cell death that is characterised by nuclear fragmentation and breakdown into apoptotic vesicles without extracellular release of the cellular contents, and consequently without eliciting an inflammatory reaction [19]. Apoptosis has an important role in IBDV pathogenesis and immunosuppression. A high level of apoptosis is evident in chicken peripheral blood lymphocytes infected with serotype I IBDV strain L [85], and peripheral blood lymphocytes showed a high apoptotic index (nuclear fragmentation and cellular breakdown into apoptotic vesicles).

Morbidity in IBDV-infected flocks can reach up to 100 %, and mortality rates can be as high as 90 % [61]. The most severe clinical manifestations are seen in chicks of 3 to 6 weeks of age, when the BF approaches its maximal stage of development. Birds of 1 to 14 days of age are less susceptible; they are usually protected by maternal Abs. Infected birds more than 6 weeks old rarely develop clinical signs of disease. However, they produce Abs to the virus. The incubation period is usually 2 to 3 days, after which birds show distress, depression, ruffled feathers, anorexia, diarrhoea, trembling and dehydration. The clinical disease lasts for 3 to 4 days, followed by rapid recovery in surviving birds.

Macroscopically, the infected birds are dehydrated, with haemorrhages frequently seen in the thigh and pectoral muscles [52]. The mucous content of the intestine increases. A yellowish transudate starts to cover the BF at 2 or 3 dpi. The BF begins to increase in size and weight at 3 dpi. It reaches double its normal weight by 4 dpi, begins to decrease in size, and returns to its normal weight by 5 dpi. The transudate disappears as the BF returns to its normal size. The BF starts to atrophy at 8 dpi.

Microscopically, degeneration and necrosis of lymphocytes start in the medullary area of the bursal follicles as early as 1 dpi [52]. Lymphocytes become replaced by heterophils. By day 3 or 4 postinfection, all of the lymphoid follicles are affected. Severe oedema, hyperaemia, and marked accumulation of heterophils are evident, which cause the increased bursal weight. Cystic cavities develop in the follicular medulla. These cystic cavities are caused by necrosis and phagocytosis by heterophils and plasma cells. During the stage of bursal atrophy, fibroplasia of the bursal tissue becomes evident. In addition, the bursal epithelium becomes proliferative, forming a glandular-like structure, which consists of the bursal columnar epithelium containing globules of mucin. In the late stages, scattered lymphocyte foci appear without the ability to form functional follicles.

Immune response towards IBDV

The role of the BF in IBDV infection was revealed when bursectomized birds survived infection with lethal doses of IBDV without showing any clinical illness [61]. The stage of B cell differentiation in the BF is important for viral replication, as stem cells and peripheral B cells do not support replication of the virus. The acute phase of the disease lasts for about 7-10 days [76]. Within this phase, bursal follicles are depleted of B cells and the BF becomes atrophic, which results in a diminished Ab response and increased susceptibility to secondary infection in recovered birds [61, 76]. The resulting immunosuppression leads to diminished Ab production following vaccination against other viral diseases, leading to subsequent outbreaks. Recovered birds show high anti-IBDV Ab titres.

Following IBDV infection, rapid progressive loss of B cells occurs in the bursal cortex and medulla (Fig. 2), peripheral blood and thymic medulla [66]. Bursal epithelial cells are also affected through the loss of some surface antigens, and it has been reported that T cells are not susceptible to infection. Kim et al. [39] revealed the infiltration of IBDV-infected BF with $CD4^+$ and $CD8^+$ T cells starting from 4 dpi and increasing until T cells represented 65 % of the bursal cell population at 7 dpi. In a more recent report by Withers et al. [90], the numbers of $CD4^+$ cells, $CD8^+$ cells, and macrophages increased in the BF following IBDV infection as early as 1 dpi. Moreover, the bursal cells and the splenocytes up-regulated IFN-γ transcription. IBDV-infected $CD4^+$ and $CD8^+$ T cells inhibited the mitogenic response of splenocytes to ConA treatment [38]. Chemical induction of T cell depletion caused an increase in the bursal viral load [39]. In addition, a combined surgical and chemical induction of T cell depletion produced the same effect and decreased the transcriptional level of interferon (IFN)-γ and interleukin (IL)-2 [67]. It also delayed the recovery of IBDV-infected BF.

The protection against IBDV infection does not depend solely on the induction of virus-neutralising Abs [68]; T cell involvement is critical. IgM^+ cells serve as targets for IBDV [76]. Viral particles are detected in the BF and other peripheral lymphoid organs, such as the caecal tonsils, spleen, and thymus [69, 76, 89]. In these reports, $CD4^+$ and $CD8^+$ T cells accumulated at areas of virus replication. After recovery, the BF becomes repopulated with IgM^+ B cells. Depletion of Bu-1^+, IgM^+, and IgY^+ (the avian equivalent of IgG) cells from the BF, spleen, and thymus of chickens infected with vvIBDV strain UK611 was evident [89]. The BF was repopulated with small numbers of Bu-1^+ cells 14 days postinfection. Few of these cells expressed IgM or IgY. Bursal macrophages increased for the first 3 to 5 days after infection, and this was followed by an influx of $CD4^+$ and $CD8^+$ T cells. Depletion of cortical thymocytes was evident during the acute phase of infection. Withers et al. [90] reported a bursal recovery as early as one week after infection with vIBDV strain F52/70. Such recovery was associated with the development

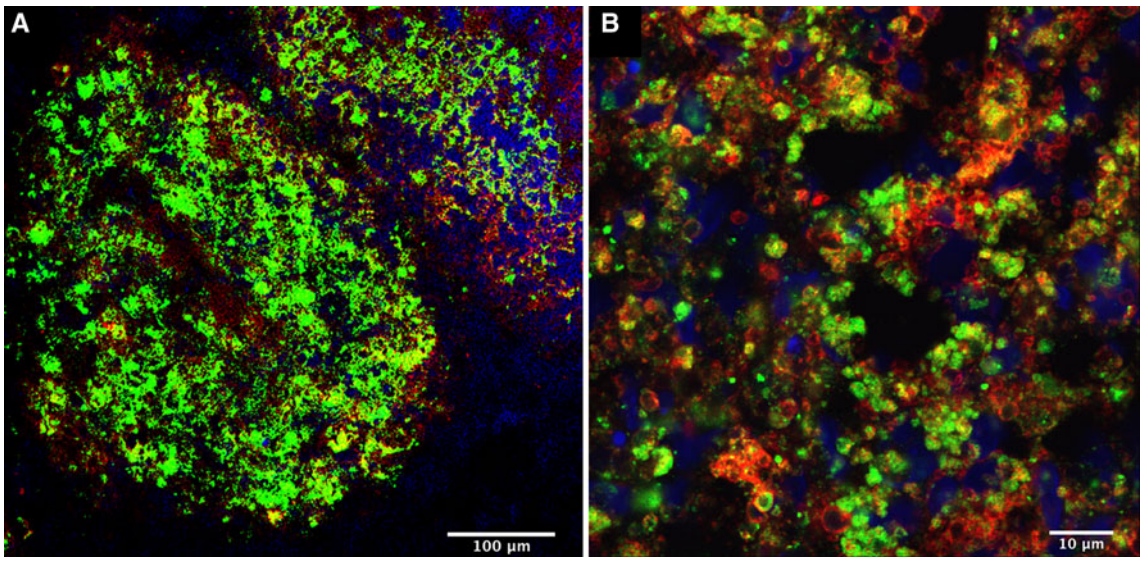

Fig. 2 The interaction between B cells and vIBDV strain F52/70 in an IBDV-infected BF at 4 dpi [54]. Rhode Island red (RIR) birds (3 weeks of age) were infected intranasally with vIBDV strain F52/70 at egg infectious dose $(EID)_{50}$ $10^{1.6}$. (**A**) A section of an infected BF showing the interaction between B cells (red) and IBDV (green). (**B**) A higher magnification showing the same features. Some of the B cells have lost their cellular integrity. IBDV particles can be seen co-localising with and inside most of the B cells. Areas of co-localisation are seen in yellow. The B cell marker (IgG1 isotype) was used with a secondary conjugate (Alexafluor 568). The IBDV marker (IgG2a isotype) was used with a secondary conjugate (Alexafluor 488) (color figure online)

of two types of follicles, differentiated (resembling the uninfected control BF) and undifferentiated (lacking a recognisable cortex and medulla). The abundance of undifferentiated follicles in the BF structure was associated with an inability to mount an Ab response against IBDV. IBDV infection caused reduction in the number of chicken splenic macrophages at 3 and 5 dpi [63]. Such cells contained IBDV particles. IBDV infection was associated with the up-regulation of CD8$\alpha\alpha^+$ TCR2$^-$cells, CD4$^-$ CD8$\alpha\alpha^-$ TCR2$^+$ cells, CD8$\alpha\alpha^+$ TCR2$^+$ cells, CD4$^+$ TCR2$^-$ cells, and CD4$^+$ TCR2$^+$ cells in the BF and the CT [54]. In addition, IBDV particles were seen co-localised with and contained by CD8$\alpha\alpha^+$ TCR2$^-$ cells, CD4$^+$ TCR2$^-$ cells, CD4$^-$ CD8$\alpha\alpha^-$ TCR2$^+$ cells, CD8$\alpha\alpha^+$ TCR2$^+$ cells, and CD4$^+$ TCR2$^+$ (Figs. 3, 4) in the BF and co-localised with CD8$\alpha\alpha^+$ TCR2$^-$ cells, CD4$^-$ CD8$\alpha\alpha^-$ TCR2$^+$ cells, and CD4$^+$ TCR2$^-$ cells in the CT.

The role of cell-mediated immunity in IBDV infection was made clear by the increased bursal mRNA transcription of the pro-inflammatory cytokines IL-1β, IL-6, CXCLi2, and IFN-γ following *in vivo* infection of chickens with vIBDV strain F52/70 and vvIBDV strain UK661, together with down-regulation of transforming growth factor-β4 [24]. The up-regulation of IFN-γ was correlated with an up-regulation of IL-12α but not IL-12β or IL-18. In a different study, an increased level of IL-18 mRNA in splenic macrophages was detected five days after infection with IBDV strain Irwin Moulthrop [63]. The levels of mRNA transcripts of other pro-inflammatory mediators, including IL-1β, IL-6, and inducible nitric oxide synthase, were also increased. In addition, *in vivo* challenge with vvIBDV UK661 strain up-regulated the transcriptional levels of type I, II, and III IFN as well as IL-18, IL-4, and IL-13 [54].

Detection and control of IBDV in commercial flocks

The differentiation between field and vaccine strains of IBDV is crucial and can be achieved by a number of techniques, including enzyme-linked immunosorbent assay [7], reverse transcription polymerase chain reaction (RT-PCR) directed towards single nucleotide polymorphisms in the VP2 region [36], and restriction fragment length polymorphism (where viral-genotype-specific restriction enzyme sites are analysed) combined with the use of RT-PCR [5]. Avian viruses [avian influenza virus, infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and IBDV] have also been distinguished using microarrays coupled with RT-PCR [81]. In addition, pan-viral detection microarrays have been developed and tested successfully for sero-/genotyping of key veterinary viruses with a potential use in the field or as a surveillance tool [30].

IBDV vaccines include live attenuated, inactivated/killed, and immune complex vaccines (a mixture of hyper-immune sera from specific-pathogen-infected chickens and embryo-adapted live pathogens [74]. The application route varies from *in ovo* administration to addition to drinking

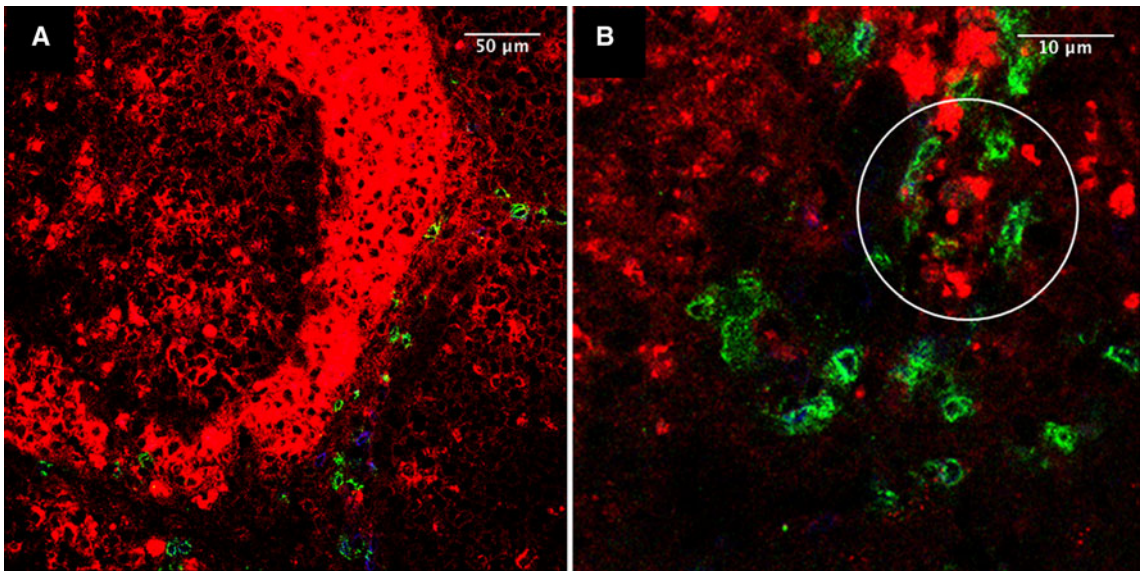

Fig. 3 The interaction between CD8αα$^+$ TCR2$^+$ cells and IBDV in a BF of infected with vvIBDV strain UK661 at 4 dpi [54]. RIR birds (3 weeks of age) were infected intranasally with vvIBDV (strain UK661) at a dose of $10^{1.3}$ EID_{50}. (**A**) A section of an infected BF showing CD8αα$^+$ cells (green) and TCR2$^+$ cells (blue) surrounding IBDV-infected areas (red). (**B**) A higher magnification showing areas of co-localisation between CD8αα and TCR2 markers (cyan). Areas showing the localisation of IBDV particles in CD8αα$^+$ TCR2$^+$ cells are circled. The TCR2 marker (IgG1 isotype) was used with a secondary conjugate (Alexafluor 633). The CD8αα marker (IgG2b isotype) was used with a secondary conjugate (Alexafluor 488). The IBDV marker (IgG2a isotype) was used with a secondary conjugate (Alexafluor 568) (color figure online)

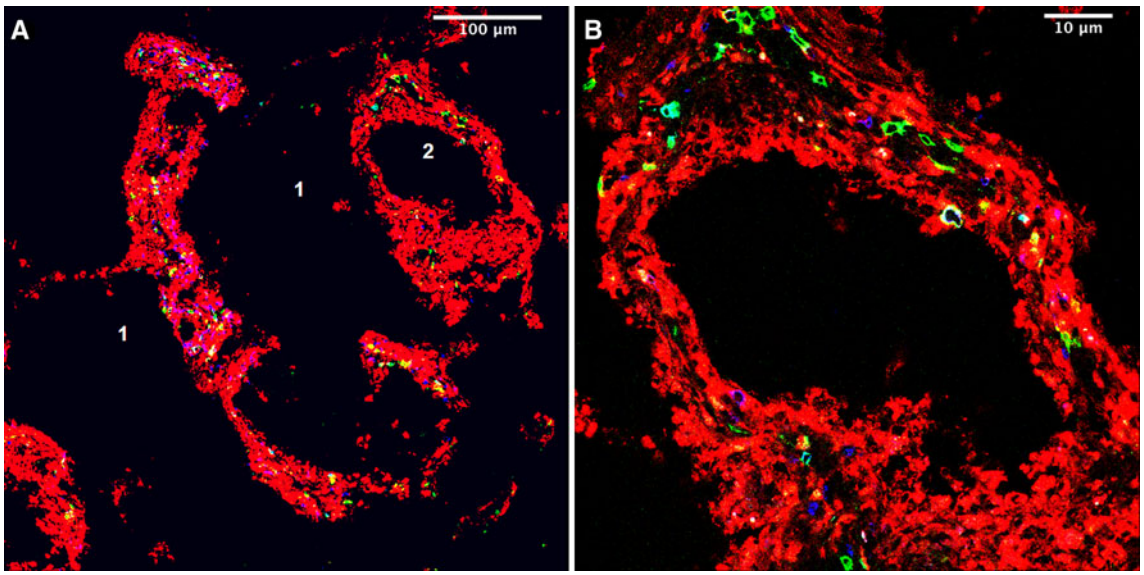

Fig. 4 The interaction of CD4$^+$ and TCR2$^+$ cells with vvIBDV strain UK661 in the BF at 4 dpi [54]. RIR birds (3 weeks of age) were infected intranasally with vvIBDV (strain UK661) at a dose of $10^{1.3}$ EID_{50}. (**A**) A section of an IBDV-infected BF showing CD4$^+$ (green) and TCR2$^+$ cells (blue) in IBDV-infected areas (red). The image was taken using ×10 magnification. (**B**) A higher magnification showing the same features. The TCR2 marker (IgG1 isotype) was used with a secondary conjugate (Alexafluor 633). The CD4 marker (IgG2b isotype) was used with a secondary conjugate (Alexafluor 488). The IBDV marker (IgG2a isotype) was used with a secondary conjugate (Alexafluor 568) 1 = destroyed bursal tissue. 2 = cystic cavity at the centre of an infected lymphoid follicle (color figure online)

water or intramuscular injection. Breeder chickens are vaccinated by adding IBDV vaccine to drinking water to prevent infection of newly hatched chicks or by oral live-virus vaccination of breeding stock at 18 weeks of age, together with injection of inactivated vaccine in oil adjuvant just before laying. This is repeated a year later. This results in a well-maintained high level of neutralizing Ab throughout the laying life of the birds. Maternal Abs

provide effective protection for chicks for 4 to 7 weeks after hatching. If chicks have low or inconsistent levels of maternal Abs, an attenuated virus vaccine is given at 1-2 weeks of age. Maternal Abs are transferred from the mother to the chick via the egg yolk. IgY begins to be absorbed from the yolk in the late stages of embryonic development until shortly after hatching [58].

Recombinant DNA-IBDV vaccines

There is growing concern that intensive use of live attenuated vaccines against IBDV could be driving this pathogen to increasing virulence due to potential mutation. IBDV is immunosuppressive, and the response of the poultry industry to disease outbreaks in vaccinated flocks has been through the use of more-virulent (hot) vaccines. With immunosuppressive pathogens, there is a risk that such hot vaccines could be harmful to susceptible genotypes or chicks that are poorly protected by maternal Abs. Therefore, these problems have raised increasing interest in the development of subunit vaccines, where one or more genes encoding specific pathogen antigens are expressed by a recombinant DNA vaccine.

DNA vaccination offers several advantages for delivering protective antigens; DNA vaccines mimic a natural viral infection in that the antigens they encode are produced in their native structure and are presented in the context of MHC class I and II, evoking a balanced immune response. In addition, it is possible to administer multicomponent vaccines in a single dose. There is no evidence of injection-site reactions and no risks resulting from reversion to the wild-type. And finally, neonates can be immunized with minimal interference from maternal Abs. However, it still remains to be determined whether DNA vaccines can always overcome maternal Abs. DNA vaccines are stable at high ambient temperatures, removing the need for maintaining a cold chain. Finally, constructing and purifying plasmid DNA is relatively quick and easy compared to conventional vaccines.

New approaches to immunization have sprung from the understanding of DNA and the ability to construct expression plasmids, recombinant viruses and recombinant bacteria. Recently, genetically engineered vaccines have been developed to elicit cell-mediated as well as humoral immunity. DNA vaccines expressing either VP2 or the VP4-2-3 polyprotein (segment A) of IBDV have been used as plasmid DNA vaccines for IBDV [17, 18, 27, 41, 47, 48, 55, 56, 71, 72, 88]. The resulting vaccines produced variable levels of protection, ranging from partial to complete protection, against IBDV challenge. Complete protection, against clinical disease, mortality and damage to the BF was only observed when the DNA vaccine was applied in a prime-boost regimen [17, 18, 47]. Of the several administration routes tested (the intramuscular, intraperitoneal, oral and eyedrop routes), the intramuscular route was the only one that provided protection and an anti-viral Ab response [47]. In contrast, topical administration of DNA vaccines has been reported to induce IgA and IgM anti-viral Abs [34]. When compared, DNA vaccines containing the polyprotein gene are generally more protective than DNA vaccines containing the single VP2 gene [27, 47]. However, Chang et al. [18] reported that a VP2-containing DNA plasmid induced complete protection.

In ovo injection of a 40-μg DNA vaccine (pCI-VP2) based on the plasmid vector pCI-neo carrying the coding sequence for VP2 from the vIBDV strain F52/70, in the amniotic fluid at 18 days of incubation, accompanied by a boost with *fpIBD1* post-hatch, produced complete protection against IBDV-induced mortality and bursal pathology [31]. This protection was not evident with the use of either vaccine on its own. An Ab response against IBDV was not detected after the prime-boost vaccination, even after chicks were challenged with IBDV. Moreover, the *in vitro* incubation of empty vector (pCI-neo) with HD11 cells, a chicken macrophage cell line, and monocyte-derived macrophages stimulated the release of nitric oxide and IL-6 from both, proving the immunostimulatory effect of unmethylated CpG motifs (DNA motifs, prevalent in bacteria and viral DNA but heavily suppressed and methylated in vertebrate genomes, are also present in plasmid DNA) in the form of plasmid DNA [32]. This immunostimulatory effect was inhibited when the plasmid was digested with DNase and methylated with cytosine, confirming the immunomodulatory role of CpG motifs in the plasmid DNA and suggesting the potential role of CpG motifs in plasmid DNA vaccines as vaccine adjuvants.

Particularly powerful among T cell vaccines have been combinations of DNA and live vectors in which a live vector vaccine is used to boost the response to a DNA prime, or in which one recombinant viral vector is used for priming and a second viral vector for boosting [75]. These heterologous prime-boost immunizations elicit stronger immune responses than can be achieved by priming and boosting with the same vector. The first vaccination initiates memory cells; the second vaccination expands the memory response. Outside of the immune response to the common vaccine insert, which undergoes a tremendous boost, the two agents do not raise responses against each other and thus do not interfere with each other's activity. DNA vaccines used alone induce T cell responses in animals, as can antigen with many adjuvants. Various strategies have been considered to improve DNA vaccines, such as cytokine augmentation.

Cytokines as vaccine adjuvants with recombinant DNA-IBDV vaccines

The successful elimination of pathogens following prophylactic immunization depends to a large extent on the ability of the host's immune system to recognize when it is necessary to become activated and how to respond most effectively, preferably with minimal injury to healthy tissue. In the design of effective, non-replicating vaccines, immunological adjuvants serve as critical components that instruct and control the selective induction of the appropriate type of antigen-specific immune response. Hence, vaccine adjuvants are essential to stimulate the host's immune response to antigens that lack immunogenicity. Theoretically, adjuvants can be divided into either facilitators of signal 1 (enhancing the duration or magnitude of either whole antigen or its peptide fragments presented by MHC molecules on APC in lymphoid organs) and/or inducers of endogenous signal 2 molecules (cytokines, membrane-bound co-stimulatory molecules or other host-derived natural adjuvants).

Effective vaccine adjuvants, like Freund's adjuvant, have limited clinical use due to their potential toxicity, as they mediate their action through non-specific induction of several cytokines; e.g., Freund's adjuvant induces T cell proliferation and the production of IL-2 and IFN-γ [37]. Therefore, the use of specific cytokines as vaccine adjuvants should augment immunogenicity without the side effects exerted by nonspecific cytokine induction. A number of such host-derived immunostimulatory factors have been described as immunopotentiators of vaccines when co-administered either as a heterologous expression product or following delivery by a viral vector [33, 40, 87]. They likely act by providing the second signal directly to adaptive effector T or B cells or by regulating indirectly the generation of other essential signal 2 molecules. Based on their biological activity, mammalian and avian cytokines have been used as adjuvants to enhance antigen presentation (granulocyte macrophage-colony stimulation factor (GM-CSF), macrophage-CSF, IL-1α/β, and IFN-γ), enhance T cell immune responses (IL-2, IL-12, IL-6, IFN-γ), and enhance B cell humoral immune responses (GM-CSF, IL-1α/β, IL-2, IL-6, IFN-γ, IFN-α, and IFN-β) [23, 33, 42, 48, 51, 80].

Co-administration of recombinant chicken IFN-γ with antigen resulted in enhanced secondary Ab responses that persisted at higher levels and for longer periods compared to antigen injected in the absence of IFN-γ [51]. In addition, IFN-γ treatment allowed lower doses of antigen to be more effective. Recombinant IL-18 was used as a vaccine adjuvant with inactivated NDV, IBV, and *Clostridium perfringens* α-toxoid [21]. Raised Ab titres were observed for NDV and *C. perfringens* α-toxoid but not for IBV. Protection against challenge with virulent IBDV was enhanced when a DNA plasmid expressing chicken IL-2 was co-administered with a DNA vaccine encoding VP2 [35, 48]. Plasmid vectors carrying chicken IL-6 and IBDV VP2-4-3 were used by Sun et al. [80] to immunize chickens against vvIBDV strain SH95. Immunization with a mixture of both genes conferred protection on 90 % of chickens. In addition, partial protection and raised Ab titre were observed when chicken IL-2 was used as a vaccine adjuvant with VP2 [42, 48] when compared to the effect of VP2 alone. The combined *in ovo* administration of IL-12 or IL-18 with or without pCI-VP2 protected bursal cells and splenocytes from *in vitro* infection with vvIBDV strain UK661 [54].

Viral-vectored IBDV vaccines

Fowlpox IBD1 (fpIBD1)

Various viruses have been used as vectors for IBDV, e.g., Marek's disease virus [83, 84], Semliki Forest virus [64], baculoviruses [57], avian adenovirus [28, 78], and recently, herpesvirus of turkey [14]. The use of Fowlpox virus (FPV) as a recombinant vector for use in poultry started in the 1980s. Recombinant FPV vectors have been successful in protection against a wide variety of diseases, e.g., NDV [9], IBV, avian influenza virus, and Marek's disease virus [10]. Moreover, recombinant FPV vaccines have been used for non-avian species [10, 86]. FP9 is the best characterized of the FPV strains used for recombinant vaccine purposes. FP9 was isolated in the late 1980s at the Houghton Poultry Research Station, U.K. [79].

The FPV genome is 288 kb in length and contains 260 ORFs [1]. The genome consists of a central coding region bounded by two identical inverted terminal repeat (ITR) regions of approximately 9.5 kb each. The boundaries between the ITRs and the central coding region are marked by the 3′ 148 codons of ORF FPV010 and FPV251. Poxviruses have immunomodulatory strategies; one of them is the ability to bind IL-18 [1, 44, 91]. Eldaghayes [25] proved that FPV-IL-18 binding protein is encoded by ORF 214.

Bayliss et al. [4] constructed a recombinant FPV vaccine, fpIBD1 (FPV strain FP9, a tissue-culture adapted strain of the parent FPV strain HP-1, encoding VP2 from vIBDV strain F52/70 as a β-galactosidase fusion protein, which was inserted in the ITR of the FPV genome). fpIBD1 (in a prime-boost vaccination regimen) protected outbred Rhode Island red (RIR) chickens from mortality induced by vIBDV strain F52/70 or the highly virulent strain CS89, although not vIBDV-induced bursal damage. Successful vaccination with fpIBD1 was revealed to be dependent on the titre of the challenge virus, as high titres of challenge virus were able to overcome protection induced by fpIBD1, whereas challenge with a low titre of virus did not [77]. Protection by fpIBD1

was induced in the absence of detectable serum Abs [4, 16, 77], suggesting a significant role for cell-mediated immunity in protection from IBDV challenge. In contrast, Ab responses to influenza virus and FPV were detected in chickens vaccinated with a recombinant FPV expressing VP2 and influenza virus haemagglutinin [11]. fpIBD1 also protected against IBDV-induced immunosuppression, as shown by measuring the chicken's Ab response [16].

Eldaghayes [25] manipulated fpIBD1 to express chicken IL-18 (fpIBD1::IL-18) and investigated the potential of chicken IL-18 to act as a vaccine adjuvant against vIBDV (F52/70). Chicken IL-18 was inserted into the ORF FPV030 locus (FPV homologue of human PC-1, plasma cell membrane glycoprotein, which has alkaline phosphodiesterase and nucleotide pyrophosphatase activities), which was found to be non-essential for FPV replication in tissue culture [1, 15, 43]. fpIBD1::IL-18 provided complete protection against challenge with vIBDV; no viral RNA or bursal damage was detected in vaccinated challenged birds. In a different study, both fpIBD1 and fpIBD1::IL-18 protected RIR from challenge with the vvIBDV UK661 strain [54]. Both vaccines raised the humoral antibody response. In addition, they both raised transcriptional levels of type I, II, and III IFN as well as T helper 1 and T helper 2 cytokines, IL-18 and IL-4 and IL-13, respectively.

Conclusion

Various vaccination strategies have been tested experimentally against IBD. In comparison with the traditional live attenuated and inactivated vaccines, recombinant vaccines have proved a success in providing protection against IBDV infection, depending on the route of administration, the amount or titre of the vaccine, and the challenge dose of the virus, without the risk of reverting to virulence or inducing bursal pathology. While vaccination regimens using the traditional live attenuated vaccine starts at day 14 of bird's life (in the case of the presence of maternal Abs), DNA vaccines can be applied at an earlier age without any interference from maternal Abs. In addition, recombinant vaccines can provide protection against multiple infectious agents (through the insertion of their specific immunogenic genes in the vaccine construct in a single carrier construct), saving labour costs, vaccination costs, and stress to the vaccinated flock. However, it is still in question if these vaccines are cost competitive.

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