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Infectious Bursal Disease: A complex host–pathogen interaction

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ABSTRACT

Infectious Bursal Disease (IBD) is caused by a small, non-enveloped virus, highly resistant in the outside environment. Infectious Bursal Disease Virus (IBDV) targets the chicken's immune system in a very comprehensive and complex manner by destroying B lymphocytes, attracting T cells and activating macrophages. As an RNA virus, IBDV has a high mutation rate and may thus give rise to viruses with a modified antigenicity or increased virulence, as emphasized during the last decades. The molecular basis of pathogenicity and the exact cause of clinical disease and death are still poorly understood, as it is not clearly related to the severity of the lesions and the extent of the bursal damage. Recent works however, pointed out the role of an exacerbated innate immune response during the early stage of the infection with upregulated production of promediators that will induce a cytokine storm.

In the case of IBDV, immunosuppression is both a direct consequence of the infection of specific target immune cells and an indirect consequence of the interactions occurring in the immune network of the host. Recovery from disease or subclinical infection will be followed by immunosuppression with more serious consequences if the strain is very virulent and infection occurs early in life. Although the immunosuppression caused by IBDV is principally directed towards B-lymphocytes, an effect on cell-mediated immunity (CMI) has also been demonstrated therefore increasing the impact of IBDV on the immunocompetence of the chicken. In addition to its zootechnical impact and its role in the development of secondary infections, it may affect the immune response of the chicken to subsequent vaccinations, essential in all types of intensive farming. Recent progress in the field of avian immunology has allowed a better knowledge of the immunological mechanisms involved in the disease but also should give improved tools for the measurement of immunosuppression in the field situation. Although satisfactory protection may be provided by the induction of high neutralizing antibody titres, interference from parental antibodies with vaccination has become the most important obstacle in the establishment of control programs. In this context, recombinant HVT and immune complex vaccines show promising results.

1. Introduction: Poultry production, a risky business

Immunosuppression is recognized by the poultry industry in Europe as a problem that appears to be increasing with intensive poultry production. Viruses which specifically infect and destroy immune cells are in most cases, the primary agents responsible, although other factors like stress might also play a role. Affected flocks show increased susceptibility to infection with opportunistic pathogens, often leading to chronic disease situations, and suboptimal vaccine responses. This often results in lower than expected economic outputs and downgrading of carcasses, and antibiotic therapy is frequently used as the means of control.

The immunosuppressive viral diseases have an important economical and societal impact due to the direct losses they provoke but also to the indirect losses as consequence to immunosuppression or to the interaction they might have together or with other factors. The direct economic losses are due to specific mortality, depending on the virulence and the dose of the inoculum, the age and breed of the birds and the presence or absence of a passive immunity. The indirect losses are due to acquired immunodeficiency, impaired growth and condemnation of carcasses. This situation increases the economic impact of the diseases but might also have an important impact on Public Health by favoring the development of several poultry infection of zoonotic importance like Salmonellosis, Campylobacter and Avian influenza. For instance, in the latter case, higher multiplication and circulation of low pathogenic influenzas in immunocompromised chickens is a high risk to the poultry industry and a threat for human health taking into account that low pathogenic influenzas can readily turn to high pathogenic influenzas which can be transmitted to humans (as demonstrated by the recent Asian crisis). In addition, the increased use of antibiotics and chemicals to fight against opportunistic (secondary) infections is a major concern for human health, if we consider the risks linked to the presence of residues in meat product, the release of residues into the environment and the increasing

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antibiotic resistances. Viral diseases represent the predominant pathology in commercial flocks and vaccination is therefore essential for control. Furthermore, immunosuppression is one of the most common causes for a failure of the general vaccination program and successful IBD vaccination is considered as a key factor in the establishment of a satisfactory control schedule.

Very recent reviews have focussed on the disease and its causative agent (Maghoub, 2012) and vaccination (Muller et al., 2012). General information can be found in the IBDV specific chapter of the 12th edition of Diseases of Poultry (Eterradossi and Saif, 2008) and other textbooks. In this review, according to the general topic of this special issue, we will concentrate on the immune response to IBDV that is responsible for the pathogenesis and the acquired immunosuppression, with a focus on the host response and its measurement.

2. Short description of the etiological agent: When a virus attacks the defenses of the host

Infectious Bursal Disease (IBD), or Gumboro disease, is a viral infection that was described for the first time in the 60's in Gumboro, Delaware, United States (Eterradossi and Saif, 2008) and now occurs worldwide. Two serotypes have been recognized so far by the use of monospecific neutralizing antisera: serotype 1 causing disease in chicken and serotype 2 which is apathogenic (McFerran et al., 1980). Only chickens (*Gallus gallus*), develop an infectious and highly contagious disease after infection with pathogenic serotype 1 virus.

IBDV is highly infectious in young chickens, and is characterized by the destruction of the lymphoid organs, and in particular, the Bursa of Fabricius (BF), which is the site where B-lymphocyte maturation and differentiation occurs in birds. Indeed, the target cell is the B lymphocyte in an immature stage, and the infection, when not fatal, causes an immunosuppression, in most cases temporary, the degree of which is often difficult to determine. Until 1987, the isolated strains were of low virulence and caused only 1–2% of specific mortality in the field. As of 1987, however, an increase in specific mortality was described in different parts of the world. In the United States, strains with new antigenic properties and responsible for up to 5% specific mortality were described (Jackwood and Saif, 1987). At the same time, in Europe and subsequently in Japan, mortality rates of up to 100% in SPF (specific pathogen-free) chickens, 50–60% in laying hens, and 25–30% in broilers were observed and reproduced in isolators, due to very virulent (vvIBDV) strains isolated in the field (Chettle et al., 1989; Nunoya et al., 1992; van den Berg et al., 1991). These strains have now spread all over the world, with the exception of Australia. The first cases of vvIBDV infections in the United States were only identified at the end of 2008 (Stoute et al., 2009) but apparently, they did not spread throughout California.

The virus responsible for IBD is a member of the family *Birnaviridae*, within the *Avibirnavirus* genus (Muller et al., 1979). It is non-enveloped with a single-shelled icosahedral capsid and has a diameter between 55 and 60 nm (Fig. 1). This relatively simple structure confers the virus high resistance in the outside environment, and represents a key issue in the control of the disease. The genome of the virus consists of two segments, A and B, of double-stranded RNA. Segment A encodes the viral structural proteins VP2, VP3 and VP4 as well as VP5 a protein of regulatory function. As the external capsid protein, VP2 elicits neutralizing antibody and represents the molecular basis for antigenicity with variation in the encoding nucleotide sequences resulting in antigenic variants. As the internal capsid protein, VP3 induces group-specific antibodies. Segment B encodes VP1, the viral polymerase, involved in replication and transcription of the virus and thus plays a role in the virulence of the virus (for a review on the viral structure and gene organization, see Coulibaly et al., 2005; Delmas et al., 2004; van den Berg, 2000).

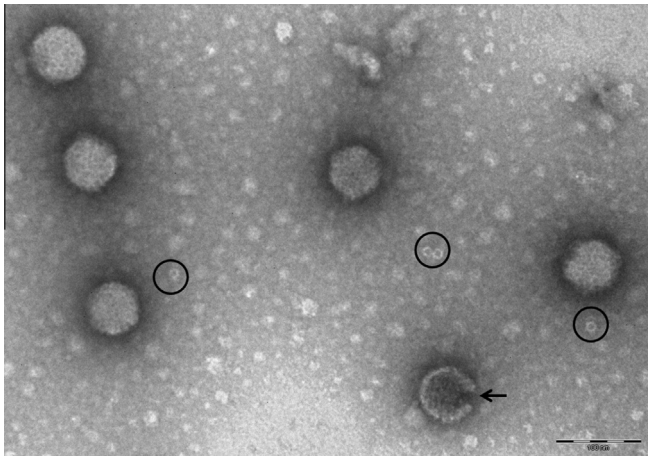

Fig. 1. Electron micrograph of negatively stained IBDV. Scale bar 100 nm. One of the nucleocapsids is damaged (arrow) explaining the presence of isolated capsomers (circles). Courtesy of Dr. J. Mast (CODA-CERVA).

The molecular basis for IBDV pathogenicity is still poorly understood. To illustrate this, nine strains of IBDV, isolated at different times and from different geographic regions of Europe and China, were characterized. Batches of all strains were prepared following standardized protocols and checked for the absence of contaminating viruses. Criteria used for their characterization were: (i) the nucleotide sequence of the VP2 variable region, (ii) binding to a panel of neutralizing monoclonal antibodies in antigen capture enzyme-linked immunosorbent assays (ELISA), and (iii) virulence in specific pathogen free chickens after infection with a standardized number of median embryo infective doses. Based on the first two criteria, two of nine strains were classified as classical virulent (cv) IBDV and five as very virulent (vv) IBDV strains. Remarkably, although a clear-cut difference was demonstrable between European cvIBDV and vvIBDV strains, there was a continuum in the pathogenicity of Chinese vvIBDVs. These results indicated the likely existence of differences in virulence within IBDV lineages determined on the basis of antigenic typing using monoclonal antibodies and the alignment of the VP2 sequences. This emphasized limitation in the analysis of IBDV pathotypes based on the VP2 gene and indicated that virulence is a polygenic trait (van den Berg et al., 2004).

The polygenic nature of IBDV pathogenicity has been further demonstrated by the isolation of a vvIBDV strain with reduced pathogenicity in a rare natural segment-B-reassorted isolate (Le Nouen et al., 2006) and, by using reverse genetics, a clear role of the VP1 polymerase could be established confirming that both genome segments influence vvIBDV pathogenicity and may provide new targets for the attenuation of vvIBDVs (Escaffre et al., 2012; Le Nouën et al., 2012). Interestingly, the latter work suggested possible interactions between VP1 and another, as yet unidentified, host molecule.

Recent progress regarding our understanding of IBDV epidemiology have illustrated that it is probably only a matter of time until vvIBDVs are replaced by an emerging strain with new antigenic or pathotypic properties. This raises the question of possible sources for the introduction of new IBDV strains.

3. Pathogenesis: A growing evidence for a role of proinflammatory cytokines in acute IBD

Pathogenesis can be defined as the method used by IBDV to cause injury to the host with mortality, disease and/or

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immunosuppression as a consequence. These injuries can be evaluated at different levels: the host, the organ and the cell, and are exacerbated in the acute forms of the disease. The main characteristic of vvIBDVs is their increased virulence. A better understanding of the mechanisms of pathogenicity is thus essential for the control of the disease.

Host range. IBDV is highly host specific. The selected host for IBDV is the young chicken where a clinical disease occurs whilst in older birds the infection is essentially subclinical. As already mentioned, only chickens (*Gallus gallus*) develop IBD after infection by serotype 1 viruses and inoculation of IBDV in other avian species fails to induce disease. Turkeys (*Meleagris gallopavo*) and Peking ducks (*Anas peking*) may be asymptomatic carriers of serotype 1 viruses whose pathogenicity is ill defined (McFerran et al., 1980). Experimental inoculation of game birds (quails, partridges, pheasants and guinea fowls) with serotype 1 viruses failed to induce any clinical sign or disease, even when a very virulent IBDV strain was used for challenge (van den Berg et al., 2001).

Varying susceptibility of different chicken breeds has been described with higher mortality rates in light than in heavier breeds (Bumstead et al., 1993; Nielsen et al., 1998). Studies with vvIBDV demonstrated the exacerbated susceptibility of Leghorn-type SPF chickens (Rauw et al., 2007). More recent studies have confirmed a significant influence of chicken's genetic background on IBD outcome and, more closely, its association with the early immune response during the acute phase after infection with vvIBDV (Aricibasi et al., 2010; Ruby et al., 2006). Furthermore, the differential immunopathogenesis of IBDV was investigated in different conventional layer and broiler type chickens in comparison to highly susceptible SPF layers often used for experimental studies. Layer-type chickens of all genetic backgrounds showed significantly higher IBDV antigen loads in the BF, clinical signs and death rate compared to broiler type birds (Tippenhauer et al., 2012).

Symptomatology & lesions. Disease severity and clinical signs depend on the age and sensitivity of the infected birds, the virulence of the strain, and the degree of passive immunity transmitted by the parents. Initial infection in a given farm is generally very acute, with very high mortality rates if a very virulent strain is involved. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are then gradually replaced by subclinical forms. Nonetheless, acute episodes may still occur (van den Berg, 2000). Moreover, a primary infection may also be unapparent if maternal antibodies are present. Acute IBDV infections are characterized by severe clinical signs and high mortality. Indeed, vvIBDVs produce disease signs similar to classical type 1 infections but the acute phase is exacerbated and more generalized in the affected flock. The incubation period is very short: 2–3 days. In acute cases, the animals are exhausted, prostrated, dehydrated, suffer from aqueous diarrhea, and their feathers are ruffled. Mortality commences on the third day of infection, reaches a peak, then drops rapidly, and the surviving chickens recover a state of apparent health after 5–7 days. In the case of vvIBDV infection, the incubation period seems to be shortened (60 h instead of 72) which would be consistent with a faster replication in the affected animals (van den Berg, unpublished observations). The age susceptibility is extended, covering the entire growing period in broilers and the peaks of mortality show sharp death curves followed by rapid recovery (Chettle et al., 1989; Nunoya et al., 1992; Tsukamoto et al., 1992; van den Berg et al., 1991).

Compared with classical virulent strains, vvIBDVs induce higher mortality rates in fully susceptible chickens. But the exact cause of clinical disease and death is still unknown, as it is not clearly related to the severity of the lesions and the extent of the bursal damage. Indeed, after infection, some birds with few bursal lesions can be found dead while others can survive despite extensive bursal damage. Moreover, mortality rates are often variable and the establishment of the lethal dose 50 (LD50) for the standardization of challenge has always been difficult. This variability must be related to host factors.

On post mortem examination of birds that died during the acute phase of vvIBD, the BF is the principal diagnostic organ: it is turgid, oedematous and sometimes haemorrhagic and turns atrophic within 7–10 days. In addition, dehydration and nephrosis with swollen kidneys are common and ecchymotic hemorrhages in the muscle and the mucosa of the proventriculus are observed in many affected birds (Eterradossi and Saif, 2008). The damage is transient and the structure of the BF is restored and repopulated with lymphocytes; the duration of the recovery process depends on the age at infection and the virulence of the strain (Sharma et al., 2000; Vervelde and Davison, 1997). Depletion of lymphoid cells is observed not only in the BF but also in the non-bursal lymphoid tissues. Pathogenicity of vvIBDV has been associated with virus distribution in the BF but also in non-bursal lymphopoietic and hematopoietic organs. Indeed, using various immunostaining methods, a higher frequency of antigen-positive cells could be demonstrated after infection of birds with vvIBDV than with other strains, in the thymus (Inoue et al., 1994; Nunoya et al., 1992; Sharma et al., 1993), the spleen and the bone marrow (Inoue et al., 1999; Tanimura et al., 1995; Tsukamoto et al., 1995). In particular, atrophy of the thymus has been associated with the acute phase of the disease and might be indicative of the virulence of the isolate, although it is not associated with extensive viral replication in thymic cells (Sharma et al., 1993).

Target cells. All compartments of the bird's immune system will be affected during infection with IBDV. IBDV targets the chicken's immune cells in a very comprehensive and complex manner by destroying B lymphocytes, attracting T cells and activating macrophages (Fig. 2).

The target organ for IBDV is the Bursa of Fabricius (BF) at its maximum development, which is a specific source for mature B-lymphocytes in avian species. Bursectomy can prevent illness in chicks infected with virulent virus (Hiraga et al., 1994). The severity of the disease is directly related to the number of susceptible cells present in the BF; therefore, the highest age susceptibility is between 3 and 6 weeks, when the BF is at its maximum rate of development. This age susceptibility is extended in the case of vvIBDV infection (Nunoya et al., 1992; van den Berg et al., 1991). Depletion of lymphoid B cells in the Bursa of Fabricius after IBDV infection is due to both necrosis and apoptosis. Actively dividing, surface immunoglobulin M-bearing B cells are lysed by IBDV infection (Hirai and Calnek, 1979; Hirai et al., 1981; Rodenberg et al., 1994). Apoptosis, characterized by nuclear fragmentation and cellular breakdown into apoptotic vesicles also plays an important role in IBDV pathogenesis. A high level of apoptosis can be evidenced in peripheral blood lymphocytes of chickens infected with serotype 1 IBDV (Vasconcelos and Lam, 1994). Very virulent strains causing increased pathology and earlier mortality also induce also a higher level of chIFN-γ mRNA in bursal tissue (Eldaghayes et al., 2006; Liu et al., 2010). The viral proteins VP2 and especially VP5 have been suspected to play a crucial role in IBDV replication by inducing cell death (Fernandez-Arias et al., 1997; Liu and Vakharia, 2006). It was shown *in vitro* that IBDV infection activates effector caspase 3 and the initiation caspase 9 as well as nuclear factor-κB (NFκB), likely through the accumulation of oxygen reactive species, resulting in apoptosis late in the infective cycle (Liu and Vakharia, 2006). More recently, VP5 was confirmed to be a major apoptosis inducer by interacting with the voltage-dependent anion channel 2 (VDAC2) in the mitochondrion (Li et al., 2012). Additionally, the RNA-binding VP3 polypeptide likely ensures the continuity of the IBDV replication cycle by inhibiting PKR-mediated apoptosis (Busnadiego et al., 2012). On the other hand, apoptosis has also been observed in viral antigen-negative bursal cells

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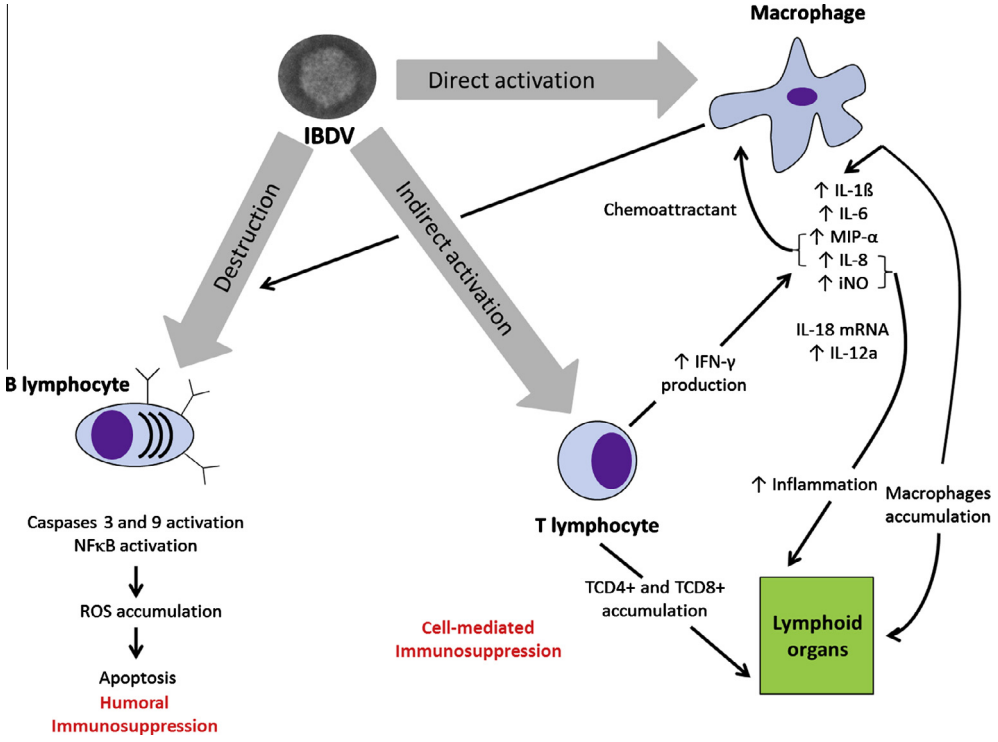

Fig. 2. Interactions between IBDV and the host immune cells (IBDV = infectious bursal disease virus, ROS = reactive oxygen species, iNOS = inducible nitric oxide synthetase, IL = interleukine, IFN = interferon, MIP = macrophage inflammatory protein).

(Nieper et al., 1999; Tanimura and Sharma, 1998), reinforcing the role of immunological mediators in the process.

The interaction between IBDV and the host cell has become clearer over the years. It was first pointed out that the virus prerequisites a certain stage of cell differentiation for its replication. The old hypothesis was that this fact may be due to special receptors or to a potential synthesis apparatus being present in such cells (Burkhardt and Muller, 1987). It was then demonstrated that IBDV could be mainly controlled by the presence of a virus receptor composed of a *N*-glycosylated protein on the surface of IgM-bearing cells (Ogawa et al., 1998). A decrease in the IgM B-cell population relative to IgA and IgG B-cell following IBDV infection was observed and, afterwards, two distincts IgM B-cell subpopulations were identified (Petkov et al., 2009). More recently, it was also suggested that the IBDV might use the α4β1 integrin as a specific binding receptor in avian cells (Delgui et al., 2009). Although membrane perforation was suggested as the means of penetration mediated by IBDV, the cellular mechanism being hijacked to facilitate its entry is still largely unknown. Recent result suggests that the intact IBDV particle is transported to the V-ATPase positive vesicles for uncoating and implicates an essential role of clathrin independent endocytosis during the viral entry (Yip et al., 2012).

Cells of the monocyte-macrophage lineage can also be infected in a persistent and productive manner and play a crucial role in the dissemination of the virus (Burkhardt and Muller, 1987; Inoue et al., 1992) as well as in the onset of the disease (Kim et al., 1998; Sharma and Lee, 1983). In bursal macrophages, viral RNA was detected by RT-PCR and viral proteins by immunochemistry between 1 and 7 dpi (Khatri et al., 2005). Confocal microscopic examination revealed cells that were positive for both KUL01 (macrophage surface marker) and R63 (IBDV-VP2 marker), thus confirming the presence of the virus in macrophages (Palmquist et al., 2006). As a consequence, the macrophage functions, notably the phagocytic activity, are modified by the infection with IBDV (Lam, 1998; Sharma et al., 2000) and cytokine gene expression is upregulated (see next §), therefore influencing normal immune responsiveness of the affected birds (Sharma et al., 2000).

Finally, although they are not susceptible to infection, T cells and IFNγ play an important indirect role in the pathogenesis of IBD (see Fig. 3 and next §). Indeed, there is an influx and infiltration of CD4+ and CD8+ cells into the BF between 1 and 10 dpi, most probably enhancing cellular damage (Sharma et al., 2000; Vervelde and Davison, 1997).

Host immune response. As for any infection, the immune response against IBDV infection consists in a first, early, non-specific innate immune reaction followed by the induction of an active adaptive immune response that does not depend solely on the induction of virus-neutralizing antibody, as T cell involvement is also critical. During infection, a complex network of cytokines controls both inflammatory and specific immune responses. As regulators for the initiation and maintenance of host defenses, cytokines ultimately determine the type of response and the effector mechanisms generated to mediate resistance. As effector molecules, cytokines are produced transiently and locally to control the amplitude and duration of the immune response. Therefore, cytokines play pivotal roles in the regulation of both inflammation and immunity. Likewise, excessive or insufficient production of cytokines may contribute significantly to the pathophysiology of the disease.

There is growing evidence for a role of innate immunity, particularly proinflammatory mediators, in the pathogenesis of IBD. Indeed, during the acute phase of IBD and as early as 1 day post-infection (dpi), there is a dramatic infiltration of CD4 cells, CD8+ cells and macrophages at and near the site of virus replication, mainly in the BF (Sharma et al., 2000; Withers et al., 2005). Bursal T cells are activated and exhibit up-regulation of gene transcription of pro-inflammatory cytokines e.g. ChIL-1β, ChIL-6, CXCi2 and ChIFN-γ (Eldaghayes et al., 2006). High levels of systemic ChIFNγ and ChIL-6 were also observed during the acute phase following vvIBDV challenge demonstrating the role of an exacerbated innate

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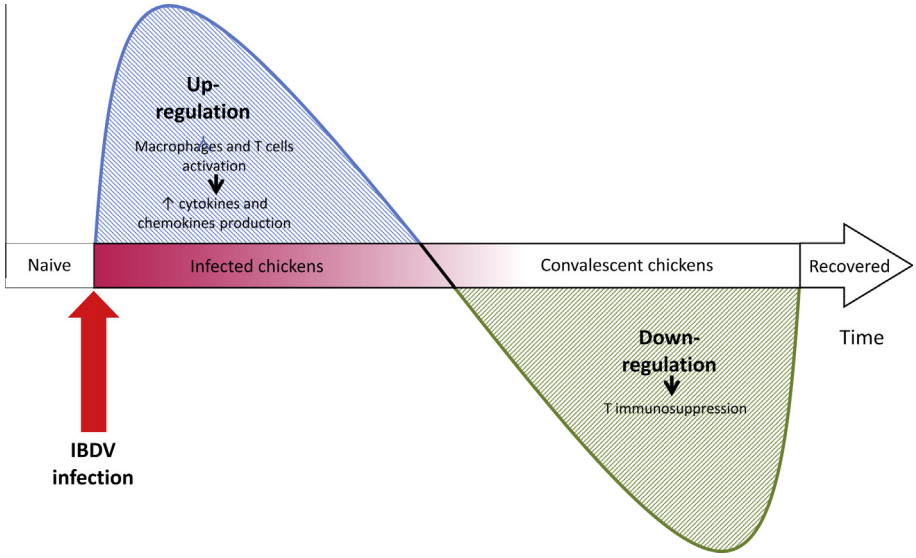

Fig. 3. The pivotal role of ChIFNγ in the immunopathology of IBDV.

immune response in the acute phase of the disease, leading to a so-called "cytokine storm" (Rauw et al., 2007). The ChIFN-γ up-regulation was correlated with production of IL12a, an increased level of IL18 mRNA in splenic macrophages and pro-inflammatory factors including ChIL-1β, ChIL-6, and inducible nitric oxide synthetase (iNOS) (Palmquist et al., 2006) that may promote cellular dysregulation and accentuate tissue destruction (Digby and Lowenthal, 1995; Karaca et al., 1996; Kim et al., 1998). Moreover, macrophages and monocytes infected by IBDV are directly activated producing high levels of mediators such as proinflammatory cytokines, interleukin-1 (IL-1) and IL-6, chemokines (IL-8 and MIP-α and nitric oxide (NO) (Kim et al., 1998; van den Berg, 2000; Khatri et al., 2005; Rauf et al., 2011a). The signal transduction pathways involved in macrophage activation have also been examined (Khatri and Sharma, 2006). The role of mitogen-activated protein kinases (MAPKs) and NF-κB was tested by using specific pharmacological inhibitors. The addition of p38 MAPK inhibitor, SB-203580 and NF-κB inhibitor Bay 11-7082, suppressed IBDV-induced NO production and mRNA expression of iNOS, IL-8 and COX-2 (Khatri and Sharma, 2006). These results suggest that IBDV uses cellular signal transduction machinery, in particular the p38 MAPK and NF-κB pathways, to elicit macrophage activation. The increased production of NO, IL-8 and COX-2 by macrophages may contribute to bursa inflammatory responses commonly seen during the acute phase of IBDV infection (Khatri et al., 2005). This was confirmed in a more recent study (Rauf et al., 2011b), where the overexpression of chemokines genes, IL-8 and MIP-α was also higher in IBDV-infected chickens during the early phase of infection (chicken IL-8 acts as a chemoattractant for heterophils and monocytes). In summary, IBDV appears to trigger both direct (T cell activation) and indirect (macrophage activation) pathways to induce a "cytokine storm" during the acute phase of the disease and the individual susceptibility must be related to the variable intensity of the innate immune response (van den Berg, 2000; Rauw et al., 2007; Rauf et al., 2011b). In a very recent study, an Agilent microarray was used to investigate different transcriptional profiles of the TLR pathway and related genes of chicken bursa at 48 h after infection with IBDV, compared with simulated infection. Expression of 58 genes changed significantly. Forty-six genes associated with chicken bursa proinflammatory effects, chemotactic effects, and T-cell stimulation were upregulated, which meant enhancement of these features. Twelve genes that are related to proliferation and differentiation of bursal cells were downregulated, implying suppression of these features. These results revealed that genes of the TLR pathway play an important role in the pathogenicity of IBDV infection (Guo et al., 2012).

Recovery from the acute phase of the disease will be accompanied by the acquisition of a strong specific acquired immunity against IBDV but immunosuppression against other pathogens (see next §). This paradoxical response is related to the fact that the main IBDV target cell is the immature B cell and that mature IBDV-specific B cells will expend after contact with the antigen, inducing a strong anamnestic response. More recently, Withers et al. (2005, 2006) described two types of follicles emerging after recovery of chicks from IBDV infection: large follicles with a normal structure and rapidly proliferating B cells that were derived from a small proportion of surviving bursal stem B cells still capable of undergoing gene conversion and small follicles lacking distinct structure deriving from mature B cells that had already undergone gene conversion. These data suggest that the proportion of small versus large follicles in the BF after recovery might give an indication about the severity of the infection and about the level of immunosuppression. The marked influx of T cells into the infected bursa and the linked production of cytokines and chemokines indicate that cell-mediated immunity, although also associated to the immunopathogenesis of the virus, plays an important role in the clearance of IBDV and recovery (Williams and Davison, 2005). In a recent study, Rauf et al. (2011b) evaluated the molecular mechanisms of cytotoxic T cell responses in the pathogenesis of IBD in chickens. Infection of chickens with IBDV was accompanied by the infiltration of CD4(+) and CD8(+) T cells into the bursa. There was an upregulation in the gene expression of important cytolytic molecules; perforin (PFN), granzyme-A (Gzm-A), DNA repair and apoptotic proteins; high mobility group proteins (HMG) and poly(ADP-ribose) polymerase (PARP) in the Bursa of Fabricius (BF) whereas expression of NK (natural killer) lysin was downregulated. Importantly, PFN producing CD4+ and CD8+ T cells were also detected in the bursa of IBDV-infected chickens by immunohistochemistry. The expression of Th1 cytokines, IL-2 and IFN-γ was also strongly upregulated, suggesting the activation of T cells. The findings of this study highlighted the role of cytotoxic T cells in the clearance of virus-infected cells (Rauf et al., 2011a).

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4. Immunosuppression: The hidden enemy

Immunosuppression has been defined as a "state of temporary or permanent dysfunction of the immune response resulting from damage to the immune system and leading to increased susceptibility to disease" (Dohms and Saif, 1984) and often a suboptimal antibody response (Lutticken, 1997). However, this definition considers more the consequences (increased disease incidence) than the causes, of which mechanisms, beyond the destruction of a specific cell type, are still not fully understood.

The destruction of the BF by IBDV creates an immunosuppression, which will be all the more serious the younger the infected bird. In addition to its zootechnical impact and its role in the development of secondary infections, it may affect the immune response of the chicken to subsequent vaccinations, essential in all types of intensive farming.

The immunosuppression has been most often evidenced using experimental models based on the measurement of humoral responses induced by different antigens such as *Brucella abortus*, sheep red blood cells, or Newcastle disease vaccines (Allan et al., 1972; Giambrone et al., 1976; Giambrone, 1979). The best assessment is clearly the measurement of vaccinal protection against a challenge infection by the Newcastle virus as described in the OIE *Manual of Standards for Diagnostic Tests and Vaccines* since it constitutes a measurement of both humoral and cellular immunity. However, they only give a partial picture of the immunosuppression as, according to the clonal nature of immunity, it will depend on the number of NDV-specific clones that will be destroyed. Using such tests, the most serious and longest-lasting immunosuppression was described when day-old chicks were infected by cIBDV (Allan et al., 1972; Faragher et al., 1974) with duration up to the age of 6 weeks (Giambrone et al., 1976). In field conditions, chickens tend to become infected toward the age of 2–3 weeks, when maternal antibodies decline. Unfortunately, these techniques are time-consuming, laborious, costly, and contrary to animal welfare. Thus, they are usually confined to the evaluation of safety in the IBDV vaccine registration procedures (Guittet et al., 1992).

Recent developments in the field of avian immunology should allow moving in the future from a purely descriptive definition of immunosuppression to a more analytical analysis of the immunocompetence with a closer relationship between structure and function of the implicated immune cells and organs. Structural tests (e.g. relative organ weight, lesions scores, bursametry, bursal imaging, cells counting, etc.) are now more and more completed by functional tests dissecting the different compartments of the immune response (innate, humoral, cell-mediated). These new tools, although still limited to well-equipped laboratories, could, in a near future, be used as pen-side tests to assess the immunocompetence of flocks. Different tests, ranging from structural to functional, are presented in Table 1.

In the case of IBDV, immunosuppression is a direct consequence of the infection of different types of chicken immune cells (B cells, macrophages) that is then followed by the induction of necrosis and apoptosis (Vasconcelos and Lam, 1994; Tham and Moon, 1996; Rodriguez-Lecompte et al., 2005; Wang et al., 2009; Li et al., 2012) or an indirect consequence of the virus-induced changes in the regulation of the immune responses (Schat and Skinner, 2008). In any case, recovery from IBD or subclinical infection will be followed by immunosuppression with more serious

Table 1
Different structural to functional tests for the assessment of immunosuppression.

At level of	Structural tests		Functional tests	
	Descriptive	Analytic	Direct	Indirect
Host	• Uneven growth and stunting • Tired, depressed birds • Increased respiratory reactions • Poor weight gain and feed conversion – birds do not reach performance objectives • Increased incidence of secondary infections			Humoral response: decreased IgM and IgG antibodies response after administration of a heterologous antigen (SRBC, *Brucella abortus*, NDV)
Organ	• BF is turgid, oedematous, and haemorrhagic • Inflammation of non bursal lymphoid tissues (spleen, thymus) • Swollen kidneys • Ecchymotic hemorrhages in the muscle and the mucosa of the proventriculus	• Histochemistry: depletion of lymphoid cells in the BF and non-bursal lymphoid tissues • Relative organ weights and lesions scores (bursa or thymus) (E.g.: bursameter) • Nephrosis	Detection of immune related gene expression in BF, spleen or blood highlights a decreased or increased production of cytokines	
Cell		• Evaluation of immune responses to vaccination realized on the field (ei NDV antibody titers) > vaccination failures • Cell counting: depletion or infiltration of lymphocytes and/or macrophages in lymphoid tissues (Mabs, FACS, Immunohistochemistry, confocal microscopy, etc.)	Innate immunity: reduction of monocytes/macrophages functions (phagocytosis) and of NK-like activity	Cellular response: decreased mitogenic activation of lymphocytes measured by proliferation tests or IFNg detection

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consequences if the strain is very virulent and infection occurs early in life.

Although the immunosuppression caused by IBDV is principally directed towards B-lymphocytes, an indirect effect on cell-mediated immunity (CMI) has also been demonstrated (Cloud et al., 1992a,b; Sharma and Fredericksen, 1987; Sharma et al., 1989) therefore increasing the impact of IBDV on the immunocompetence of the chicken. The infiltrated T cells constituting the majority of the bursal population after IBDV infection are unresponsive to mitogen activation at days 4 and 9 days pi (McNeilly et al., 1999; Rauw et al., 2007). Moreover splenocytes from IBDV-infected chickens were also shown to be deficient in secretion of ChIL-2 (Kim et al., 1998; Sharma and Fredericksen, 1987). It is now well accepted that macrophages are the central effector cells of the innate immune system and influence the nature of the adaptive immune response. In a study performed at 3 and 5 dpi, spleens of virus-exposed chickens had fewer macrophages than those of virus-free controls; the robust expression of proinflammatory cytokine transcripts, along with a decrease in macrophage numbers, suggest that IBDV activates and may lead to a reduction of resident macrophages *in vivo* (Palmquist et al., 2006). However, it remains unclear how these changes play a role in immunosuppression (Schat and Skinner, 2008). Mechanisms like the development of suppressor macrophages and the impairment of helper T-cells have been suggested to explain this clear impairment of the activation capability (Sharma and Fredericksen, 1987; Vervelde and Davison, 1997). As described previously, IBDV is a potent inductor of ChIFNγ production, which has been demonstrated as able to inhibit the *in vitro* mitogenic proliferation of T cells (Sharma et al., 2000) and ChIFNγ production of splenocytes from naive chickens (Rauw et al., 2007). In mice, it has been shown that IFNγ is responsible for the decrease of IL-2 production by splenocytes (Gajewski et al., 1988; Bradley et al., 1996). Thus, IBDV modulates T cell functions: activated T cells will overproduce ChIFN-γ that, in turn, will stimulate macrophages for the production of pro-chemokines and cytokines. Afterwards, this up-regulation in T cell activation is followed by a feedback down-regulation in convalescent chickens, leading to T cell immunosuppression, of which the duration and importance will depend on the strain and dose of virus as well as on the age and breed susceptibility of the chickens. It can thus reasonably be concluded that overproduction of ChIFN-γ plays a pivotal role in the pathogenesis and immunosuppression induced by IBDV (Rauw et al., 2007) (Fig. 3). The acquired immunosuppression can thus also be demonstrated *ex vivo* by using proliferation tests (Confer et al., 1981; Confer and MacWilliams, 1982; Karaca et al., 1996; McNeilly et al., 1999; Sharma and Lee, 1983) or by measuring cytokine release after mitogen activation of T cells (Lambrecht et al., 2000). The measurement of ChIFN-γ after mitogen or antigen recall stimulation might thus be a good indicator of immunosuppression in chicken after IBDV infection or immunocompetence of flocks in general (Fig. 4).

5. Control & vaccination: A key role of IBD vaccination in the vaccination programme

An important characteristic of IBDV is its high stability in the environment, even after disinfection. Indeed, the virus can persist in installations for 54–122 days (Benton et al., 1967). Due to the stable nature of the virus and the large amounts excreted following infection, it is practically impossible to remove all sources of infection once a rearing site has been contaminated. There is evidence, however, that thorough cleaning and disinfection of houses between flocks and the practice of all-in all-out management reduces the challenge virus. It may also delay challenge thus allowing more time for vaccines to induce immunity.

In practice, control of IBD is greatly dependent upon the use of vaccines. Taking all the previous considerations into account, a satisfactory vaccine should protect against the disease, especially the acute phase, and the consequences of the disease, namely immunosuppression. Humoral immunity plays a decisive role in protection against IBD. There is indeed a close correlation between titres in neutralizing antibodies on the one hand and protection on the other hand. This is borne out by the excellent passive protection provided by maternal antibodies against mortality, lesions of the bursa and immunosuppression, respectively. The half-life of the passive antibodies, depending on blood volume, varies between 3 (for broilers) and 5 days (for laying hens). However, vaccination can be disturbed by the interference of maternal-derived antibodies (MDA) (Block et al., 2007). Indeed, MDA are transmitted from the mother to her offspring through the yolk and protect the chicks until the development of the adaptive immune response (Davison, 2008). Thus, if one knows the antibody titre of a chick at birth, one

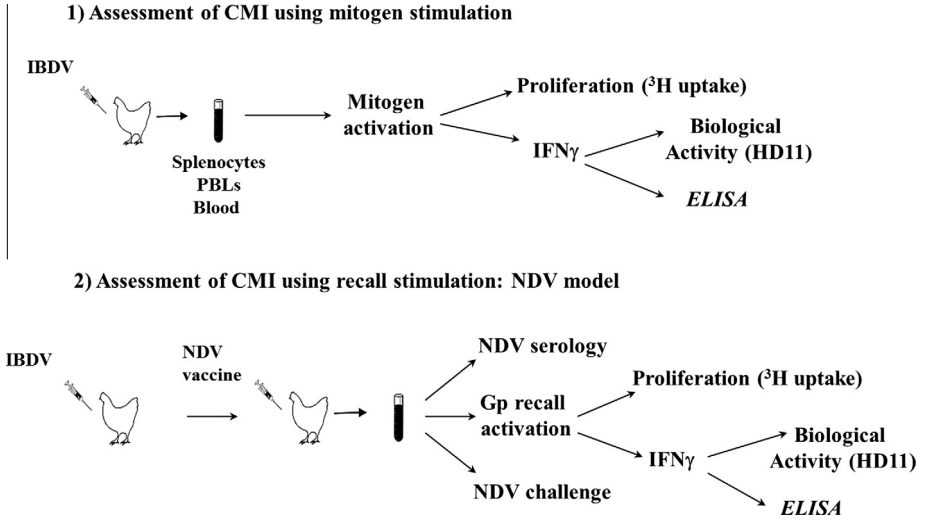

Fig. 4. Assessment of cell-mediated immune response after *ex-vivo* stimulation of T lymphocytes. Although the immunosuppression caused by IBDV is principally directed towards B lymphocytes, an effect on cell-mediated immunity has also been demonstrated. Therefore, immunosuppression can be measured *in vitro* by using proliferation tests or by measuring cytokine (ChIFNγ) release after mitogen (1) or antigen recall (2) activation of T cells using either the HD11 biological assay or a specific capture enzyme-linked immunosorbent assay (ELISA) for chicken IFNγ.

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can determine the time of maximum susceptibility to the vaccine. This determination is very important when establishing vaccination programs (De Herdt et al., 2005). Neutralizing antibodies are protective and these can be provided by immunizing chickens with live attenuated vaccines given in the drinking water. In addition, a live vaccine will induce a strong CMI response, the role of which is still unclear but important. Indeed, protection in the absence of virus-specific antibodies and studies with T cell compromised chickens have indicated that functional T cells are needed to control IBDV replication during the acute phase of infection (Yeh et al., 2002; Rautenschlein et al., 2002). It has been recently shown that a dominant fragment of VP2 can induce humoral and cellular immunity against IBD and elicits a protective immune response in chickens better than the available attenuated viral strains and thus could be used as or in a vaccine (Pradhan et al., 2012).

To obtain high levels of MDA in the progeny, parent stock are vaccinated between 4 and 10 weeks of age with live vaccine and again at approximately 16 weeks with inactivated oil-adjuvanted vaccine. In the progeny, the MDA levels wane with time, but may protect against virulent challenge up to between 2, 5 and 3, 5 weeks of age. Mild vaccine strains that cause no bursal lesions cannot be used effectively in chicks with MDA until about 4 weeks of age as they are neutralized. Moderately virulent vaccine ('intermediate') strains that are less affected by MDA can be given with some success as early as 2–3 weeks of age, depending upon MDA titres. As MDA levels may vary within a flock and between flocks repeated vaccination is practiced by some in order to ensure that chicks are actively immunized as soon as the MDA levels have waned to a level at which they do not neutralize the vaccine. Intermediate and hot vaccine strains can induce bursal lesions and cause immunosuppression (Mazariegos et al., 1990). The intensive use of this kind of vaccines increased preoccupations about residual pathogenicity and about the decreasing effectiveness of vaccines against other diseases.

The ideal IBDV vaccine should thus be safe and capable of priming an immune response after a single inoculation *in ovo* or at hatching in the presence of MDA. Different approaches have been investigated to achieve these goals but, so far, only two kinds of new generation vaccines have been successfully developed and commercialized: herpes virus of turkey (HVT) recombinant vector vaccines and immune complexes.

The first successful new concept was the establishment of immune complex vaccines (Icx). These vaccines consist of a mixture of specific hyperimmune neutralizing antiserum (or "virus neutralizing factor") with a vaccine virus under conditions that are not sufficient to neutralize the vaccine virus but which are sufficient for delaying the pathological effects of the vaccine alone. This allows young chicks to be vaccinated even with a strain that would be too virulent for use *in ovo* or at hatching (Whitfill et al., 1995). Other advantages of the Icx vaccines are that they are effective in the presence of maternally derived antibodies (Giambrone et al., 2001) and can be delivered by subcutaneous injection at 1 day old in the hatchery (Ivan et al., 2005) or, alternatively when the egg injection equipment is available, they are also suitable for *in ovo* vaccination at day 18 of incubation (Haddad et al., 1997). Although the mechanism of action is still poorly understood, such IBDV complexing with specific antibodies cause a delay in virus detection of approximately 5 days, thus decreasing the viral load in the bursa and therefore, the bursal lesions, with a remarkable low level of depletion of bursal and splenic B lymphocytes (Ivan et al., 2005; Jeurissen et al., 1998).

The second concept is based on the widely used Marek's disease vaccine made of the serotype 3 HVT, which is well known to be safe and poorly sensitive to MDA interference, and this is why it was developed as a vector for IBD (Le Gros et al., 2009). Indeed, it has been shown in different comparative studies that MDA interfere with humoral response in vaccination with intermediate live vaccines but do not have any impact on immunization with HVT recombinant vector vaccines containing the VP2 sequence (Bublot et al., 2007; Le Gros et al., 2009; Zorman Rojs et al., 2011). Remarkably, it was also observed that this vector vaccine provided protection from a challenge with variant IBDV (Perozo et al., 2009). This vectored vaccine removes the dilemma of considering safety versus efficacy for IBD vaccination that poultry veterinarians currently face with the use of classical IBDV vaccines. Furthermore, it also protects against MD, and its use in the hatchery, where the vaccination procedure is very well controlled, reduces the need to administer vaccine by drinking water on poultry farms.

Other approaches are still in experimental phase and have not been brought to practice so far (for review, see Maghoub, 2012; Muller et al., 2012). Among these, other vectors have been engineered to express VP2 or the polyprotein VP4-2-3 such as Newcastle disease virus (NDV), fowlpox (FPV) or adenovirus but they suffer from safety or efficacy often related to interference of MDA issues. Indeed, although recombinant vaccines possess the advantage of a bivalent vaccination when the vector is a vaccine strain, they have the limitations of the vector in terms of safety and efficacy and, if the vector is sensitive to MDA, so will the recombinant be. In addition, depending on the site of insertion and expression, some interference against the insert might also be observed. This might be particularly critical and is probably one of the reasons why the only recombinant vaccine commercialized so far is the HVT vector expressing VP2. Likewise, sub-unit vaccines (produced in prokaryotes, yeast or baculovirus) and DNA vaccines have shown promises but also limitations. Although the first were expected to advantageously replace inactivated vaccines, they cannot be used for priming and they still suffer from the costs of production. DNA vaccines are safe and insensitive to MDA but they are not sufficiently immunogenic to replace attenuated vaccines. Indeed, in addition to individual variability in the immune response and general low humoral response, the results showed that a single DNA delivery without a boost vaccination was not always sufficient to induce protective immunity and therefore necessitate multiple administrations or boost with another vaccine, thus raising the cost issue.

6. Conclusion: "There is nothing permanent except change" (Heraclitus)

A clinical picture of IBD has dominated the field in different parts of the world since more than two decades, with high mortality rates and considerable economic losses. Antigenically and genetically homogeneous vvIBDV-like strains have apparently spread in most countries and are now frequently isolated worldwide from acute cases. This sudden and dramatic emergence has stimulated research in IBDV due to the need of new adequate control measures. Important progress has been made in the range of molecular virology, epidemiology of the disease, pathogenesis and development of new vaccines.

The role of T cells, macrophages and dendritic cells in the disease has been demonstrated. Particularly, T lymphocytes have recently been shown to contribute to the onset of the acute disease. Although their role in clearance of the virus and recovery from the disease is essential, their function is exacerbated after infection with vvIBDVs. Hopefully, current and future research in the field of avian immunology will allow a better understanding of the immunological mechanisms involved in the disease but also give tools for the measurement of immunosuppression in the field situation and, therefore, a better identification of protective criteria. This would allow early recognition of problem flocks and will facilitate further studies to determine the cause of the condition.

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Recent increases in understanding of immune modulation and the rapid development of recombinant products for manipulation of immune responses could potentially offer a means of treatment of these conditions, once an accurate diagnosis has been reached.

However, additional research is still needed to overcome some of the current obstacles. Particularly, the identification of a virulence marker, through a better understanding of the host–pathogen interactions and of the underlying molecular mechanisms, is essential. In this regards, the reverse genetic system, providing the tool to construct chimeric viruses, will be decisive for the identification of virulence markers and the genetic attenuation of strains. Likewise, future research needs to be focusing on the molecular mechanisms between viral proteins and host cells, and particularly cytokine regulation. This should allow the development of more appropriate control measures in order to afford a higher degree of protection to young birds that carry maternal immunity.

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