Cellular and Molecular Life Sciences (2019) 76:2779–2788
https://doi.org/10.1007/s00018-019-03117-1



REVIEW

# Progress on chicken T cell immunity to viruses

Manman Dai[1,3] · Chenggang Xu[1,3,4,5] · Weisan Chen[2] · Ming Liao[1,3,4,5]

Received: 18 December 2018 / Revised: 14 April 2019 / Accepted: 24 April 2019 / Published online: 17 May 2019


## Abstract

Avian virus infection remains one of the most important threats to the poultry industry. Pathogens such as avian influenza virus (AIV), avian infectious bronchitis virus (IBV), and infectious bursal disease virus (IBDV) are normally controlled by antibodies specific for surface proteins and cellular immune responses. However, standard vaccines aimed at inducing neutralizing antibodies must be administered annually and can be rendered ineffective because immune-selective pressure results in the continuous mutation of viral surface proteins of different strains circulating from year to year. Chicken T cells have been shown to play a crucial role in fighting virus infection, offering lasting and cross-strain protection, and offer the potential for developing universal vaccines. This review provides an overview of our current knowledge of chicken T cell immunity to viruses. More importantly, we point out the limitations and barriers of current research and a potential direction for future studies.

**Keywords** Chicken · $CD8^+$ T cell response · $CD4^+$ T cell response · Virus · Epitope

## Introduction

It is well known that avian-related viruses such as avian influenza virus (AIV), Newcastle disease virus (NDV), avian infectious bronchitis virus (IBV), and infectious bursal disease virus (IBDV) have been causing enormous economic losses in the global poultry industry. Although vaccines have been used for a long time to prevent epidemics in China, these viruses have still been identified and found to induce disease in livestock in recent years [1–4]. The protection provided by commercial vaccines, such as inactivated influenza vaccines, is largely dependent on stimulated neutralizing antibodies, which directly bind to the virus to prevent its entry into host cells [5]. As immune-selective pressure results in the continuous mutation of viral surface proteins, the protective effects of such vaccines against current or upcoming epidemic strains can be significantly decreased.

Vaccines aimed at stimulating T cell responses are currently the subject of intense interest for mammalian studies because they promise both broader strain coverage and longer-lasting protection than current antibody-based vaccines. Therefore, it may also be possible to develop safe and more effective T cell-based vaccines for the poultry industry in the future.

Here, we summarize the published data and provide an overview of progress in understanding chicken T cell

✉ Weisan Chen
Weisan.Chen@latrobe.edu.au

✉ Ming Liao
mliao@scau.edu.cn

Manman Dai
daimanman1229@163.com

Chenggang Xu
chgangxu@scau.edu.cn

1 College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, People's Republic of China

2 T Cell Lab, Department of Biochemistry and Genetics, La Trobe Institute of Molecular Science, La Trobe University, Bundoora, Australia

3 National and Regional Joint Engineering Laboratory for Medicament of Zoonosis Prevention and Control, Guangzhou, People's Republic of China

4 Key Laboratory of Veterinary Vaccine Innovation of the Ministry of Agriculture, Guangzhou, People's Republic of China

5 Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, Guangzhou, People's Republic of China

immunity against the above-mentioned viruses. In addition, this review aims to highlight the limitations and barriers of current research and a potential direction for future studies.

## T cell response

T cells proliferate and differentiate into effector T cells after recognizing an antigen on the surface of an antigen-presenting cell (APC), in the context of major histocompatibility complex (MHC) molecules and costimulation. The recognition of cognate antigen–MHC complexes by T cells requires a "second signal" generated via numerous costimulatory and inhibitory receptors to regulate the extent, quality, and duration of their activation. Structurally, these receptors consist of the immunoglobulin super family (IgSF) and tumor necrosis factor (TNF) family members. Some costimulatory and inhibitory receptor ligand pairs have been characterized in chickens (Table 1), but thus far the vast majority have not been analyzed. Interestingly, the activating receptor CD28 and the inhibitory receptor CTLA-4 bind to identical ligands designated as CD80 and CD86, but deliver opposing signals. Triggering CD28 leads to T cell activation, whereas CTLA-4 ligation inhibits further T cell activation and promotes T cell tolerance [6].

### $CD8^+$ T cells

Peptides derived from endogenous antigen processing are loaded on to MHC class I molecules. Almost all nucleated cells express class I heterodimer molecules on their surface and make use of the specialized MHC peptide binding cleft to display peptides derived from tumor and pathogen antigens, mostly from intracellular locations. $CD8^+$ T cells first recognize the peptide–MHC class I complex via the T cell receptor (TCR), and then proliferate and differentiate into armed antigen-specific cytotoxic T lymphocytes (CTLs). Activated CTLs are able to kill other cells displaying the same peptide–MHC complex. CTLs exert their protective effect via a range of effector mechanisms, including the release of cytotoxic granules that contain perforin and granzymes and the induction of apoptosis via Fas/Fas-L interactions, and via TNF-related apoptosis-inducing ligands and the production of various pro-inflammatory cytokines, including interferon gamma (IFN-γ), macrophage inflammatory protein-1β, interleukin (IL)-2, and TNF-α, which can either directly inhibit viral function or help recruit other immune cells to assist with viral clearance [12, 13]. In this manner, infected cells are eliminated, thereby limiting the replication and spread of invading pathogens. Based on detection of the gene transcription levels of cytolytic molecules in the immune organ, chicken $CD8^+$ T cells are thought to use the Fas/Fas-L and/or perforin–granzyme A cytolytic pathways to clear viruses, such as IBDV, IBV and Marek's disease virus (MDV) [14–17]. The cytotoxic activity of CTLs has been detected in response to MDV-infected splenocytes and cell lines expressing MDV antigens [18]. However, still very few researchers have further studied the potential mechanisms of specific chicken $CD8^+$ T cell responses to virus infection in vitro via monitoring CTL activity, which is a more accurate method, because this assay is expensive, technically challenging, and requires the use of inbred chickens and MHC-matched target cells.

**Table 1** Chicken costimulatory molecules

| Function | Family | Receptor | Ligand | References |
|---|---|---|---|---|
| Activating | IgSF | CD28 (chr.7) | CD80/86 | [6, 7] |
| Activating | IgSF | ICOS (chr.7) | Y08823 | [6, 7] |
| Inhibitory | IgSF | CTLA-4 (chr.7) | CD80/86 | [6, 7] |
| Inhibitory | IgSF | PD1 (chr.9) | ? | [6, 7] |
| Activating | TNFR | CD30 (chr.21) | CD30L | [8, 9] |
| Activating | TNFR | RANK/0PG(chr.2) | RANKL | [10] |
| Activating | TNFR | CD40(chr.20) | CD40L | [11] |

### $CD4^+$ T cells

MHC class II molecules are mainly expressed on the surface of APCs such as macrophages, dendritic cells (DC), and B cells. Exogenous antigens derived from pathogens can be endocytosed or phagocytosed and processed by APCs, and the resulting peptides are presented on the surface of the APC in the context of MHC class II molecules. $CD4^+$ T cells recognize the peptide–MHC-II complex via their TCRs and then exert their protective effect indirectly by "helping" both $CD8^+$ T cells and B cells to eliminate virus and virus-infected cells via cytotoxic and antibody neutralization mechanisms, respectively, although some $CD4^+$ T cells are capable of direct killing [19–21]. For example, $CD4^+$ T cells expressing the cytolytic molecule perforin were detected in IBDV-infected chickens [15]. Effector $CD4^+$ T cells can differentiate into many T helper (Th) subsets, resulting in the production of different cytokine patterns and effector functions. The development of various effector subsets is largely determined by cytokines present during $CD4^+$ T cell priming. The two best characterized effector subsets are Th1, promoted by IL-12 and IFN-γ and inhibited by IL-4, and Th2, promoted by IL-4 and suppressed by IFN-γ. Cytotoxic Th1 cells produce the "signature" cytokines IL-2, IFN-γ, and TNF-α, and Th2 cells produce the "signature" cytokines IL-4, IL-5, IL-13 and IL-10, which that are crucial for regulating immune responses to intra- and extracellular pathogens, respectively [22, 23]. The existence of the Th1–Th2 paradigm in chickens has also been known [24]. However, whether this paradigm holds true

at the cellular and molecular levels and whether chicken Th cells can become terminally polarized to a Th1 or Th2 phenotype remain to be determined. Additionally, chicken $CD4^+$ T cells can be polarized into Th17 cells that produce IL-17A, as determined by genomic analyses [25], infectious studies [26], and phenotype analyses [27, 28]. Additionally, two subpopulations of chicken regulatory T cells (Treg cells) have been identified including TGF-beta$^+$CD4$^+$ T cells and $CD4^+CD25^+$ T cells [29, 30]. Chicken $CD4^+CD25^+$ Treg cells have been shown to express high level of CTLA-4, produce high concentrations of IL-10, TGF-β4, and suppress T cell proliferation in vitro [29]. Chicken TGF-beta$^+$ Treg cells are reportedly involved in the pathogenesis and immunosuppression of MDV infection [30].

### Limitations of studying chicken $CD4^+$ and $CD8^+$ T cell responses

Our knowledge of T cell immunity stems mainly from human and mouse studies, and avian species have their own unique characteristics. Until recently, the majority of studies on the avian T cell response were limited to detecting the proliferation of splenocytes and IFN-γ production due to limited reagent availabilities, detection method, and T cell culture techniques [31–33]. In addition, the lack of an appropriate mAb against chicken cytokines has also hampered the phenotypic and functional identification of chicken T cells. As shown in Table 2, technologies of intracellular cytokine staining (ICS) and T cell line generation have been rarely established and used in chicken T cell immunity research due to limited reagent availability, which prohibits studies on the chicken T cell phenotype, and dominant viral antigen and epitope identification. The current bottleneck regarding our knowledge of chicken $CD4^+$ and $CD8^+$ T cell responses at the cellular and molecular levels must be surpassed.

## Chicken MHC

Compared to the mammalian MHC, the chicken MHC, also called the "B locus", is more compact and organized differently. The B-F/B-L region within the B locus contains the genes encoding classical class I and class $II^\beta$ chains, which present antigens to stimulate immune responses, such as allograft rejection, antiviral and antitumoral immunity, and cellular help for high affinity antibody production [39–43]. Peptide binding motifs have been discovered for a number of common chicken MHC class I alleles (Table 3). The anchor residues involved in binding to the MHC class I molecules of these different alleles were found to be just as fastidious as those described for mammalian MHC class I molecules [39, 44, 45]. Regretfully, there have been no studies on the structure or peptide binding motifs of chicken MHC class II molecules to date. There are several reports showing that chicken MHC class II molecules determine the responses to the inactivated vaccines tested, which might be largely related to stimulation by the binding epitopes [46–49]. Therefore, to better understand the role of chicken MHC class II molecules during virus infection, it is critical to conduct side-by-side structural and functional studies on these molecules.

X represents any amino acids. Anchor residues specific for the different MHC alleles are shown in bold. '/' indicates that either one of the amino acids could be chosen.

**Table 3** Known peptide binding motifs for chicken class I molecules [32]

| MHC class I allele | Anchor residues |
|---|---|
| MHC B12 | X–X-X–X-**V/I-**X-X-(X)-**V/L/I** |
| MHC B4 | X-**D/E**-X-X-**D/E**-X-X-(X)-**E/L/I** |
| MHC B15 | X-**R**-X–X-X–X-X-(X)-**Y** |
| MHC B19 | X-**R**-X–X-X–X-X–**Y/P/L/F** |
| MHC B21 | X–**H/K/R**-X-X–X-X–X-(X)-**E/D-**X-**A/V/L/I/F/M** |

**Table 2** Major methods of detecting T cell responses used in research on mammals and chickens

| Species | Method | Purpose | References |
|---|---|---|---|
| Mammal | CD107 assay | CTL function degranulation | [34] |
| Mammal | IFN-γ-secreting ELISPOT assay | T cell activation | [35] |
| Mammal | ICS assay | $CD4^+$ and $CD8^+$ T cell enumeration and phenotype | [35, 36] |
| Mammal | Generation of virus- or peptide-specific T cell line | Dominant viral antigen or minimal T cell epitope identification | [35, 37] |
| Chicken | Cr release assay | CTL killing | [18] |
| Chicken | IFN-γ-secreting ELISPOT assay | Splenic lymphocyte response | [38] |

'(X)' represents a variable number of amino acids between the anchor residues.

In chickens, two types of class I and class II genes exist; class I genes include the BF2 and BF1 genes, meanwhile class II genes consist of the BLB2 and BLB1 gene. However, it seems most likely that there will be a single dominantly expressed class I (BF2) and class II molecule (BLB2) on the surface of chicken cells, which determines either resistance or susceptibility to a particular pathogen [50]. The peptide binding motifs shown above could therefore potentially be used to predict the corresponding MHC-determined resistance and susceptibility. Chicken MHC B haplotypes have been shown to display differential resistance to several viruses, including Marek's disease virus, avian leukosis virus, NDV, Rous sarcoma virus (RSV), IBV, and avian influenza virus (AIV), as well as *Salmonella* [51–60]. This phenomenon is most likely related to the neutralizing antibody and $CD4^+$ and $CD8^+$ T cell responses stimulated by viral peptides presented by specific MHC B alleles. However, in contrast to what has been described for humans and mice, knowledge of virus epitope-specific $CD4^+$ and $CD8^+$ T cells in chickens is limited.

## Importance of T cell immunity in avian virus infection

In avian virus infection, animal studies clearly indicate a protective role for $CD8^+$ T cells, as summarized in Table 4. For instance, $CD8^+$ T cells can eliminate infectious viruses such as IBV, RSV, and AIV [38, 61, 62]. Moreover, adoptive transfer of activated H9N2 AIV-specific $CD8^+$ T cells into naïve chickens reduced morbidity and enhanced survival following subsequent lethal H5N2 AIV challenge [63, 64]. The latter studies are particularly important because they demonstrate that memory $CD8^+$ T cells protect against AIV and therefore provide a direct rationale for the development of T cell-based vaccines that elicit cross-protective $CD8^+$ T cells with the potential to combat novel AIV strains whose surface hemagglutinin glycoproteins are not recognized by pre-existing antibodies.

Data for chicken $CD4^+$ T cells are almost nonexistent compared to those for their $CD8^+$ counterparts in the context of AIV, IBV, and IBD infection, but they are nevertheless important. A key period for developing immunosuppression of avian leukosis virus subgroup J (ALV-J) infection was identified at 3–4 weeks post-infection, when $CD4^+$ T cell numbers were significantly reduced [65]. In additon, a potential vaccine for ALV-J has been reported to increase the numbers of $CD4^+$ and $CD8^+$ T cells as well as the IL-4 and IFN-γ levels in immunized chickens [66]. Moreover, chicken biliary exosomes significantly inhibited ALV-J replication while promoting the proliferation of $CD4^+$ T cells [67]. Therefore, these findings imply that $CD4^+$ T cells mediate protection against ALV-J.

Taken together, these studies highlight the importance of $CD4^+$ and $CD8^+$ T cells in avian virus infection and strongly suggest that an optimal T cell-based vaccine needs to induce both protective $CD4^+$ and $CD8^+$ T cells.

## Known chicken $CD4^+$ and $CD8^+$ T cell epitopes

To date, 22 $CD8^+$ T cell epitopes and one $CD4^+$ T cell epitope for AIV, five $CD8^+$ T cell epitopes and 10 $CD4^+$ T cell epitopes for IBV, one $CD8^+$ T cell epitope for IBD, and one $CD8^+$ T cell epitope for RSV have been identified according to the Immune Epitope Database (IEDB, http://www.iedb.com, see Table 5).

The published minimal $CD8^+$ T cell and $CD4^+$ T cell epitopes, including those from all linked references in the IEDB, are listed in Tables 6 and 7, respectively. In Table 6, four $CD8^+$ T cell epitopes derived from IBV and one $CD8^+$ T cell epitope derived from RSV have been identified against viral infection by animal experimentations [38, 61],and the detailed information is shown in Table 4. Besides, one $CD8^+$ T cell epitope derived from IBDV was verified that conferred protection to IBDV challenge, as assessed by bursal damage and viremia [68]. In ex vivo functional verification experimentations, 21 $CD8^+$ T cell epitopes in AIV including H5N8, H5N1, and H7N1 subtypes and one $CD8^+$ T cell epitope in IBV (NQFYIKLT) were found to stimulate the activation of avian lymphocytes [31, 32, 69–71]. However, one $CD8^+$ T cell epitope in H9N2 AIV (KILTIYSTV) has just been identified via binding detection analysis instead of functional experiments. In Table 7, the $CD4^+$ T cell epitope derived from AIV (WTILKPSDTINFESN) has been identified to induce $CD4^+$ T cell activation [31]. But, the $CD4^+$ T cell epitopes derived from IBV need to be further identified by modern and more accurate methods.

Almost all these epitopes were pre-screened via either the peptide elution-based method or peptide prediction algorithms. The elution-based method identifies epitopes by eluting peptides from MHC complexes and analyzing them via sequencing, mass spectrometry, or both. Although it works well for identifying many peptide sequences, the peptide elution-based method does not indicate whether a particular peptide is actually immunogenic. Peptide prediction algorithms assign the likelihood of a particular peptide being presented by an MHC molecule based on the peptide sequence and known MHC binding motifs. However, given the polymorphism of MHC alleles and the fact that many immunogenic peptides do not even contain typical binding motifs, this method could be fallible, does not predict epitopes that could possibly

**Table 4** Relevant publications showing that $CD8^+$ T cells are important in avian virus infection

| Key observation | Model | Experimental design | Major findings | Method of detection | References |
|---|---|---|---|---|---|
| $CD8^+$ T cell epitopes were effective against IBV challenge | Specific pathogen-free (SPF) chickens | 1. Spleen lymphocytes from SPF chickens immunized with the S1 protein were stimulated by synthesized peptides and reactivity was tested | Four peptides efficiently stimulated $CD8^+$ T cell proliferation and IFN-γ secretion | ELISpot assay and flow cytometry | [38] |
| | | 2. Chickens were vaccinated with the poly-CTL-epitope DNA vaccine (pV-S1T), and the protection efficacy was analyzed | Ninety percent of immunized chickens were protected after challenge with $10^6$ $ELD_{50}$ of IBV. | Survival | |
| $CD8^+$ T cell epitope from v-Src is immunoprotective against Rous sarcoma virus growth | Chickens with MHC class I allele B-F12 | Chickens were vaccinated with peptides in liposomes. T cell proliferation was detected in vitro and protection efficacy was analyzed in vivo | The tumor incidence in immunized chickens was reduced from 100% to 55.6% after challenge with PR-RSV-C | [$^3$H] thymidine uptake assay, flow cytometry and tumor incidence | [61] |
| Cross-reactive cellular immunity induced by H9N2 influenza viruses protected chickens from lethal infection with H5N1 influenza viruses | B2 syngeneic SPF chickens ($B^2$/$B^2$) | Adoptive transfer of T lymphocytes or $CD8^+$ T cells from H9N2-infected chickens protected naïve chickens from lethal H5N1 influenza virus | Most chickens primed with H9N2 survived lethal challenge; H5N1- and H9N2- specific $CD8^+$ T cells recognized target cells infected with H5N1 | Flow cytometry and CTL assay | [63, 64] |
| T cells primed with H9N2 can react to homologous (H9N2) and heterologous (H7N2) AIV-infected target cells | B2 syngeneic SPF chickens ($B^2$/$B^2$) | Splenic lymphocytes from infected and naïve birds were examined for cross-reactivity against homologous and heterologous low pathogenic avian influenza (LPAIV) infection by ex vivo stimulation | Splenic lymphocytes derived from H9N2-infected birds displayed lysis of both homologous and heterologous isolates of AIV-infected target cells | CTL and proliferation assays | [62] |

**Table 5** Source antigens of the chicken T cell epitopes recorded in IEDB

| Virus | Source antigens | $CD8^+$ T cell epitopes (n) | $CD4^+$ Tcell epitopes (n) |
|---|---|---|---|
| AIV | Hemagglutinin | 2 | 1 |
| | Nucleocapsid protein | 2 | 0 |
| | Nucleoprotein | 13 | 0 |
| | Matrix | 5 | 0 |
| | Total | 22 | 1 |
| IBV | S1 glycoprotein | 5 | 5 |
| | S2 protein | 0 | 1 |
| | Nucleocapsid protein | 0 | 4 |
| | Total | 5 | 10 |
| IBD | VP2 | 1 | 0 |
| RSV | v-src | 1 | 0 |

be post-translationally modified, and is unable to predict immunodominant epitopes [12]. In addition, the above chicken T cell epitope functional verification assay is limited to detecting the peptide-stimulated lymphocyte proliferation or IFN-γ production determined by the IFN-γ ELISPOT assay. Detailed information about these detection methods is described in "T cell response" and is listed in Table 2.

Furthermore, researchers do not systematically identify peptides from each viral protein. In fact, targeted efforts to identify T cell epitopes derived from partial viral proteins might have biased antigen selection. For example, some researchers might have initially stereotypically thought that the HA protein of influenza A virus (IAV) might be the dominant target of the $CD4^+$ T cell response. However, M1 and NP were reported to be dominant antigens recognized by IAV-specific $CD4^+$ T cells after screening 11 individual IAV proteins in humans [37, 72]. Interestingly, in chicken studies, AIV HA protein is still chosen, often the only antigen, for identifying $CD4^+$ T cell epitopes as shown in Table 5.

A systematic screening approach using antigen-specific T cells is a more accurate and robust method for identifying immunodominant peptides [73, 74]. This approach identifies highly immunogenic peptides with different MHC combinations and provides the most direct and accurate identification of potentially useful vaccine candidates. In the future, we believe that this method could potentially be used to identify immunodominant epitopes recognized by chicken T cells. The main barriers to this effort are the culture of chicken antigen-specific T cells in vitro and the establishment of infection models for the analysis of T cell function in vivo. Researchers should commit to breaking these technical barriers to allow future discoveries in the field of chicken T cell research. For instance, researchers need to identify the phenotypes and functions of various types of chicken T cells, the dominant viral antigens, and the minimal epitopes to establish a chicken T cell epitope database.

## Concluding remarks

Avian T cell immunity plays an important protective role against avian virus infection, and T cell-based vaccines represent an important new development in worldwide efforts to combat virus infection. However, research on avian T cell immunity is still in its infancy. A few areas of the avian virus-specific T cell response remain unknown, especially the identification of immunodominant T cell epitopes. Therefore, we summarized comprehensive information from previous studies and highlighted a potential direction for future studies. Particularly, an urgent need exists for the development of major tools, including different kinds of antibodies for intracellular cytokine staining, various APC lines expressing different MHC alleles, in vitro culture methods to maintain chicken T cell lines or even T cell clones, infection models for the analysis of T cell function in vivo, and immune evasion mechanisms.

Mammalian studies indicate that T cell-based vaccines might have the potential to be universal vaccines and address the limitations of antibody-based approaches concerning broader virus strain protection [12]. Additionally, in chicken studies, H9N2 AIV-specific T cells could reportedly provide cross-protection against heterogenous H9N2 and H5N2 AIV infection [63, 64, 78], implying that chicken T cell-based vaccines eliciting cross-protective T cell responses could potentially combat various AIV strains, especially novel strains whose surface hemagglutinin glycoproteins are not recognized by pre-existing antibodies. In addition, the future implementation of T cell-based universal vaccines is likely beneficial for decreasing the usage of different vaccine subtypes in livestock, reducing stress to the animals, and lowering labor and production costs. However, it should be noted that vaccine, especially live vaccine, usage may induce silent infection, thus perpetuating the disease [79]. In general, chicken T cell-based vaccines would be the research and development trend of future vaccines.

Based on the limited reference availability, we have summarized the chicken T cell immunity against common avian viruses in this review to assist in further studies. We hope that a better understanding of avian T cell immunity will enable researchers in the field to develop future vaccines capable of stimulating a wider range of $CD8^+$ and $CD4^+$ T cell responses.

**Table 6** Chicken minimal $CD8^+$ T cell epitopes recorded in IEDB

| Virus | Antigen | Epitope sequence | Position | MHC restriction | References |
|---|---|---|---|---|---|
| Influenza A virus (A/chicken/Guangdong/11/97(H9N2)) | Hemagglutinin | KILTIYSTV | 523–531 | BF2*2101 | [75] |
| Influenza A virus (A/turkey/Ireland/1378/1983(H5N8)) | Hemagglutinin | WTILKPSDTINFESN | 246–260 | chicken class I | [31] |
| Influenza A virus (A/goose/Guangdong/1/1996(H5N1)) | Nucleocapsid protein | KRGINDRNF | 198–206 | B19 | [69] |
| Influenza A virus (A/goose/Guangdong/1/1996(H5N1)) | Nucleocapsid protein | PKKTGGPIY | 89–97 | B19 | [69] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | VMELIRMI | 189–196 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | AVKGVGTMV | 181–189 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | DGKWVRELI | 100–108 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | EDLRVSSFI | 338–346 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | AEIEDLIFL | 250–258 | B4 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | NATEIRASV | 20–28 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | PTFSVQRNL | 409–417 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | VERMVGGI | 28–35 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | VGTMVMEL | 185–192 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | VGTMVMELI | 185–193 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | YDKEEIRRI | 110–118 | B4 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Nucleoprotein | YEQMETGE | 10–17 | B4 | [32] |
| Influenza A virus (A/goose/Gongdong/1/96 (H5N1)) | Nucleoprotein | RRRDGKWV | 69–76 | BF2*1501 | [70] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Matrix | KTRPILSPL | 47–55 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Matrix | ILGFVFTL | 59–66 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Matrix | VETYVLSI | 7–14 | B12 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Matrix | MRTIGTHP | 216–223 | B19 | [32] |
| Influenza A virus (A/chicken/Italy/1067/1999(H7N1)) | Matrix | KDDLIENL | 230–237 | B12 | [32] |
| Avian infectious bronchitis virus (strain Holte) | S1 glycoprotein | GAYAVVNV | 45–52 | Chicken class I | [38] |
| Infectious bronchitis virus | Spike glycoprotein S1 subunit, partial | SRIQTATDP | 412–420 | Chicken class I | [38] |
| Avian infectious bronchitis virus (strain Holte) | Spike glycoprotein S1, partial | SRIQTATQP | 413–421 | Chicken class I | [38] |
| Infectious bronchitis virus | S1 glycoprotein, partial | SRNATGSQP | 516–524 | Chicken class I | [38] |
| Infectious bronchitis virus | S1 glycoprotein | NQFYIKLT | 527–534 | Chicken class I | [71] |
| Infectious bursal disease virus 52/70 | VP2 | GNVLVGEGV | 156–164 | BF2*1201 | [68] |
| Rous sarcoma virus | v-src | LPACVLEV | 517–524 | B12 | [61] |

**Table 7** Chicken minimal $CD4^+$ T cell epitopes recorded in IEDB

| Virus | Antigen | Epitope sequence | Position | MHC restriction | References |
|---|---|---|---|---|---|
| Avian infectious bronchitis virus (strain Vic S) | S1 glycoprotein | KAVSAAGVHFKAGGPI | 186–201 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | S1 glycoprotein | ITYKVMREVRALAYFVNGTA | 201–220 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | S1 glycoprotein | QYNTGNFSDGLYPFTN | 237–252 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | S1 glycoprotein | PPNSGGVNTIQLYQTKTAQ | 286–304 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | S1 glycoprotein | GSQAIENQFYIKLTNGS | 521–537 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | S2 protein | NCPYVSYGKFCIKPDGSIST | 8–27 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | Nucleocapsid protein | GYWRRQARYKPGKSG | 69–83 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain Vic S) | Nucleocapsid protein | PAADLNWGENQDGIVWV | 100–116 | Chicken class II | [76] |
| Avian infectious bronchitis virus (strain M41) | Nucleocapsid protein | QHGYWRRQARFKPGKGG | 67–83 | Chicken class II | [77] |
| Avian infectious bronchitis virus (strain M41) | Nucleocapsid protein | WRRQARFKPGKGG | 71–83 | Chicken class II | [77] |
| Influenza A virus (A/turkey/Ireland/1378/1983(H5N8)) | Hemagglutinin | WTILKPSDTINFESN | 246–260 | Chicken class II | [31] |

**Author contributions** MMD drafted the manuscript. WSC, ML, and CGX revised the manuscript.

**Funding** This work was supported by the National Natural Science Foundation Grants (31802174 and 31830097) and a China Postdoctoral Science Foundation Grant (2018M630956).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of financial interest.

## References

1. Xu C, Ye H, Qiu W, Lin H, Chen Y, Zhang H, Liao M (2018) Phylogenetic classification of hemagglutinin gene of H9N2 avian influenza viruses isolated in China during 2012–2016 and evaluation of selected candidate vaccine strains. Poult Sci 97:3023–3030
2. Yang HM, Zhao J, Xue J, Yang YL, Zhang GZ (2017) Antigenic variation of LaSota and genotype VII Newcastle disease virus (NDV) and their efficacy against challenge with velogenic NDV. Vaccine 35:27–32
3. Feng K, Wang F, Xue Y, Zhou Q, Chen F, Bi Y, Xie Q (2017) Epidemiology and characterization of avian infectious bronchitis virus strains circulating in southern China during the period from 2013–2015. Sci Rep 7:6576
4. Li K, Courtillon C, Guionie O, Allee C, Amelot M, Qi X, Gao Y, Wang X, Eterradossi N (2015) Genetic, antigenic and pathogenic characterization of four infectious bursal disease virus isolates from China suggests continued evolution of very virulent viruses. Infect Genet Evol J Mol Epidemiol Evolut Genet Infect Dis 30:120–127
5. McKinstry KK, Dutton RW, Swain SL, Strutt TM (2013) Memory CD4 T cell-mediated immunity against influenza A virus: more than a little helpful. Arch Immunol Ther Exp 61:341–353
6. Greenwald RJ, Freeman GJ, Sharpe AH (2005) The B7 family revisited. Annu Rev Immunol 23:515–548
7. Bernard D, Hansen JD, Du Pasquier L, Lefranc MP, Benmansour A, Boudinot P (2007) Costimulatory receptors in jawed vertebrates: conserved CD28, odd CTLA4 and multiple BTLAs. Dev Comp Immunol 31:255–271
8. Abdalla SA, Horiuchi H, Furusawa S, Matsuda H (2004) Molecular cloning and characterization of chicken tumor necrosis factor (TNF)-superfamily ligands, CD30L and TNF-related apoptosis inducing ligand (TRAIL). J Vet Med Sci 66:643–650
9. Burgess SC, Young JR, Baaten BJ, Hunt L, Ross LN, Parcells MS, Kumar PM, Tregaskes CA, Lee LF, Davison TF (2004) Marek's disease is a natural model for lymphomas overexpressing Hodgkin's disease antigen (CD30). Proc Natl Acad Sci USA 101:13879–13884
10. Sutton KM, Hu T, Wu Z, Siklodi B, Vervelde L, Kaiser P (2015) The functions of the avian receptor activator of NF-kappaB ligand (RANKL) and its receptors, RANK and osteoprotegerin, are evolutionarily conserved. Dev Comp Immunol 51:170–184
11. Tregaskes CA, Glansbeek HL, Gill AC, Hunt LG, Burnside J, Young JR (2005) Conservation of biological properties of the CD40 ligand, CD154 in a non-mammalian vertebrate. Dev Comp Immunol 29:361–374
12. Grant EJ, Chen L, Quinones-Parra S, Pang K, Kedzierska K, Chen W (2014) T-cell immunity to influenza A viruses. Crit Rev Immunol 34:15–39
13. Kreijtz JH, Fouchier RA, Rimmelzwaan GF (2011) Immune responses to influenza virus infection. Virus Res 162:19–30

14. Rauf A, Khatri M, Murgia MV, Saif YM (2012) Fas/FasL and perforin-granzyme pathways mediated T cell cytotoxic responses in infectious bursal disease virus infected chickens. Results Immunol 2:112–119
15. Rauf A, Khatri M, Murgia MV, Saif YM (2011) Expression of perforin-granzyme pathway genes in the bursa of infectious bursal disease virus-infected chickens. Dev Comp Immunol 35:620–627
16. Wang X, Rosa AJ, Oliverira HN, Rosa GJ, Guo X, Travnicek M, Girshick T (2006) Transcriptome of local innate and adaptive immunity during early phase of infectious bronchitis viral infection. Viral Immunol 19:768–774
17. Sarson AJ, Abdul-Careem MF, Read LR, Brisbin JT, Sharif S (2008) Expression of cytotoxicity-associated genes in Marek's disease virus-infected chickens. Viral Immunol 21:267–272
18. Garcia-Camacho L, Schat KA, Brooks R Jr, Bounous DI (2003) Early cell-mediated immune responses to Marek's disease virus in two chicken lines with defined major histocompatibility complex antigens. Vet Immunol Immunopathol 95:145–153
19. Babon JA, Cruz J, Ennis FA, Yin L, Terajima M (2012) A human CD4+ T cell epitope in the influenza hemagglutinin is cross-reactive to influenza A virus subtypes and to influenza B virus. J Virol 86:9233–9243
20. DiPiazza A, Richards KA, Knowlden ZA, Nayak JL, Sant AJ (2016) The role of CD4 T cell memory in generating protective immunity to novel and potentially pandemic strains of influenza. Front Immunol 7:10
21. Brown DM, Lampe AT, Workman AM (2016) The differentiation and protective function of cytolytic CD4 T cells in influenza infection. Front Immunol 7:93
22. Jelley-Gibbs DM, Strutt TM, McKinstry KK, Swain SL (2008) Influencing the fates of CD4 T cells on the path to memory: lessons from influenza. Immunol Cell Biol 86:343–352
23. Mosmann TR, Coffman RL (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145–173
24. Degen WG, Daal N, Rothwell L, Kaiser P, Schijns VE (2005) Th1/Th2 polarization by viral and helminth infection in birds. Vet Microbiol 105:163–167
25. Kaiser P, Poh TY, Rothwell L, Avery S, Balu S, Pathania US, Hughes S, Goodchild M, Morrell S, Watson M, Bumstead N, Kaufman J, Young JR (2005) A genomic analysis of chicken cytokines and chemokines. J Interferon Cytokine Res 25:467–484
26. Zhang L, Liu R, Song M, Hu Y, Pan B, Cai J, Wang M (2013) *Eimeria tenella*: interleukin 17 contributes to host immunopathology in the gut during experimental infection. Exp Parasitol 133:121–130
27. Walliser I, Gobel TW (2018) Chicken IL-17A is expressed in αβ and γδ T cell subsets and binds to a receptor present on macrophages, and T cells. Dev Comp Immunol 81:44–53
28. Walliser I, Gobel TW (2017) Generation of glycosylphosphatidylinositol linked chicken IL-17 to generate specific monoclonal antibodies applicable for intracellular cytokine staining. Dev Comp Immunol 73:27–35
29. Shanmugasundaram R, Selvaraj RK (2011) Regulatory T cell properties of chicken CD4+ CD25+ cells. J Immunol 186:1997–2002
30. Gurung A, Kamble N (2017) Association of Marek's disease induced immunosuppression with activation of a novel regulatory T cells in chickens. PLoS Pathog 13:e1006745
31. Haghighi HR, Read LR, Haeryfar SM, Behboudi S, Sharif S (2009) Identification of a dual-specific T cell epitope of the hemagglutinin antigen of an h5 avian influenza virus in chickens. PLoS One 4:e7772
32. Reemers SS, van Haarlem DA, Sijts AJ, Vervelde L, Jansen CA (2012) Identification of novel avian influenza virus derived CD8+ T-cell epitopes. PLoS One 7:e31953
33. Tan L, Zhang Y, Liu F, Yuan Y, Zhan Y, Sun Y, Qiu X, Meng C, Song C, Ding C (2016) Infectious bronchitis virus poly-epitope-based vaccine protects chickens from acute infection. Vaccine 34:5209–5216
34. Mittendorf EA, Storrer CE, Shriver CD, Ponniah S, Peoples GE (2005) Evaluation of the CD107 cytotoxicity assay for the detection of cytolytic CD8+ cells recognizing HER2/neu vaccine peptides. Breast Cancer Res Treat 92:85–93
35. Zhao M, Liu K, Luo J, Tan S, Quan C, Zhang S, Chai Y, Qi J, Li Y, Bi Y, Xiao H, Wong G, Zhou J, Jiang T, Liu W, Yu H, Yan J, Liu Y, Shu Y, Wu G, Wu A, Gao GF, Liu WJ (2018) Heterosubtypic protections against human-infecting avian influenza viruses correlate to biased cross-T-cell responses. mBio 9:e01408–e01418
36. Du CL, Xu K, Min ZH, Li DD, Yuan HL, Liu C, Chen ZH (2017) Cytokine profiles of CD4(+) T memory cells in asthma and their relationship with asthma severity. Zhonghua yi xue za zhi 97:2333–2337
37. Chen L, Anthony A, Oveissi S, Huang M, Zanker D, Xiao K, Wu C, Zou Q, Chen W (2017) Broad-Based CD4(+) T cell responses to influenza a virus in a healthy individual who lacks typical immunodominance hierarchy. Front Immunol 8:375
38. Tan L, Liao Y, Fan J, Zhang Y, Mao X, Sun Y, Song C, Qiu X, Meng C, Ding C (2016) Prediction and identification of novel IBV S1 protein derived CTL epitopes in chicken. Vaccine 34:380–386
39. Kaufman J, Volk H, Wallny HJ (1995) A "minimal essential Mhc" and an "unrecognized Mhc": two extremes in selection for polymorphism. Immunol Rev 143:63–88
40. Vainio O, Veromaa T, Eerola E, Toivanen P, Ratcliffe MJ (1988) Antigen-presenting cell-T cell interaction in the chicken is MHC class II antigen restricted. J Immunol 140:2864–2868
41. Guillemot F, Billault A, Pourquie O, Behar G, Chausse AM, Zoorob R, Kreibich G, Auffray C (1988) A molecular map of the chicken major histocompatibility complex: the class II beta genes are closely linked to the class I genes and the nucleolar organizer. EMBO J 7:2775–2785
42. Vainio O, Koch C, Toivanen A (1984) B-L antigens (class II) of the chicken major histocompatibility complex control T-B cell interaction. Immunogenetics 19:131–140
43. Briles WE, Mc GW, Irwin MR (1950) On multiple alleles effecting cellular antigens in the chicken. Genetics 35:633–652
44. Koch M, Camp S, Collen T, Avila D, Salomonsen J, Wallny HJ, van Hateren A, Hunt L, Jacob JP, Johnston F, Marston DA, Shaw I, Dunbar PR, Cerundolo V, Jones EY, Kaufman J (2007) Structures of an MHC class I molecule from B21 chickens illustrate promiscuous peptide binding. Immunity 27:885–899
45. Wallny HJ, Avila D, Hunt LG, Powell TJ, Riegert P, Salomonsen J, Skjodt K, Vainio O, Vilbois F, Wiles MV, Kaufman J (2006) Peptide motifs of the single dominantly expressed class I molecule explain the striking MHC-determined response to Rous sarcoma virus in chickens. Proc Natl Acad Sci USA 103:1434–1439
46. Zhou H, Lamont SJ (2003) Chicken MHC class I and II gene effects on antibody response kinetics in adult chickens. Immunogenetics 55:133–140
47. Liu W, Miller MM, Lamont SJ (2002) Association of MHC class I and class II gene polymorphisms with vaccine or challenge response to Salmonella enteritidis in young chicks. Immunogenetics 54:582–590
48. Juul-Madsen HR, Dalgaard TS, Rontved CM, Jensen KH, Bumstead N (2006) Immune response to a killed infectious bursal disease virus vaccine in inbred chicken lines with different major histocompatibility complex haplotypes. Poult Sci 85:986–998
49. Juul-Madsen HR, Nielsen OL, Krogh-Maibom T, Rontved CM, Dalgaard TS, Bumstead N, Jorgensen PH (2002) Major histocompatibility complex-linked immune response of young chickens vaccinated with an attenuated live infectious bursal disease virus vaccine followed by an infection. Poult Sci 81:649–656

50. Miller MM, Taylor RL Jr (2016) Brief review of the chicken major histocompatibility complex: the genes, their distribution on chromosome 16, and their contributions to disease resistance. Poult Sci 95:375–392
51. Banat GR, Tkalcic S, Dzielawa JA, Jackwood MW, Saggese MD, Yates L, Kopulos R, Briles WE, Collisson EW (2013) Association of the chicken MHC B haplotypes with resistance to avian coronavirus. Dev Comp Immunol 39:430–437
52. Briles WE, Briles RW (1982) Identification of haplotypes of the chicken major histocompatibility complex (B). Immunogenetics 15:449–459
53. Lambrecht B, Gonze M, Meulemans G, van den Berg TP (2004) Assessment of the cell-mediated immune response in chickens by detection of chicken interferon-gamma in response to mitogen and recall Newcastle disease viral antigen stimulation. Avian Pathol J WVPA 33:343–350
54. Dunnington EA, Larsen CT, Gross WB, Siegel PB (1992) Antibody responses to combinations of antigens in white Leghorn chickens of different background genomes and major histocompatibility complex genotypes. Poult Sci 71:1801–1806
55. Heinzelmann EW, Clark KK, Collins WM, Briles WE (1981) Host age and major histocompatibility genotype influence on Rous sarcoma regression in chickens. Poult Sci 60:2171–2175
56. Joiner KS, Hoerr FJ, Ewald SJ, van Santen VL, Wright JC, van Ginkel FW, Toro H (2007) Pathogenesis of infectious bronchitis virus in vaccinated chickens of two different major histocompatibility B complex genotypes. Avian Dis 51:758–763
57. Kim DK, Lillehoj HS, Hong YH, Park DW, Lamont SJ, Han JY, Lillehoj EP (2008) Immune-related gene expression in two B-complex disparate genetically inbred Fayoumi chicken lines following *Eimeria maxima* infection. Poult Sci 87:433–443
58. Lamont SJ (1998) Impact of genetics on disease resistance. Poult Sci 77:1111–1118
59. Mays JK, Bacon LD, Pandiri AR, Fadly AM (2005) Response of white leghorn chickens of various B haplotypes to infection at hatch with subgroup J avian leukosis virus. Avian Dis 49:214–219
60. Yoo BH, Sheldon BL (1992) Association of the major histocompatibility complex with avian leukosis virus infection in chickens. Br Poult Sci 33:613–620
61. Hofmann A, Plachy J, Hunt L, Kaufman J, Hala K (2003) v-src oncogene-specific carboxy-terminal peptide is immunoprotective against *Rous sarcoma* growth in chickens with MHC class I allele B-F12. Vaccine 21:4694–4699
62. Kapczynski DR, Liljebjelke K, Kulkarni G, Hunt H, Jiang HJ, Petkov D (2011) Cross reactive cellular immune responses in chickens previously exposed to low pathogenic avian influenza. BMC Proc 5(Suppl 4):S13
63. Seo SH, Peiris M, Webster RG (2002) Protective cross-reactive cellular immunity to lethal A/Goose/Guangdong/1/96-like H5N1 influenza virus is correlated with the proportion of pulmonary CD8(+) T cells expressing gamma interferon. J Virol 76:4886–4890
64. Seo SH, Webster RG (2001) Cross-reactive, cell-mediated immunity and protection of chickens from lethal H5N1 influenza virus infection in Hong Kong poultry markets. J Virol 75:2516–2525
65. Wang F, Wang X, Chen H, Liu J, Cheng Z (2011) The critical time of avian leukosis virus subgroup J-mediated immunosuppression during early stage infection in specific pathogen-free chickens. J Vet Sci 12:235–241
66. Xu Q, Cui N, Ma X, Wang F, Li H, Shen Z, Zhao X (2016) Evaluation of a chimeric multi-epitope-based DNA vaccine against subgroup J avian leukosis virus in chickens. Vaccine 34:3751–3756
67. Wang Y, Wang G, Wang Z, Zhang H, Zhang L, Cheng Z (2014) Chicken biliary exosomes enhance CD4(+)T proliferation and inhibit ALV-J replication in liver. Biochem Cell Biol 92:145–151
68. Butter C, Staines K, van Hateren A, Davison TF, Kaufman J (2013) The peptide motif of the single dominantly expressed class I molecule of the chicken MHC can explain the response to a molecular defined vaccine of infectious bursal disease virus (IBDV). Immunogenetics 65:609–618
69. Hou Y, Guo Y, Wu C, Shen N, Jiang Y, Wang J (2012) Prediction and identification of T cell epitopes in the H5N1 influenza virus nucleoprotein in chicken. PLoS One 7:e39344
70. Zhang W, Huang Q, Lu M, Zhu F, Huang YY, Yang SH, Kong Z, Zhang XM, Xu CT (2016) Exploration of the BF2*15 major histocompatibility complex class I binding motif and identification of cytotoxic T lymphocyte epitopes from the H5N1 influenza virus nucleoprotein in chickens. Adv Virol 161:3081–3093
71. Zhu FZ, Lu M, Huang QH, Huang YY, Yang SH, Cui YS, Liu C, Tan L, Kong Z, Xu CT (2016) Interactive mechanism between avian infectious bronchitis S1 protein T cell peptide and avian MHC I molecule. Virus Res 215:76–83
72. Chen L, Zanker D, Xiao K, Wu C, Zou Q, Chen W (2014) Immunodominant CD4+ T-cell responses to influenza A virus in healthy individuals focus on matrix 1 and nucleoprotein. J Virol 88:11760–11773
73. Grant E, Wu C, Chan KF, Eckle S, Bharadwaj M, Zou QM, Kedzierska K, Chen W (2013) Nucleoprotein of influenza A virus is a major target of immunodominant CD8+ T-cell responses. Immunol Cell Biol 91:184–194
74. Wu C, Zanker D, Valkenburg S, Tan B, Kedzierska K, Zou QM, Doherty PC, Chen W (2011) Systematic identification of immunodominant CD8+ T-cell responses to influenza A virus in HLA-A2 individuals. Proc Natl Acad Sci USA 108:9178–9183
75. Yan RQ, Wu ZM, Fang QM, Zhang ZL, Zhang J, Li XS, Hao HF, Xia C (2008) Reconstruction of a chicken BF2 protein complex and identification of binding nonamer peptides derived from avian influenza virus hemagglutinin. Vet Immunol Immunopathol 126:91–101
76. Ignjatovic J, Sapats S (2005) Identification of previously unknown antigenic epitopes on the S and N proteins of avian infectious bronchitis virus. Adv Virol 150:1813–1831
77. Boots AM, Kusters JG, van Noort JM, Zwaagstra KA, Rijke E, van der Zeijst BA, Hensen EJ (1991) Localization of a T-cell epitope within the nucleocapsid protein of avian coronavirus. Immunology 74:8–13
78. Wei Y, Qi L, Gao H, Sun H, Pu J, Sun Y, Liu J (2016) Generation and protective efficacy of a cold-adapted attenuated avian H9N2 influenza vaccine. Sci Rep 6:30382
79. Spackman E, Pantin-Jackwood MJ (2014) Practical aspects of vaccination of poultry against avian influenza virus. Vet J (Lond, Engl: 1997) 202:408–415