Deciphering desirable immune responses from disease models with resistant and susceptible chickens

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ABSTRACT Coccidiosis and necrotic enteritis (NE) are among the most significant diseases affecting the poultry industry. These diseases have become more prominent in the wake of policies to reduce the use of antibiotics in animal production. This has led to more research focused on better understanding the immune system and its responses to pathogen challenge, and thus developing informed strategies to exploit immune responses that can support enhanced disease resistance and growth performance. Some chicken breeds and lines show greater resistance or susceptibility to various diseases, and thus these birds maybe able to shed light on immune processes or pathways that contribute to the more resistant/susceptible state. This review attempts to identify potentially important genes that show some consistency in (relative) up or downregulation in key tissues between the resistant and susceptible chickens. For coccidiosis and NE, relative downregulation of IL-10 and (slightly less consistently) upregulation of IFN-γ appear to be features of more resistant birds. Data for IFN-α, IL-12, and IL-17D are currently less consistent. Gene expression data from NE studies have identified some potentially interesting, perhaps less well understood, immune-related genes (e.g., TCF12, BCL2, IRF2, TRAF3, TAB3, etc.,) that maybe associated with the resistant and/or susceptible phenotype. *Salmonella* and *Campylobacter* are important foodborne pathogens harbored by the chicken intestinal tract, while infectious bursal disease and infectious bronchitis are also important viral diseases of poultry. We, therefore, consider whether there are consistent features from resistant/susceptible disease models with these pathogens that relate to findings from the coccidiosis and NE studies. It is not anticipated that ideal immune responses to these pathogens will be identical but rather that consistent elements maybe identified that could help inform breeding or alternative strategies to support general disease resistance and enhanced (and efficient) flock productivity.

INTRODUCTION

Coccidiosis and necrotic enteritis (**NE**) are major intestinal disorders of chickens and were identified as the top two issues affecting broiler production by US poultry veterinarians in a recent survey (Hofacre and Mathis, 2015). It is estimated that coccidiosis and NE cost the poultry industry around US$ 3 and 6 billion per annum, respectively, through lost productivity, prevention strategies, and/or therapeutic interventions (Wade and Keyburn, 2015). The true cost to the industry may,

however, be significantly greater due to subclinical or undiagnosed disease. In addition, there is a close association between coccidiosis and NE, with coccidiosis being a recognized predisposing factor for the development of NE (Williams, 2005).

Following the European Union ban on the use of antibiotics for growth promotion in 2006, there has been a global focus on reducing overall antibiotic use in animal production and reserving their use for appropriate therapeutic purposes. Even here, there is a desire to review production practices and evaluate strategies that may facilitate reduced therapeutic use. The US has also seen significant changes with major producers implementing "no antibiotics ever" or "reduced use" production systems. However, these changes can increase the occurrence of coccidiosis and NE (Gaucher et al., 2015), the significance of other, for example immunosuppressive, diseases, the need to fundamentally review the way in which birds are raised and managed, and the thorough consideration of alternative strategies that can underpin future production practices.

These significant developments in the poultry industry are necessitating a holistic overview and approach to disease prevention and control, and profitable productivity. The immune system is obviously fundamental to bird health, and there is much focus on optimizing immunity, ideally in ways that do not compromise, but rather enhance, overall bird/flock performance. Achieving this requires a good understanding of immune processes and their relationship with productive parameters. In this regard, some chicken breeds and lines naturally demonstrate increased relative resistance or susceptibility to common poultry pathogens, influenced by genetics, including genes within the major histocompatibility complex (**MHC**) region of the genome (Kim et al., 2009). Understanding the key, perhaps even subtle, differences between more resistant or susceptible birds could be fundamental to exploiting key immune pathways or processes to improve bird health without compromising, or even enhancing, (efficient) performance.

COCCCIDOSIS

Coccidiosis is caused by species of the *Eimeria* parasite and is probably the most significant enteric disease affecting chickens. *Eimeria* are ubiquitous and can persist in the environment for long periods (Blake and Tomley, 2014). There are seven species of *Eimeria* that are known to infect chickens, with *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* being the most prevalent and pathogenic for broilers (McDougald, 1998). Each *Eimeria* species is recognized for targeting specific regions of the gut. *E. acervulina* affects the proximal small intestine, *E. maxima* affects the mid-intestine, and *E. tenella* affects the ceca (Hammond and Long, 1973). Following ingestion of a sporulated *Eimeria* oocyst, mechanical disruption and digestive processes release four sporocysts and subsequently their sporozoites, which attach to, and invade, epithelial cells in the susceptible region of the intestine (Blake and Tomley, 2014). The sporozoite then proceeds through further developmental phases (e.g., trophozoite, merozoite, gamete, zygote, etc.,) in the host before the formation of an unsporulated oocyst that is excreted into the environment, where it eventually sporulates. Invasion of epithelial cells causes damage to the epithelium and leads to haemorrhagic or malabsorptive disease, which results in poor growth performance or even death. Whilst good husbandry can play a part in managing coccidiosis, anticoccidial drugs and vaccination are necessary options for coccidiosis control, but each has their own drawbacks. Resistance to anticoccidial drugs has been a problem for many years, and vaccination costs can be prohibitive for the broiler industry (Blake and Tomley, 2014).

Inbred lines of chickens have been reported to have differing susceptibility to *Eimeria*, with relatively resistant lines having higher *in-vitro* peripheral blood lymphocyte and T-cell proliferative responses to sporozoite antigen, before and after *Eimeria* infection, than more susceptible lines (Lillehoj, 1986; Bumstead et al., 1995). Subsequent studies with bird lines differing in their susceptibility to *Eimeria* have shown that more resistant birds had higher serum and duodenal IL-2 (after secondary infection; Li et al., 2002) and greater cecal nitric oxide (**NO**; Lillehoj and Li, 2004) following *E. tenella* infection, while downregulation of jejunal liver-expressed antimicrobial peptide 2 has been associated with greater susceptibility to *E. maxima* infection when comparing two commercial broiler lines (Casterlow et al., 2011). More recently, a chicken line more susceptible to *E. maxima* infection (15I) had a greater increase in serum IL-10 than a more resistant line (C.B12), while there were earlier increases in the expression of IFN-γ and IL-10 (and IL-21) in the gut of the more resistant line (Bremner, 2018). Table 1 outlines some of the key observations from studies employing quantitative RT-PCR to compare immune-related gene expression in inbred White Leghorn (**WL**) (Rothwell et al., 2004) and Fayoumi (**F**) (Kim et al., 2008) lines differing in susceptibility to *E. maxima*. In resistant lines of both breeds, expression of IFN-γ was, generally, relatively increased in the spleen and intestine (F) of resistant birds on various days up to 6 d postinfection (**dpi**), while IL-10 was relatively reduced (constitutive and 2 to 9 dpi), compared to more susceptible birds. Other notable features of more resistant F birds were relative upregulation of IFN-α (4 and 5 dpi) and IL-17D (4 dpi) in jejunal intraepithelial lymphocytes (**IEL**) and IFN-α (4 dpi) in the spleen, and relative downregulation of IL-12 (5 dpi), IFN-α (5 dpi), and IL-17D (4 and 5 dpi) in the spleen and IL-17D (5 dpi) in jejunal IEL.

NECROTIC ENTERITIS

Pathogenic strains of *Clostridium perfringens* are responsible for NE, with those expressing the NetB toxin a definitive cause in disease models (Keyburn et al., 2008). *C. perfringens* normally inhabit the gastrointestinal tract (**GIT**) but these are typically nonpathogenic strains. Compromised intestinal health allows pathogenic, toxin-secreting strains of *C. perfringens* to become established and proliferate. The toxin(s) causes pore formation in the plasma membrane of cells, leading to epithelial cell death and the formation of necrotic lesions in the (small) intestine (Timbermont et al., 2011). Simple infection with pathogenic *C. perfringens* alone is not sufficient to cause NE (Moore, 2016), and thus other factors contribute to the proliferation of pathogenic strains, increased production of toxin(s) and the development of disease. As mentioned previously, coccidiosis is a key predisposing factor, along with excess protein, nonstarch polysaccharides, wet litter, and mycotoxins. Birds are typically affected between 2 and 6 wk of age, which likely reflects diminishing passive immunity, while the bird's immune system remains functionally suboptimal. As noted, NE

Table 1. Gene expression changes for more resistant relative to susceptible phenotype for relevant coccidiosis and NE studies.

	Eimeria (coccidiosis)				*Eimeria/C. perfringens* (necrotic enteritis)			
	Rothwell et al., 2004		Kim et al., 2008		Truong et al., 2015a,b		Truong et al., 2017a, b	
Breed	White Leghorn		Fayoumi		White Leghorn		White Leghorn	
Lines	C.B12 (R), 15I (S)		M5.1 (R), M15.2 (S)		6.3 (S), 7.2 (R)		6.3 (S), 7.2 (R)	
Age	21 doa		28 doa		14 doa		14 doa	
Organism	*E. maxima*		*E. maxima*		*E. maxima/C. perfringens*		*E. maxima/C. perfringens*	
Infection phase	3, 6, 9 dpi		3, 4, 5 dpi		2 (to 6) dpi		2 (to 6) dpi	
Tissue/cell	Small intestine	Spleen	Jejunal IEL	Spleen	Intestinal mucosa	Spleen	Ileal mucosa	Spleen
IFN-α			↑(4 and 5 dpi)	↑(4), ↓(5 dpi)		↓	↓	
IFN-γ		↑(6 dpi)	↑(3 and 5), ↓(4 dpi)	↓(0), ↑(3, 4, 5 dpi)		↑	↑	↓
IL-10	↓(6 and 9 dpi)	↓(C and 6 dpi)		↓(0 dpi)	↓			↓
IL-12				↓(5 dpi)	↑	↑		
IL-17D			↑(4), ↓(5 dpi)	↓(4 and 5 dpi)	↑			

(R) = resistant; (S) = susceptible; doa = days of age; dpi = days post infection; C = constitutive; IEL = intraepithelial lymphocytes.
↑↓ Significant or >2-fold difference in expression for more resistant phenotype vs. susceptible.

has emerged as a significant intestinal disease since the use of antibiotics as growth promoters began to be phased out (Van Immerseel et al., 2016). Thus, vaccination could be an attractive alternative to antibiotic use but there remain many gaps in our knowledge, particularly with regards to the pathogenesis of NE and immunity. While NetB seems key to the onset of NE, the target cell(s), cell receptor, or how this toxin causes necrotic lesions with inflammation are currently unknown (Van Immerseel et al., 2016), and this lack of knowledge currently undermines attempts to develop effective vaccines.

Studies with chicken breeds/lines seemingly differing in their susceptibility to experimental NE have identified some differences in immune parameters. Hong et al., (2012) reported that a commercial broiler line more resistant to NE (Jang et al., 2013) showed increased relative expression of jejunal mucosal IL-17F and various avian β-defensins (**AvBD**; AvBD 1, 3, 4, 6, 8, and 10), while a few AvBD (11 and 13) were relatively decreased, compared to a more susceptible broiler line at 2 dpi with *C. perfringens* (6 dpi with *E. maxima*). Other studies have utilized the highly inbred WL Avian Disease and Oncology Laboratory lines 6.2 and 7.3 or F chicken lines M5.1 and M15.2, which were originally identified/selected based on their susceptibility to Marek's disease and/or avian leukosis retroviruses (Bacon et al., 2000; Kim et al., 2008), respectively. These lines have demonstrated some differences in susceptibility to experimental NE (Kim et al., 2014; Kim et al., 2015) and reported differences in the relative expression of various immune-related genes (e.g., BCL2, TAB3, TCF12, VEGFA, etc.,) in the intestine and spleen between the lines (Dinh et al., 2014; Kim at al., 2014; Kim et al., 2015), as well as the expression of some of their regulating microRNAs (Dinh et al., 2014). Table 1 again highlights the relative expression of some specific genes in the intestinal mucosa and spleen of the WL lines (6.3 and 7.2). Following *E. maxima* challenge at 14 doa and *C. perfringens* at 18 doa, IL-12 and IL-17D were relatively upregulated in the spleen or intestinal mucosa or both at 20 doa in the seemingly more resistant line, while IFN-α and IL-10 were both shown to be relatively downregulated in both sites. Data for IFN-γ were less consistent with relatively increased expression reported in the intestinal mucosa and spleen of the more resistant line, but also a relative decrease in the spleen in another study at the same timepoint (20 doa).

INTERPRETATION OF IMMUNE-RELATED GENE EXPRESSION IN BIRDS MORE OR LESS RESISTANT TO COCCIDIOSIS AND NE

Although gene expression data has its limitations due to only being a (n initial) step in the production of a functional protein, these data are, nevertheless, informative. It is of interest to consider whether there are common immune-related features that may contribute to greater resistance to coccidiosis and necrotic enteritis. Of course, such interpretation must keep in mind that time (e.g., day) post infection that samples for analysis are obtained only provide a snapshot in time of (relative) gene expression in a dynamic environment. For example, Kim et al., (2008) reported that at 3 dpi IFN-γ was relatively upregulated, downregulated 4 dpi, and upregulated again 5 dpi in jejunal IEL of the more resistant line. In addition, site of investigation is also an important consideration. Most studies have interrogated the primary site of infection (intestine) or a key secondary lymphoid organ central to systemic immune responses (spleen). The most consistent feature from Table 1 is that IL-10 is either constitutively or in response to infection relatively down-regulated/less upregulated in chicken lines apparently more resistant to coccidiosis and NE. IL-10 is generally considered an anti-inflammatory cytokine that helps regulate inflammatory responses to various pathogens (Couper

et al., 2008) and contributes to intestinal homeostasis (Manzanillo et al., 2015). IL-10 maybe produced by a variety of innate (e.g., dendritic cells, macrophages, monocytes, etc.,) and acquired (various T cell subsets and B cells) immune cells (Wu et al., 2016). chIL-10 has been shown to inhibit the expression of IFN-γ by mitogen-activated splenocytes (Rothwell et al., 2004), and thus it is not surprising to see that more resistant lines, with relatively reduced expression of IL-10, generally have relatively increased expression of IFN-γ in the spleen and intestine (or cells; Table 1). In addition, neutralization of chIL-10 by monoclonal antibodies upregulated the production of NO (cytotoxic towards various pathogens) by lipopolysaccharide-stimulated chicken bone marrow-derived macrophages (Wu et al., 2016). It is also interesting to note that IL-10 was not detected in the serum of healthy (uninfected) birds, whereas a substantial increase in serum IL-10 was reported 5 dpi in birds challenged with both a high and low dose of *E. tenella*, leading to the suggestion that *Eimeria* infections may cause systemic immunosuppression and that serum IL-10 could be a marker for infection (Wu et al., 2016). Moreover, IL-10 inhibits the proliferation of Th cells and production of various associated cytokines (Couper et al., 2008), and, in conjunction with the observations outlined above, the immunoregulatory activities of IL-10 may compromise vaccine efficacy and anti-IL-10 interventions may prove effective methods to improve vaccine responses (Darrah et al., 2010). Whilst concern is often expressed about the potential negative effects of inflammation on the growth performance of animals (Broom and Kogut, 2017), it is important to note that, where provided, better growth performance is a parameter used to determine the more resistant chicken line to coccidiosis or NE challenge in the studies discussed above. Therefore, (relative) reduction of IL-10 can be associated with improved indicators of gut status (e.g., reduced lesions; Kim et al., 2015) and growth performance when birds are confronted with ubiquitous intestinal pathogens. With this in mind, it has been reported that feeding antibodies against IL-10 improves the performance of *E. acervulina*, *E. maxima*, and *E. tenella* challenged broiler chickens and thus may offer an alternative strategy to alleviate the impact of coccidiosis (Arendt et al., 2016; Sand et al., 2016).

An (excessively) immunoregulatory cellular environment may not, therefore, be beneficial, while the relative enhancement of IFN-γ (type II interferon) appears to contribute to the resistance of birds to coccidiosis and NE. IFN-γ is regarded as a key effector of cell-mediated immunity and numerous antimicrobial functions. IFN-γ is produced by various innate and acquired immune cells and increases antigen processing/presentation, leukocyte trafficking, natural killer (**NK**) cell and macrophage activity, ROS and NOS production, autophagy, secretion of proinflammatory cytokines, and influences antibody responses (Kak et al., 2018). These functions are coordinated to control

and eliminate the pathogen (and minimize associated tissue injury), notably intracellular pathogens such as *Eimeria*. The data available for IFN-α, IL-12, and IL-17D are somewhat less consistent. IFN-α is a prominent type I interferon (along with IFN-β) predominantly produced by hematopoietic cells, notably plasmacytoid dendritic cells, in mammals (Ivashkiv and Donlin, 2014). When type I IFNs bind to their cognate receptor (interferon-alpha receptor), it leads to activation of pathways regulating interferon-stimulated genes (**ISG**) and enhanced antigen presentation, regulated inflammasome activation, and upregulation of pro-inflammatory cytokines (Garrido et al., 2018). Garrido et al., (2018) reported that IFN-α induced the expression of ISG (at 6h post stimulation) by chicken macrophages to a greater extent than IFN-β (while IFN-β was a stronger inducer of pro-inflammatory cytokine gene expression (IL-1β, IL-6, and IL-8)). IL-12, produced by various immune cells (e.g., dendritic cells, macrophages, neutrophils), drives inflammatory Th1 responses, production of IFN-γ, and the cytotoxic activity of NK and CD8+ T cells (Issaranggun Na Ayuthaya et al., 2018). IL-17D is a member of the IL-17 family of cytokines produced by Th17 cells, is expressed in a wide range of tissues and organs, and stimulates the expression of IL-6 and IL-8 by various cells (Min et al., 2013). Therefore, the various identified cytokines contribute to determining the (degree of) inflammatory milieu, with various interactions, that influence effector cells and their functions in response to pathogens.

The focus so far has been on those genes/cytokines that have, historically, been regarded as of particular interest and/or have been selected for interrogation. The advent of global transcriptome profiling has helped to identify genes whose function maybe less well understood. However, in time, some of these genes and their respective pathways could be particularly important in determining the susceptibility or resistance of chickens to diseases. Table 2 outlines some genes, and their functions, from NE studies that maybe particularly relevant in determining disease susceptibility/resistance based on consistent (relative) up or downregulation in more resistant birds.

SALMONELLA AND *CAMPYLOBACTER*

Salmonella and *Campylobacter*, specifically *C. jejuni*, are the leading causes of foodborne illness in humans and can be carried at high numbers in the GIT of poultry. *Salmonella enterica* are typically divided into broad host range and host-adapted serovars. Broad host range serovars (e.g., *S. Typhimurium* and *S. Enteritidis*) may cause limited, low-level systemic infection in healthy chickens over 1 wk of age but principally colonize the GIT where they may persist, asymptomatically, for several weeks/months (carrier state; Wigley,

Table 2. Genes potentially of particular interest in the intestine (and spleen) from NE studies.

Gene symbol	Description	Function	General response[2]
TCF12[1]	Transcription factor 12	Direct DNA (E-box) binding and suppresses E-cadherin expression	↓
BCL2[1]	B-cell CLL/lymphoma 2	Apoptosis regulator	↓
IRF2[1]	Interferon regulatory factor 2	Competitively inhibits the IRF1-mediated transcriptional activation of IFN-α and IFN-β	↓
APP	Amyloid beta (A4) precursor protein	Encodes cell surface receptor and transmembrane precursor protein	↓
TRAF3[1]	TNF receptor-associated factor 3	Crucial regulator suppressing c-Jun N-terminal kinase and NFκB signaling, but facilitates type I interferon production	↓
TAB3	TGF-beta-activated kinase 1/MAP3K7-binding protein 3	NFκB signal transduction pathway	↓
SERPINF1[1]	Serpin peptidase inhibitor, clade F, member 1	Heterophil (elastase) inhibitor	↓
ARHGEF6[1]	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	Role in cellular processes initiated by extracellular stimuli working through G protein coupled receptors.	↓
HSP90B1	Heat shock protein 90 kDa beta (Grp94)	Assists folding, maintenance, and degradation of proteins, and protects from excess heat	↓
TNFRSF11B[1]	Tumor necrosis factor receptor superfamily, member 11b	Inhibition of osteoclast differentiation and activity	↓
CALB1[1]	Calbindin 1, 28 kDa	Calcium binding and transport	↑

Based on data from Dinh et al., 2014; Hong et al., 2014; Kim et al., 2014; Kim et al., 2015.
[1]Includes spleen.
[2]Significant or >2-fold difference in expression for more resistant phenotype vs. susceptible.

2014). Host-adapted serovars (e.g., *S. Gallinarum* and *S. Pullorum*) are generally poor colonizers of the GIT but do cause systemic infection, which can lead to significant morbidity and mortality (Wigley, 2014). *Campylobacter jejuni* colonizes the chicken GIT, principally the caeca. Traditionally, *C. jejuni* was considered a harmless gut commensal for chickens and has only really been considered in the context of the risk that poultry intestinal carriage poses to humans. The bacterium can, however, be recognized by Toll-like receptors (4 and 21), initiating an innate, inflammatory response in the chicken intestine, and thus adaptive responses, and may alter the gut epithelial structure and be recovered from the liver and spleen, making the traditional perspective of *C. jejuni* somewhat inaccurate (Humphrey et al., 2014).

It is of interest to consider whether there are common features of the chicken (immune) response to coccidiosis and NE in more resistant and/or susceptible birds that also influence the susceptibility of birds to *Salmonella* and/or *Campylobacter* infection and/or carriage. The caeca are the major reservoirs of microorganisms, including *Salmonella* and *Campylobacter*, and the caecal tonsils, situated at the ileal–caecal junction, are major lymphoid aggregates of the chicken GIT and have thus been studied. At a young age (1 week of age (**woa**)), prolonged downregulation of IFN-γ expression in the caecal tonsils of one inbred line (6^1) was associated with much greater numbers of caecal *Salmonella* than another inbred line (15I) following oral inoculation with *S. enterica* serovar Enteritidis 1009 (Sadeyen et al., 2004).

Chausse et al., (2014) reported that susceptibility to carrier-state (6^1 line) is associated with a bias towards Th2 responses in caecal enterocytes at 21 dpi with *S. enterica* serovar Enteritidis 751 at 1 woa. In older birds (30 woa), increased *Salmonella* resistance was associated with increased expression of IFN-γ, AvBD1, and AvBD2 (as well as IL-8, MIM-1, TLR4 (earlier), iNOS, IL-18) by caecal tonsils, although, at this age, line 6^1 birds were more resistant to both caecal persistence and systemic colonization than line 15I (Sadeyen et al., 2006). After breaching the intestinal epithelium, *Salmonella* principally interact with macrophages (primary target) and heterophils (primary responders to infection in chickens; Wigley, 2014), and thus the characteristics of these cells, isolated from more or less resistant chicken lines, have been assessed. Peripheral blood macrophages and heterophils, derived from 8 to 12 woa and 1 doa chicken lines, respectively, demonstrated greater basal and/or more rapid expression of IL-6, CXCLi2/IL-8, and IL-18 by cells from more resistant lines following stimulation with *Salmonella* (or other related phagocytic agonists; Swaggerty et al., 2004; Wigley et al., 2006).

In *C. jejuni* inoculated chickens (1 doa) at 7 dpi, AvBD 10 and 12 were significantly upregulated in the caeca of birds more resistant to caecal colonization by *C. jejuni* (Li et al., 2010). In addition, NALP1 was also upregulated in the caeca of more resistant chickens, and the NALP1 inflammasome is an intracellular pattern recognition receptor multiprotein complex, activation of which leads to the production of the active

forms of IL-1β and IL-18, although their gene expression was not upregulated in the study at the timepoint analyzed.

INFECTIOUS BURSAL DISEASE AND INFECTIOUS BRONCHITIS

Infectious bursal disease (**IBD**) and infectious bronchitis (IB) are both significant diseases afflicting the poultry industry (Hofacre and Mathis, 2015). IBD (also known as Gumboro disease) is a highly contagious disease of young chickens that is caused by the IBD virus, which is a member of the *Birnaviridae* family (Smith et al., 2015a). The virus primarily replicates in B cells expressing Bu-1 and surface immunoglobulin, resulting in apoptosis and atrophy of the bursa of Fabricius (Tippenhauer et al., 2013). The apoptosis of the immature B cells results in severe immunosuppression with impaired antibody and vaccine responses, and increased susceptibility to other pathogens, especially when birds are infected before 3 woa (Aricibasi et al., 2010). Very virulent strains cause acute disease and high mortality in chickens less than 6 woa. IB virus, a coronavirus, replicates in tissues of the upper respiratory tract, although some strains cause more widespread issues by replicating in the kidney, oviduct, and intestinal tract (Awad et al., 2014). IBV can cause severe disease in young birds with morbidity close to 100%. Vaccination, and supporting antimicrobial therapy for complicating/secondary bacterial infections, is the primary methods to prevent and/or counteract the impact of IBD and IB but the continuous emergence of new variant viruses ensures that these diseases remain economically important (Chhabra et al., 2018).

Again, it is of interest to see whether there is commonality in terms of immune-related mediators that are associated with greater resistance/susceptibility to IBD and IB. A more rapid inflammatory response, characterized by upregulation of genes including IL-2, IL-6, CXCLi2/IL-8, IL-18, IFN-α, IFN-γ, and iNOS in the bursa at 1 dpi, was found for a chicken line considered more resistant to IBD following IBDV infection at 1 to 2 doa (Ruby et al., 2006). Other studies in young chickens (3 to 5 woa) have, however, reported that increased, perhaps less well controlled, inflammatory mediators (e.g., IL-1β, CXCLi2/IL-8, IFN-β and IFN-γ) in serum, spleen, and bursa, up to 7 dpi, enhance susceptibility to/pathogenesis of IBDV, particularly more virulent strains (Aricibasi et al., 2010; Tippenhauer et al., 2013; Smith et al., 2015a), although the overall (anti/pro) inflammatory context is not always assessed. With regards to IB, Dawes et al., (2014) evaluated some functional characteristics of macrophages derived from 5 to 12 woa birds apparently more or less resistant to IB and showed that greater resistance was associated with peripheral blood monocytes that differentiated into macrophages more readily and were more responsive (by way of NO production) to (poly I:C or IFNγ)

stimulation. From a slightly different perspective, and similar to some of the data for IBD, the greater pathogenicity and lesion severity in infected tissues of chickens inoculated with one of three different strains of IBV have been associated with upregulation of the proinflammatory cytokines IL-1β and IL-6 (as well as TLR3, MDA5, and IFN-β), indicating that differential innate immune responses to different IBV strains contribute to disease severity (Chhabra et al., 2018).

CONCLUSIONS AND PERSPECTIVES

Based on the available data, it seems that immune cells isolated from more resistant birds show enhanced functional characteristics (e.g., proliferation, NO production, etc.,) and/or more rapid responses. In addition, relevant cells/sites in more resistant chickens appear to, mainly, be supporting a more inflammatory environment as evidenced by the, generally, increased production of pro-inflammatory or relative downregulation of anti-inflammatory/regulatory mediators. Given this review primarily covers intracellular pathogens (*Eimeria*, *Salmonella*, IBDV and IBV), it is perhaps not surprising that a more pronounced proinflammatory, Th1-skewed response maybe observed for more resistant birds. In fact, a key immunoregulatory cytokine, IL-10, was not detected in the serum of healthy birds, and elevated levels were associated with *Eimeria* infection and/or disease susceptibility, leading to suggestions that IL-10 could be employed as a biomarker for (*Eimeria*) infection and/or anti-IL-10 treatment could be used to improve vaccine efficacy (Wu et al., 2016; Bremner, 2018). However, even where IL-10 was more upregulated in more susceptible birds, IL-10 was still expressed in the intestine and spleen of resistant birds, suggesting it helps temper the effector response, rather than (excessively) suppressing it. With this in mind, data proposing that a more pronounced inflammatory response to certain strains of pathogens influences/drives greater pathogenesis should not be overlooked and indicates that the balance of pro, anti-inflammatory, and regulatory mediators at a given site is probably more relevant than any in isolation. Therefore, relevant studies reporting all relevant (pro and anti) inflammatory mediator responses maybe more informative.

We have highlighted the limitations of gene expression data for interpreting important responses of more or less resistant birds. In addition, the specific study variables (e.g., disease model (strain, dose, etc.,), bird age/type, cells/site analzsed, etc.,) and, in particular, sampling point(s) post infection will strongly influence the results obtained. Age at infection may also be a very pertinent factor that explains some of the differences identified. Enhanced inflammatory responses in young birds maybe more beneficial due to their inherently, relatively impaired/suboptimal ability to respond to infection (Broom and Kogut, 2018a), whereas older, more immunologically mature birds may have the

capability to respond excessively. As shown in this review, rapid changes can occur in the dynamic infection environment and relevant genes maybe reported as up or downregulated depending on the time/day sampled. Rothwell et al., (2004) reported no differences in caecal tonsil responses of *E. maxima* infected birds, underlining region-specific responses and the need to sample/analyze relevant site(s). Moreover, clear demonstration/description that a specific study is utilising more or less resistant phenotypes is not always evident and is sometimes based on previous observations. Studies that show clear differences between phenotypes and characterize immune responses in the same study are important. Characterization of the gut microbiome could also be an important parameter influencing disease phenotype and differences in immune parameters (Broom and Kogut et al., 2018b).

It is also interesting that a chicken line described as more resistant at a younger age can be more susceptible at an older age, while greater resistance to one parameter (e.g., carriage) does not necessarily equate to resistance for another (e.g., organ infection). The former may relate to changes mentioned above in immune capability/responses as the immune system matures (Broom and Kogut et al., 2018a), while the latter may reflect differing mechanisms conferring resistance at different sites. We should also consider that fundamental mechanisms contributing to the more resistant phenotype may not be related to well-recognized immune pathways. For example, there maybe differences in the expression of host proteins that bind pathogen proteins (Smith et al., 2015b), that affect speed of expulsion/egress of the organism during functional development (e.g., *Eimeria* sporozoites; Lee et al., 2016), and the ability of (infected) cells to recover (Casterlow et al., 2011), etc. With this in mind, key genes conferring enhanced resistance maybe as yet unidentified or have less well-defined immune-related functions. Some of the genes outlined in Table 2 (related to NE) have been associated with resistance/susceptibility to other poultry pathogens (e.g., IBDV, Smith et al., 2015a; IBV, Smith et al., 2015b) and warrant further investigation.

In practical terms, recent studies have confirmed the benefit of steering bird responses towards a more proinflammatory state through, for example, breeding (for increased expression of IL-1β, IL-6, CXCLi2, and CCLi2) or feeding of antibodies (e.g., anti-IL-10) to improve resistance to *Salmonella enterica* serovar Enteritidis (Swaggerty et al., 2014), *Eimeria* (Swaggerty et al., 2015; Sand et al., 2016), and *Clostridium perfringens*-induced NE (Swaggerty et al., 2016). The use of proinflammatory cytokines as vaccine adjuvants against poultry relevant pathogens has also been investigated (Umar et al., 2015). Given coccidiosis is a primary predisposing factor for necrotic enteritis, effective prevention and control strategies for *Eimeria* should have the added benefit of reducing the incidence and/or severity of NE. As mentioned

previously, growth performance is often a parameter assessed to determine relative disease resistance, but not always. It would be much more informative if all relevant studies included growth performance analysis to better understand the relationship between immune responses, growth, and disease resistance.

Overall, this review highlights some of the genes more consistently associated with relative resistance (or susceptibility) to some of the most important diseases and foodborne pathogens of commercial poultry and helps to inform/support appropriate immune-based strategies to optimize poultry health and productivity. The optimum immune response is balanced to effectively counteract the pathogen and minimize tissue damage and associated nutrient costs. However, it does seem that steering the immune system towards a more proinflammatory capability (in different compartments) provides better protection against pathogens and (thus) improved growth performance. Global gene expression profiling is providing a greater overview of genes and pathways involved in the bird's response to pathogen challenge and mechanisms of resistance. Further studies, particularly incorporating bird growth performance, are, however, necessary to better develop our understanding and application of informed outcomes.

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