TRANSCRIPTOME PROFILE OF 28-4+ INTRAEPITHELIAL LYMPHOCYTE NATURAL KILLER CELLS OF CHICKEN INFECTED BY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS

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By

BOO SOOK YEE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2019

DEDICATION

This thesis is dedicated

to my husband and parents

for their endless love and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Noorjahan Banu Binti Mohamed Alitheen, PhD
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Infectious bursal disease (IBV) caused by infectious bursal disease virus (IBDV), has been infecting chicken flocks for more than fifty years exerting a considerable economic impact to the global poultry industry. The virus infection response by chicken natural killer (NK) cells has been inadequately studied due to the limited monoclonal antibodies that are available. Development of 28-4 monoclonal antibody has allowed the identification of chicken intraepithelial lymphocytes (IEL) NK cells. However, the role of IEL-NK cell and its involvement in initiating the innate immune response in fighting the very virulent IBDV (vvIBDV) are still unknown. Therefore, the objective of this study was to identify the transcriptome profile of uninfected CD3-/28.4+ IEL-NK cells and identify the differential expressed genes and small RNAs (sRNAs) in response to vvIBDV infection at 1 and 3 days post infection (dpi). In this study, the chicken was infected by 10^3 mean egg lethal dosage (ELD_{50}) of vvIBDV strain 0081 for 1 and 3 days. The CD3^{-}/28.4^{+} IEL-NK cells were isolated from duodenum of the infected chicken. Then, the total RNA was isolated from CD3^{-}/28.4^{+} IEL-NK cells and used for library preparation, mRNA and sRNA sequencing, and followed by differential expression and pathway analysis. The RNA sequencing (RNA-Seq) result was validated by NanoString and sRNA sequencing result was validated by RT-qPCR. A total of 12,141 genes were expressed in uninfected chicken IEL-NK cells and most of the genes with high expression are involved in metabolic pathway whereas most of the low expressed genes involved in cytokine-cytokine receptor pathway. There is a total of 1,115 and 1,266 genes differentially expressed (DE) at 1 and 3 dpi respectively. The DE genes at 1 dpi mainly involved in DNA replication, cell cycle, apoptosis whereas the DE genes at 3 dpi involved in inflammation, antiviral response and interferon stimulation. For sRNA sequencing, there are 35 and 16 sRNAs were differentially expressed at 1 and 3 dpi, respectively. The SNORD101, gga-miR-222a and gga-miR-221-3p were up-regulated on Day 1 and 3 whereas gga-miR-30a-50, gga-miR-142-5p, gga-miR-32-5p and gga-miR-146b-5p were down-regulated on both days.

Meanwhile, *GAPDH* and *YWHAZ* were the most stably expressed genes in the chicken CD3-/28.4+ IEL-NK cells infected by vvIBDV according to the RT-qPCR result. In conclusion, the immunity of chicken $CD3^{-}/28.4^{+}$ IEL-NK cells was suppressed at 1 dpi and the proliferation of NK cells was an act of a preparation step for the defense against virus at 2 or 3 dpi. At 3 dpi, the innate immune response was activated as several genes involved in inflammation, antiviral response and recruitment of NK cells to infected area were up-regulated. This is the first study which examined the whole transcriptome profile of chicken NK cells towards IBDV infection, which provides a better insight into the early immune response of chicken NK cells.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KAJIAN EKSPRESI GEN MENGENAI TINDAK BALAS IMUN INAT SEL LIMFOSIT INTRAEPITHELIAL PEMBUNUH SEMULAJADI AYAM 28-4^{+} TERHADAP VIRUS PENYAKIT BURSAL BERJANGKIT YANG SANGAT VIRULEN

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Penyakit bursal berjangkit (IBD) yang disebabkan oleh virus penyakit bursal berjangkit (IBDV) telah menjejaskan ekonomi industri ayam selama 50 tahun. Penyelidikan yang berkaitan dengan tindak balas sel pembunuh semulajadi (NK) terhadap jangkitan virus dalam ayam adalah terhad kerana antibodi monoklonal yang sasar pada sel NK di ayam adalah terhad. Penghasilan antibodi monoklonal 28.4 telah membenarkan pengenalpastian untuk sel limfosit intraepitelium (IEL) NK dalam ayam. Walau bagaimanapun, peranan dan penglibatan untuk IEL-NK sel dalam tindak balas imun semula jadi di ayam selepas dijangkiti dengan IBDV amat virulen (vvIBDV) adalah tidak diketahui. Objektif kajian ini adalah untuk mengenalpasti profil transkrip untuk sel IEL-NK 28.4^{+} / CD3^{-} dalam ayam yang tidak dijangkiti dan mengenalpasti gen dan RNA yang kecil (sRNAs) yang di ekspresi berbeza sebagai tindak balas terhadap vvIBDV selepas dijangkiti selama satu and tiga hari. Ayam berjangkit dengan vvIBDV UPM0081 menggunakan dos mematikan telur sebanyak 10^3 selama satu dan tiga hari. Sel CD3^{-}28.4^{+} IEL-NK ayam telah terpencil dari bahagian duodenum untuk ayam yang dijangkiti. Selepas itu, jumlah RNA telah dikeluarkan dari CD3^{-}28.4^{+} IEL-NK sel ayam dan digunakan untuk persediaan perpustakaan, penjujukan RNA (RNA-Seq) dan sRNA diikuti dengan analisis perbezaan transkrip dan laluan. Keputusan RNA-Seq telah disahkan oleh NanoString dan keputusan penjujukan sRNA telah disahkan oleh tindak balas rantaian polimer masa sebenar. Sebanyak 12,141 gen telah dinyatakan di sel CD3^{-}28.4^{+} IEL-NK yang tidak dijangkit. Kebanyakan gen dengan ekspresi tinggi terlibat dalam laluan metabolik manakala gen yang ekspresi rendah terlibat dalam laluan reseptor sitokin. Gen yang berjumlah 1,115 dan 1,266 ada perbezaan ekspresi pada hari yang pertama dan ketiga selepas berjangkit dengan vvIBDV. Gen dengan perbezaan ekspresi pada hari yang pertama terlibat dalam replikasi DNA, kitaran sel dan apoptosis manakala gen dengan perbezaan ekspresi pada hari yang ketiga terlibat dalam keradangan, tindak balas antivirus dan rangsangan interferon. Kajian dari

penjujukan sRNA menunjukan 35 dan 16 sRNAs dengan perbezaan ekspresi selepas berjangkit dengan vvIBDV selama satu dan tiga hari. Transkripsi gen untuk SNORD101, gga-miR-222a dan gga-miR-221-3p telah dipertingkatkan sedangkan transkripsi gen untuk gga-miR-30a-50, gga-miR-142-5p, gga-miR-32-5p dan gga-miR-146b-5p telah dikurangkan selepas berjangkit dengan vvIBDV selama satu dan tiga hari. Sementara itu, GAPDH dan YWHAZ adalah gen yang paling stabil untuk digunakan sebagai gen rujukan di $CD3^{-}28.4^{+}$ sel pembunuh semulajadi di ayam berdasarkan hasil dari tindak balas rantaian polimer masa sebenar. Kesimpulannya, imuniti untuk $CD3^{-}28.4^{+}$ sel pembunuh semulajadi di ayam ditindas pada hari yang pertama selepas berjangkit dengan vvIBDV. Percambahan sel NK bertindak sebagai langkah persediaan untuk pertahanan terhadap virus bagi hari yang kedua or ketiga. Selepas dijangkiti selama tiga hari, tindak balas keimunan inat telah diaktifkan kerana transkripsi gen yang terlibat dalam keradangan, tindak balas antivirus dan penghantaran sel NK ke kawasan yang dijangkiti telah dipertingkatkan. Ini adalah kajian pertama yang memeriksa keseluruhan profil transkrip untuk sel $CD3^{-}28.4^{+}$ IEL-NK dalam ayam yang dijangkiti dengan vvIBDV dan kajian ini memberikan pengetahuan yang lebih mendalam berkenaan tindakbalas imun awal oleh sel NK ayam.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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µL	Microlitre
µM	Micromolar
AIV	Avian Influenza virus
ALV-J	Avian Leukosis virus J
ANOVA	One-way analysis of variance
BCL	Base calling
BCR	B-cell receptor
BF	Bursa of Fabricius
BM-DC	Bone marrow-derived dendritic cell
bp	Base pairs
BRSV	Bovine respiratory syncytial virus
BSL-2	Biosafety level-2
CASP	Caspase
CCL	CC chemokine ligand
cDNA	Complementary deoxyribonucleic acid
chBM-DCs	Bone marrow-derived DCs
CHIR	Chicken Ig-like receptors
CRD	Carbohydrate recognition domain
CRT	Cyclic reversible termination
C_q	Threshold cycle
CTLD	C-type lectin-like domain
CV%	Coefficient of variation percentage

DC	Dendritic cell
DE	Differential expressed
DEPC	Diethyl Pyrocarbonate
DNA	Deoxyribonucleic acid
ddNTP	Dideoxynucleotide triphosphates
dNTP	Deoxynucleotide triphosphates
dpi	Day-post-infection
dsDNA	Double-stranded deoxyribonucleic acid
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
ELD_{50}	Mean Egg Lethal Dosage
FDR	False discovery rate
FFPE	Formalin-Fixed Paraffin-Embedded
gDNA	Genomic DNA
GEO	Gene Expression Omnibus
GO	Gene Ontology
HPgV	Human Pegivirus
HS	High sensitivity
HT1 buffer	Hybridization buffer
HTLV	Human T-cell lymphotropic virus
HVT	Herpes virus of turkey
IACUC	Institutional Animal Care and Use Committee
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus

IBS	Institute of BioScience
Icx	Immune complex
IDT	Integrated DNA Technologies
IEL-NK	Intraepithelial lymphocytes-natural killer
IELs	Intraepithelial lymphocytes
IFITs	Tetratricopeptide repeats
IFNAR	Interferon alpha receptor
IFNGR	Interferon gamma receptor
IFN-I	Type I interferons
IFN-γ	Interferon-gamma
IgM+	Immunoglobulin M
Igs	Immunoglobulins
IHC	Immunohistochemistry
IL	Interleukin
IPNV	Infectious pancreatic necrosis virus
ISGs	IFN-stimulated genes
ITIM	Immunoreceptor tyrosine-based inhibitory motifs
ITSM	Immunoreceptor tyrosine-based switch motifs
JAK-STAT	Janus activated kinase-signal transducer and activation of transcription
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIRs	Killer cell immunoglobulin-like receptors
LPS	Lipopolysaccharide
M value	Expression stability value

MACS	Magnetic Activated Cell Sorting
MAPK	Mitogen-activated protein kinase
MCM	Minichromosome maintenance
MDA	Maternal-derived antibodies
MHC	Major histocompatibility complex
miRNA	microRNA
MNC	Mononuclear cell
mRNA	Messenger ribonucleic acid
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NDV	Newcastle disease virus
NFκB	Nuclear factor-κB
ng/μL	Nanogram/microlitre
NGS	Next-Generation Sequencing
NK	Natural killer
NKC	Natural killer gene complex
nM	Nanomolar
NO	Nitric oxide
nts	nucleotides
ORFs	Open reading frames
PAGE	Polyacrylamide gel electrophoresis
PARP	Poly(ADP-ribose) polymerase
PBS	Phosphate buffer saline
PCA	Principal Component Analysis

PCR	Polymerase Chain Reaction
PE	Phycoerythrin
PF	Passing filter
pg	Picogram
pM	Picomolar
PRRs	Pattern-recognition receptors
PRRSV	Porcine reproductive and respiratory syndrome virus
PWBC	Peripheral white blood cells
RCC	Reporter code count
RdRp	RNA-dependent RNA polymerase
RIN	RNA integrity number
ROS	Reactive oxygen species
RPKM	Reads per kilobase of exon per million reads
RPMI	Roswell Park Memorial Institute
rRNA	Ribosomal Ribonucleic acid
RT	Room temperature
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Quantitative Reverse Transcription PCR
SAMHD1	Sterile alpha motif and HD-domain-containing protein 1
SBL	Sequencing by ligation
SBS	Sequencing by synthesis
SLAM	Signalling lymphocytic activation molecule
SNA	Single-nucleotide addition
snoRNA	Small nucleolar RNA

SPF	Specific-pathogen–free
SPP1	Secreted phosphoprotein 1
sRNA	Small RNA
ssDNA	Single stranded DNA
TLRs	Toll-like receptors
T_m	Melting temperature
TNF-α	Tumour necrosis factor-alpha
TNFRSF4	Tumour necrosis factor receptor superfamily member 4
UPM	Universiti Putra Malaysia
V value	Pairwise variation value
vvIBDV	Very virulent IBDV
µg	Microgram

CHAPTER 1

INTRODUCTION

1.1 Background

Infectious bursal disease (IBD) is a contagious and immunosuppressive viral disease caused by IBD virus (IBDV), which has negative impacts on the poultry industries, causing major economic losses (Berg, 2000). IBDV is categorized as an Avibirnavirus which belongs to the Birnaviridae family, which consists of two segments of double-stranded ribonucleic acid (dsRNA) (A and B) (Müller et al., 1979a). The segment A (3.17 kb) encodes the non-structural viral protein, VP5, and a polyprotein precursor, in which after the post-translational cleavage would produce the VP2, VP3 and VP4 structural proteins (Mahgoub, 2012). The segment B which is a shorter fragment (2.8 kb) than the segment A encodes VP1, an RNA-dependent RNA polymerase (RdRp) which is involved in transcription and replication of the virus and thus, plays a critical role in determining the virulence characteristic of the virus (Coulibaly et al., 2005; Delmas et al., 2005).

IBDV isolates are categorized into subset of serotypes; serotype-1 and serotype-2. Serotype-1 is classified as highly virulent IBDV or very virulent IBDV (vvIBDV) and usually associated with typical clinical diseases such as lesion in bursal organ and even mortality, as a result of chronic infection (Mahgoub, 2012). On the other hand, serotype-2 is a low pathogenic IBDV which is commonly used as vaccine (Berg, 2000). The IBDV infection starts through the oral route, followed by viral replication inside the duodenum, caecum, and jejunum, which targets the avian immune cells for example the macrophages which capable of destroying pathogens and apoptotic cells, and the lymphoid cells at these organs (Muller et al., 1979). Afterwards, IBDV spreads to the Bursa of Fabricius (BF), an organ which IBDV rapidly replicates and attacks the undeveloped B lymphocytes, which compromises the humoral immune responses of the infected avian (Berg, 2000).

The clinical severity and mortality caused by the vvIBDV toward chicken are determined based on the existing inherited maternal antibodies, age and also genetic background for chicken (Sharma et al., 2000). Acute IBDV infections (about 2 to 3 days of incubation period) could cause the animals to be exhausted, dehydrated, prostrated, and suffered from aqueous diarrhoea as well as ruffled feathers. All these severe clinical signs lead to mortality of the chicken on the third day of infection. Any surviving chicken would recover from the infection and back to normal health condition after 5 to 7 days (Ingrao et al., 2013).

The innate immune system provides the first layer of defence against pathogen, bacteria, and virus. It is interesting to know how the chicken immune cells would

respond to the vvIBDV infection as the first three days post-infection are considered as incubation period which with faster replication of virus in affected cells (Ingrao et al., 2013). It is evident that virus infections can induce aberrant cytokine production by regulating the signalling pathway. Also, virus impacts cells of the innate immune system which express essential molecules namely pattern recognition receptors (PRRs), such as Toll-like receptors (Kimura et al., 2013). Immune cells grouped in the innate immune response are dendritic cells, macrophage and natural killer (NK) cells (Portou et al., 2015).

NK cells are a group of lymphocytes that play a critical role in the early defence against pathogens such as parasites, bacteria and viruses (Jansen et al., 2010; Lanier, 2005; Trinchieri, 1989). The primary role of NK cells is to provide an initial crucial defense against pathogens, usually within a period from 0 to 5 days while the adaptive immune system is activating (Yokoyama, 2003). NK cells secrete major inflammatory cytokines like interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) to activate macrophage, dendritic cells and T cells (Mandal and Viswanathan, 2015; Vivier et al., 2008). Mammalian NK cells have been intensively characterized in previous studies whereas the studies on avian NK cells are still limited (Neulen et al., 2015; Straub et al., 2013).

The first identified and characterised avian NK cell was a $CD3^{-}CD8\alpha^{+}$ population (Göbel et al., 1994). Later, a specific mucosal NK cell population has been isolated from intestinal intraepithelial and was named intraepithelial lymphocytes-natural killer (IEL-NK) cells (Göbel et al., 2001). A monoclonal antibody known as 28-4 was developed in 2001 to specifically targeting the IEL-NK cells namely, chicken CD25 (Göbel et al., 2001; Walliser & Göbel, 2018). Notably, these chicken IEL-NK cells showed cytotoxicity towards tumour target and were expected to play an important role in mucosal immune system (Göbel et al., 2001).

There are multiple studies on the messenger ribonucleic acid (mRNA) expression profile of chicken B cells (Quan et al., 2017), macrophages (Rasoli et al., 2015), dendritic cells (Yasmin et al., 2015), and embryonic fibroblast cells (Wong et al., 2007) but the study on chicken IEL-NK cells is limited. Transcriptomic and functional evaluation of chicken NK cells are advancing at a much slower pace when compared to the mammalian NK cells due to the difference of genomic organization between chicken and human or mice and the limited choice of monoclonal antibodies. The 28-4 monoclonal antibody was being used for the isolation of IEL-NK cells isolation in Jahromi et al., (2018) study. In this study, chicken infected with the vvIBDV strain resulted in the reduced activation of the IEL-NK cells as the virus caused the down-regulation of the expression of *CD69*, *CHIR-AB1*, and *NK-lysin* receptors, especially at 1 day-post-infection (dpi). This is the first expression study on NK receptors from the isolated chicken IEL-NK cells in response to vvIBDV but notably, with limited number of genes being tested. Therefore, it is important to study the transcriptome of chicken IEL-NK cells to understand the pathways and genes involved in the chicken innate immune response following the infection by vvIBDV.

microRNAs (miRNAs) play a significant role for regulation of mRNA expression and the gene transcription, particularly in the immune cells that are involved in the defence against vvIBDV infection. Therefore, it is of great importance to understand the miRNAs expression upon infection by vvIBDV. miRNAs are small non-coding RNA molecules consisting of 21 to 25 nucleotides (nts), that are able to trigger gene silencing via translational repression or target degradation (Bartel, 2009; Eulalio et al., 2009). Based on the latest update until October 2018, there are 882 precursors and 1232 mature miRNA in miRbase 22.1 for avian. The expression of miRNA in the chicken has been shown in previous studies to be involved in several processes, such as embryo and germ cell development (Darnell et al., 2006; Hick et al., 2008; Rathjen et al., 2009; Lee et al., 2011), immune organ function (Hicks et al., 2009), and disease (Tian et al., 2012; Wang et al., 2013; Hong et al., 2014). However, there are no current reports describing the miRNA transcriptome changes associated with vvIBDV infected IEL-NK cells.

1.2 Problem Statements and Hypothesis

The role of IEL-NK cell and its involvement in initiating the innate immune response in fighting the vvIBDV infection are still unknown. To understand the transcriptome changes of the IEL-NK cells, the mRNA and miRNA from the uninfected and vvIBDV-infected chickens were examined by high throughput sequencing using Illumina technology. It was hypothesized that most of the genes in cytokine-cytokine receptor interaction and Toll-like receptor signaling pathway are either activated or repressed after the vvIBDV infection at 1 and 3 dpi. The cytokines, chemokines and interferon were expected to play important roles in the innate immune response of IEL-NK cells. Lastly, miRNA could regulate the expression levels of genes as one of the strategies to defend the host in fighting against vvIBDV infection.

1.3 Objectives

This is the first comprehensive study on the whole transcriptome profile in chicken $CD3^-/28.4^+$ IEL-NK cells infected by vvIBDV by utilizing the advance technologies such as NGS and NanoString. The four specific objectives of this study are as follows:

1) To identify and analyse the transcriptome profile of the uninfected $CD3^-/28.4^+$ IEL-NK cells from chickens;
2) To identify differential expressed genes and pathways in $CD3^-/28.4^+$ IEL-NK cells in response to vvIBDV infection;
3) To identify miRNAs in IEL-NK cells of chickens infected by vvIBDV; and
4) To validate the differential expression of genes using the RT-qPCR and NanoString nCounter System.

REFERENCES

Abdolmaleki M, Yeap SK, Tan SW, Satharasinghe DA, Bello MB, Zareian Jahromi M, Hair Bejo M, Omar AR, Ideris A. effects of newcastle Disease Virus infection on chicken intestinal intraepithelial natural Killer cells. Frontiers in Immunology. 2018;9:1386.

Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. elife. 2015 Aug 12;4:e05005.

Agaugué S, Marcenaro E, Ferranti B, Moretta L, Moretta A. Human natural killer cells exposed to IL-2, IL-12, IL-18, or IL-4 differently modulate priming of naive T cells by monocyte-derived dendritic cells. Blood. 2008 Sep 1;112(5):1776-83.

Akira S. The role of IL-18 in innate immunity. Current Opinion in Immunology. 2000 Feb 1;12(1):59-63.

Akira S. Toll-like receptors and innate immunity. Advances in Immunology. 2001 Jan 1;78:1-56.

Allan WH, Faragher JT, Cullen GA. Immunosuppression by the infectious bursal agent in chickens immunised against Newcastle disease. The Veterinary Record. 1972 Apr;90(18):511-2.

Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones SA, Marshall M, Matzke M. A uniform system for microRNA annotation. RNA. 2003 Mar 1;9(3):277-9.

Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nature Medicine. 2008 Mar;14(3):275.

Ayyagari R, Gomes XV, Gordenin DA, Burgers PM. Okazaki fragment maturation in yeast I. Distribution of functions between FEN1 and DNA2. Journal of Biological Chemistry. 2003 Jan 17;278(3):1618-25.

Azad AA, Barrett SA, Fahey KJ. The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. Virology. 1985 May 1;143(1):35-44.

Bach EA, Aguet M, Schreiber RD. The IFNγ receptor: a paradigm for cytokine receptor signaling. Annual Review of Immunology. 1997 Apr;15(1):563-91.

Baekkevold ES, Yamanaka T, Palframan RT, Carlsen HS, Reinholt FP, von Andrian UH, Brandtzaeg P, Haraldsen G. The CCR7 ligand elc (CCL19) is

transcytosed in high endothelial venules and mediates T cell recruitment. Journal of Experimental Medicine. 2001 May 7;193(9):1105-12.

Baev V, Milev I, Naydenov M, Vachev T, Apostolova E, Mehterov N, Gozmanva M, Minkov G, Sablok G, Yahubyan G. Insight into small RNA abundance and expression in high-and low-temperature stress response using deep sequencing in Arabidopsis. Plant Physiology and Biochemistry. 2014 Nov 1;84:105-14.

Balcells I, Cirera S, Busk PK. Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers. BMC Biotechnology. 2011 Dec;11(1):70.

Bartel DP. MicroRNAs: target recognition and regulatory functions. cell. 2009 Jan 23;136(2):215-33.

Barton GM. A calculated response: control of inflammation by the innate immune system. The Journal of Clinical Investigation. 2008 Feb 1;118(2):413-20.

Bas A, Forsberg G, Hammarström S, Hammarström ML. Utility of the housekeeping genes 18S rRNA, β-actin and glyceraldehyde-3-phosphate-dehydrogenase for normalization in real-time quantitative reverse transcriptase-polymerase chain reaction analysis of gene expression in human T lymphocytes. Scandinavian Journal of Immunology. 2004 Jun;59(6):566-73.

Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Davies DR, Segal DM. The molecular structure of the TLR3 extracellular domain. Journal of Endotoxin Research. 2006 Dec;12(6):375-8.

Berg TP. Acute infectious bursal disease in poultry: a review. Avian Pathology. 2000 Jun 1;29(3):175-94.

Berger A, Sommer AF, Zwarg J, Hamdorf M, Welzel K, Esly N, Panitz S, Reuter A, Ramos I, Jatiani A, Mulder LC. SAMHD1-deficient CD14+ cells from individuals with Aicardi-Goutieres syndrome are highly susceptible to HIV-1 infection. PLoS Pathogens. 2011 Dec 8;7(12):e1002425.

Bergsveinson J, Pittet V, Ziola B. RT-qPCR analysis of putative beer-spoilage gene expression during growth of Lactobacillus brevis BSO 464 and Pediococcus claussenii ATCC BAA-344 T in beer. Applied Microbiology and Biotechnology. 2012 Oct 1;96(2):461-70.

Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annual Review of Immunology. 1999 Apr;17(1):189-220.

Block H, Meyer-Block K, Rebeski DE, Scharr H, De Wit S, Rohn K, Rautenschlein S. A field study on the significance of vaccination against infectious bursal disease virus (IBDV) at the optimal time point in broiler flocks with

maternally derived IBDV antibodies. Avian Pathology. 2007 Oct 1;36(5):401-9.

Bocci V. Interleukins. Clinical Pharmacokinetics. 1991 Oct 1;21(4):274-84.

Boehme KW, Compton T. Innate sensing of viruses by toll-like receptors. Journal of Virology. 2004 Aug 1;78(15):7867-73.

Broom LJ, Kogut MH. Inflammation: friend or foe for animal production? Poultry Science. Volume 97, Issue 2, February 2018, Pages 510–514.

Brumbaugh CD, Kim HJ, Giovacchini M, Pourmand N. NanoStriDE: normalization and differential expression analysis of NanoString nCounter data. BMC Bioinformatics. 2011 Dec;12(1):479.

Budak H, Bulut R, Kantar M, Alptekin B. MicroRNA nomenclature and the need for a revised naming prescription. Briefings in Functional Genomics. 2015 Jul 6;15(1):65-71.

Campbell KS, Purdy AK. Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations. Immunology. 2011 Mar;132(3):315-25.

Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. Nature Reviews Immunology. 2001 Oct;1(1):41.

Chatterjee A, Leichter AL, Fan V, Tsai P, Purcell RV, Sullivan MJ, Eccles MR. A cross comparison of technologies for the detection of microRNAs in clinical FFPE samples of hepatoblastoma patients. Scientific Reports. 2015 Jun 3;5:10438.

Chivero ET, Bhattarai N, McLinden JH, Xiang J, Stapleton JT. Human Pegivirus (HPgV; formerly known as GBV-C) inhibits IL-12 dependent natural killer cell function. Virology. 2015 Nov 1;485:116-27.

Choe J, Kelker MS, Wilson IA. Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science. 2005 Jul 22;309(5734):581-5.

Confer AW, Springer WT, Shane SM, Donovan JF. Sequential mitogen stimulation of peripheral blood lymphocytes from chickens inoculated with infectious bursal disease virus. American Journal of Veterinary Research. 1981 Dec;42(12):2109-13.

Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends in Immunology. 2001 Nov 1;22(11):633-40.

Coulibaly F, Chevalier C, Gutsche I, Pous J, Navaza J, Bressanelli S, Delmas B, Rey FA. The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell. 2005 Mar 25;120(6):761-72.

Darnell DK, Kaur S, Stanislaw S, Konieczka JK, Yatskievych TA, Antin PB. MicroRNA expression during chick embryo development. Developmental Dynamics. 2006 Nov;235(11):3156-65.

Davison F. The importance of the avian immune system and its unique features. In Avian Immunology 2014 Jan 1 (pp. 1-9). Academic Press.

de Araújo-Souza PS, Hanschke SC, Viola JP. Epigenetic control of interferon-gamma expression in CD8 T cells. Journal of Immunology Research. 2015;2015.

De Weerd NA, Nguyen T. The interferons and their receptors—distribution and regulation. Immunology and Cell Biology. 2012 May;90(5):483-91.

Delmas B, Kibenge FS, Leong JC, Mundt E, Vakharia VN, Wu JL. Birnaviridae. Virus Taxonomy. 2005:561-9.

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013 Jan 1;29(1):15-21.

Du S, Liu X, Cai Q. Viral-Mediated mRNA Degradation for Pathogenesis. Biomedicines. 2018 Dec;6(4):111.

Dybkaer K, Iqbal J, Zhou G, Geng H, Xiao L, Schmitz A, d'Amore F, Chan WC. Genome wide transcriptional analysis of resting and IL2 activated human natural killer cells: gene expression signatures indicative of novel molecular signaling pathways. BMC Genomics. 2007 Dec;8(1):230.

Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annual Review of Biochemistry. 1999 Jul;68(1):383-424.

ENCODE Guidelines and Best Practices for RNA-Seq V2.Dec 2016.

Eterradossi N, Saif YM. Infectious bursal disease. Diseases of Poultry. 2013 Jul 11;13:219-46.

Eterradossi N, Saif YM. Infectious bursal disease. Diseases of Poultry. 2008;12:185-208.

Eulalio A, Huntzinger E, Nishihara T, Rehwinkel J, Fauser M, Izaurralde E. Deadenylation is a widespread effect of miRNA regulation. RNA. 2009 Jan 1;15(1):21-32.

Farhanah MI, Yasmin AR, Isa NM, Hair-Bejo M, Ideris A, Powers C, Oladapo O, Nair V, Khoo JS, Ghazali AK, Yee WY. Bursal transcriptome profiling of different inbred chicken lines reveals key differentially expressed genes at 3 days post-infection with very virulent infectious bursal disease virus. Journal of General Virology. 2017 Oct 23;99(1):21-35.

Farhanah MI, Yasmin AR, Khanh NP, Yeap SK, Hair-Bejo M, Omar AR. Bursal immunopathology responses of specific-pathogen-free chickens and red jungle fowl infected with very virulent infectious bursal disease virus. Archives of Virology. 2018 Aug 1;163(8):2085-97.

Fehniger TA, Wylie T, Germino E, Leong JW, Magrini VJ, Koul S, Keppel CR, Schneider SE, Koboldt DC, Sullivan RP, Heinz ME. Next-generation sequencing identifies the natural killer cell microRNA transcriptome. Genome Research. 2010 Nov 1;20(11):1590-604.

Fernandez-Arias A, Martinez S, Rodríguez JF. The major antigenic protein of infectious bursal disease virus, VP2, is an apoptotic inducer. Journal of Virology. 1997 Oct 1;71(10):8014-8.

Gao S, Jiang H, Sun J, Diao Y, Tang Y, Hu J. Integrated Analysis of miRNA and mRNA Expression Profiles in Spleen of Specific Pathogen-Free Chicken Infected with Avian Reticuloendotheliosis Virus Strain SNV. International Journal of Molecular Sciences. 2019 Jan;20(5):1041.

Geddes VE, de Oliveira AS, Tanuri A, Arruda E, Ribeiro-Alves M, Aguiar RS. MicroRNA and cellular targets profiling reveal miR-217 and miR-576-3p as proviral factors during Oropouche infection. PLoS Neglected Tropical Diseases. 2018 May 29;12(5):e0006508.

Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, Fell HP, Ferree S, George RD, Grogan T, James JJ. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nature Biotechnology. 2008 Mar;26(3):317.

Giambrone JJ, Donahoe JP, Dawe DL, Eidson CS. Specific suppression of the bursa-dependent immune system of chicks with infectious bursal disease virus. American Journal of Veterinary Research. 1977 May;38(5):581-3.

Giambrone JJ, Dormitorio T, Brown T. Safety and efficacy of in ovo administration of infectious bursal disease viral vaccines. Avian Diseases. 2001 Jan 1:144-8.

Göbel TW, Chen CL, Shrimpf J, Grossi CE, Bernot A, Bucy RP, Auffray C, Cooper MD. Characterization of avian natural killer cells and their intracellular CD3 protein complex. European Journal of Immunology. 1994 Jul;24(7):1685-91.

Göbel TW, Kaspers B, Stangassinger M. NK and T cells constitute two major, functionally distinct intestinal epithelial lymphocyte subsets in the chicken. International Immunology. 2001 Jun 1;13(6):757-62.

Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nature Reviews Genetics. 2016 Jun;17(6):333.

Grégoire C, Chasson L, Luci C, Tomasello E, Geissmann F, Vivier E, Walzer T. The trafficking of natural killer cells. Immunological Reviews. 2007 Dec;220(1):169-82.

Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annual Review of Immunology. 2014 Mar 21;32:659-702.

Guillot L, Le Goffic R, Bloch S, Escriou N, Akira S, Chignard M, Si-Tahar M. Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. Journal of Biological Chemistry. 2005 Feb 18;280(7):5571-80.

Guo L, Louis IV, Bohjanen PR. Viral manipulation of host mRNA decay. Future Virology. 2018 Feb 16;13(3):211-23.

Guo H, Topham DJ. Interleukin-22 (IL-22) production by pulmonary Natural Killer cells and the potential role of IL-22 during primary influenza virus infection. Journal of Virology. 2010 Aug 1;84(15):7750-9.

Haddad EE, Whitfill CE, Avakian AP, Ricks CA, Andrews PD, Thoma JA, Wakenell PS. Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. Avian Diseases. 1997 Oct 1:882-9.

Håvarstein LS, Kalland KH, Christie KE, Endresen C. Sequence of the large double-stranded RNA segment of the N1 strain of infectious pancreatic necrosis virus: a comparison with other Birnaviridae. Journal of General Virology. 1990 Feb 1;71(2):299-308.

Hervas-Stubbs S, Perez-Gracia JL, Rouzaut A, Sanmamed MF, Le Bon A, Melero I. Direct effects of type I interferons on cells of the immune system. Clinical Cancer Research. 2011 May 1;17(9):2619-27.

Hewson CA, Jardine A, Edwards MR, Laza-Stanca V, Johnston SL. Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. Journal of Virology. 2005 Oct 1;79(19):12273-9.

Hicks JA, Tembhurne PA, Liu HC. Identification of microRNA in the developing chick immune organs. Immunogenetics. 2009 Mar 1;61(3):231-40.

Hinson ER, Cresswell P. The antiviral protein, viperin, localizes to lipid droplets via its N-terminal amphipathic α-helix. Proceedings of the National Academy of Sciences. 2009 Dec 1;106(48):20452-7.

Hong YH, Dinh H, Lillehoj HS, Song KD, Oh JD. Differential regulation of microRNA transcriptome in chicken lines resistant and susceptible to necrotic enteritis disease. Poultry Science. 2014 May 29;93(6):1383-95.

Hu Q, Zhao Y, Wang Z, Hou Y, Bi D, Sun J, Peng X. Chicken gga-miR-19a targets ZMYND11 and plays an important role in host defense against Mycoplasma gallisepticum (HS strain) infection. Frontiers in Cellular and Infection Microbiology. 2016 Sep 14;6:102.

Hui RK, Leung FC. Differential expression profile of chicken embryo fibroblast DF-1 cells infected with cell-adapted infectious bursal disease virus. PloS One. 2015 Jun 8;10(6):e0111771.

Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols. 2009 Jan;4(1):44.

Huang Y, Li Y, Burt DW, Chen H, Zhang Y, Qian W, Kim H, Gan S, Zhao Y, Li J, Yi K. The duck genome and transcriptome provide insight into an avian influenza virus reservoir species. Nature Genetics. 2013 Jul;45(7):776.

Humphreys IR, Walzl G, Edwards L, Rae A, Hill S, Hussell T. A critical role for OX40 in T cell–mediated immunopathology during lung viral infection. Journal of Experimental Medicine. 2003 Oct 20;198(8):1237-42.

Ihle JN. Cytokine receptor signalling. Nature. 1995 Oct;377(6550):591.

Ingrao F, Rauw F, Lambrecht B, van den Berg T. Infectious Bursal disease: a complex host–pathogen interaction. Developmental & Comparative Immunology. 2013 Nov 1;41(3):429-38.

Inoue M, Yamamoto H, Matuo K, Hihara H. Susceptibility of chicken monocytic cell lines to infectious bursal disease virus. Journal of Veterinary Medical Science. 1992 Jun 15;54(3):575-7.

Iván J, Velhner M, Ursu K, Germán P, Mató T, Drén CN, Mészáros J. Delayed vaccine virus replication in chickens vaccinated subcutaneously with an immune complex infectious bursal disease vaccine: quantification of vaccine virus by real-time polymerase chain reaction. Canadian Journal of Veterinary Research. 2005 Apr;69(2):135.

Ivanyi J, Morris R. Immunodeficiency in the chicken. IV. An immunological study of infectious bursal disease. Clinical and Experimental Immunology. 1976 Jan;23(1):154.

Jahromi MZ, Bello MB, Abdolmaleki M, Yeap SK, Hair-Bejo M, Omar AR. Differential activation of intraepithelial lymphocyte-natural killer cells in chickens infected with very virulent and vaccine strains of infectious bursal disease virus. Developmental & Comparative Immunology. 2018 Oct 1;87:116-23.

Janeway Jr CA, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002 Apr;20(1):197-216.

Jansen CA, van de Haar PM, van Haarlem D, van Kooten P, de Wit S, van Eden W, Viertlböck BC, Göbel TW, Vervelde L. Identification of new populations of chicken natural killer (NK) cells. Developmental & Comparative Immunology. 2010 Jul 1;34(7):759-67.

Juul-Madsen HR, Viertlböeck B, Härtle S, Smith AL, Göbel TW. Innate immune responses. In Avian Immunology (Second Edition) 2014 (pp. 121-147).

Kang YH, Lee CH, Seo YS. Dna2 on the road to Okazaki fragment processing and genome stability in eukaryotes. Critical Reviews in Biochemistry and Molecular Biology. 2010 Apr 1;45(2):71-96.

Kaszubowska L, Wierzbicki PM, Karsznia S, Damska M, Ślebioda TJ, Foerster J, Kmieć Z. Optimal reference genes for qPCR in resting and activated human NK cells—Flow cytometric data correspond to qPCR gene expression analysis. Journal of Immunological Methods. 2015 Jul 1;422:125-9.

Käufer I, Weiss EU. Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. Infection and Immunity. 1980 Feb 1;27(2):364-7.

Kaufman J, Milne S, Göbel TW, Walker BA, Jacob JP, Auffray C, Zoorob R, Beck S. The chicken B locus is a minimal essential major histocompatibility complex. Nature. 1999 Oct;401(6756):923.

Khatri M, Palmquist JM, Cha RM, Sharma JM. Infection and activation of bursal macrophages by virulent infectious bursal disease virus. Virus Research. 2005 Oct 1;113(1):44-50.

Kibenge FS, Qian B, Cleghorn JR, Martin CK. Infectious bursal disease virus polyprotein processing does not involve cellular proteases. Archives of Virology. 1997 Dec 1;142(12):2401-19.

Kim IJ, Gagic M, Sharma JM. Recovery of antibody-producing ability and lymphocyte repopulation of bursal follicles in chickens exposed to infectious bursal disease virus. Avian Diseases. 1999 Jul 1:401-13.

Kim IJ, Karaca K, Pertile TL, Erickson SA, Sharma JM. Enhanced expression of cytokine genes in spleen macrophages during acute infection with infectious bursal disease virus in chickens. Veterinary Immunology and Immunopathology. 1998 Feb 27;61(2-4):331-41.

Kimura H, Yoshizumi M, Ishii H, Oishi K, Ryo A. Cytokine production and signaling pathways in respiratory virus infection. Frontiers in Microbiology. 2013 Sep 17;4:276.

Kong LL, Omar AR, Bejo MH, Ideris A, Tan SW. Development of SYBR green I based one-step real-time RT-PCR assay for the detection and differentiation of very virulent and classical strains of infectious bursal disease virus. Journal of Virological Methods. 2009 Nov 1;161(2):271-9.

Kumar PA, Das SK, Rao JR. Effect of immunostimulation on cytotoxic activity of intestinal intraepithelial lymphocytes of chickens in infectious bursal disease and Eimeria tenella infections. Acta Veterinaria Hungarica. 1998;46(1):1-1.

Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Ségéral E, Yatim A, Emiliani S, Schwartz O, Benkirane M. SAMHD1 is the dendritic-and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature. 2011 Jun;474(7353):654.

Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, Pancino G. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nature Immunology. 2012 Mar;13(3):223.

Lam KM. Alteration of chicken heterophil and macrophage functions by the infectious bursal disease virus. Microbial Pathogenesis. 1998 Sep 1;25(3):147-55.

Lam KM. Morphological evidence of apoptosis in chickens infected with infectious bursal disease virus. Journal of Comparative Pathology. 1997 May 1;116(4):367-77.

Lanier LL, Testi RO, Bindl JA, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. Journal of Experimental Medicine. 1989 Jun 1;169(6):2233-8.

Lanier LL. NK cell receptors. Annual Review of Immunology. 1998 Apr;16(1):359-93.

Lanier LL. NK cell recognition. Annual Review Immunology. 2005 Apr 23;23:225-74.

Lasher HN, Shane S. Infectious bursal disease. World's Poultry Science Journal. 1994 Jul;50(2):133-66.

Le Gros FX, Dancer A, Giacomini C, Pizzoni L, Bublot M, Graziani M, Prandini F. Field efficacy trial of a novel HVT-IBD vector vaccine for 1-day-old broilers. Vaccine. 2009 Jan 22;27(4):592-6.

Lee HJ, Palkovits M, Young WS. miR-7b, a microRNA up-regulated in the hypothalamus after chronic hyperosmolar stimulation, inhibits Fos translation. Proceedings of the National Academy of Sciences. 2006 Oct 17;103(42):15669-74.

Lee SI, Lee BR, Hwang YS, Lee HC, Rengaraj D, Song G, Park TS, Han JY. MicroRNA-mediated posttranscriptional regulation is required for maintaining undifferentiated properties of blastoderm and primordial germ cells in chickens. Proceedings of the National Academy of Sciences. 2011 Jun 28;108(26):10426-31.

Leichter AL, Purcell RV, Sullivan MJ, Eccles MR, Chatterjee A. Multi-platform microRNA profiling of hepatoblastoma patients using formalin fixed paraffin embedded archival samples. GigaScience. 2015 Nov 25;4(1):54.

Li J, Donath S, Li Y, Qin D, Prabhakar BS, Li P. miR-30 regulates mitochondrial fission through targeting p53 and the dynamin-related protein-1 pathway. PLoS Genetics. 2010 Jan 8;6(1):e1000795.

Li P, Fan W, Li Q, Wang J, Liu R, Everaert N, Liu J, Zhang Y, Zheng M, Cui H, Zhao G. Splenic microRNA expression profiles and integration analyses involved in host responses to Salmonella enteritidis infection in chickens. Frontiers in Cellular and Infection Microbiology. 2017 Aug 24;7:377.

Li Z, Zhang J, Su J, Liu Y, Guo J, Zhang Y, Lu C, Xing S, Guan Y, Li Y, Sun B. MicroRNAs in the immune organs of chickens and ducks indicate divergence of immunity against H5N1 avian influenza. FEBS Letters. 2015 Feb 13;589(4):419-25.

Lian L, Qu L, Chen Y, Lamont SJ, Yang N. A systematic analysis of miRNA transcriptome in Marek's disease virus-induced lymphoma reveals novel and differentially expressed miRNAs. PLoS One. 2012 Nov 30;7(11):e51003.

Liang J, Yin Y, Qin T, Yang Q. Chicken bone marrow-derived dendritic cells maturation in response to infectious bursal disease virus. Veterinary Immunology and Immunopathology. 2015 Mar 15;164(1-2):51-5.

Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2013 Nov 13;30(7):923-30.

Lim W, Jeong W, Kim J, Ka H, Bazer FW, Han JY, Song G. Differential expression of secreted phosphoprotein 1 in response to estradiol-17β and in ovarian tumors in chickens. Biochemical and Biophysical Research Communications. 2012 Jun 8;422(3):494-500.

Lin JD, Feng N, Sen A, Balan M, Tseng HC, McElrath C, Smirnov SV, Peng J, Yasukawa LL, Durbin RK, Durbin JE. Distinct roles of type I and type III interferons in intestinal immunity to homologous and heterologous rotavirus infections. PLoS Pathogens. 2016 Apr 29;12(4):e1005600.

Liu M, Vakharia VN. Nonstructural protein of infectious bursal disease virus inhibits apoptosis at the early stage of virus infection. Journal of Virology. 2006 Apr 1;80(7):3369-77.

Liu X, Wang Y, Sun Q, Yan J, Huang J, Zhu S, Yu J. Identification of microRNA transcriptome involved in human natural killer cell activation. Immunology Letters. 2012 Apr 30;143(2):208-17.

Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell. 2001 Aug 10;106(3):259-62.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001 Dec 1;25(4):402-8.

Lo KM, Vivier E, Rochet N, Dehni G, Levine H, Haseltine WA, Anderson P. Infection of human natural killer (NK) cells with replication-defective human T cell leukemia virus type I provirus. Increased proliferative capacity and prolonged survival of functionally competent NK cells. The Journal of Immunology. 1992 Dec 15;149(12):4101-8.

Long EO. Regulation of immune responses through inhibitory receptors. Annual Review of Immunology. 1999 Apr;17(1):875-904.

Lopez-Botet M, Carretero M, Bellon T, Perez-Villar JJ, Llano M, Navarro F. The CD94/NKG2 C-type lectin receptor complex. InSpecificity, Function, and Development of NK Cells 1998 (pp. 41-52). Springer, Berlin, Heidelberg.

Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 2014 Dec;15(12):550.

Mahgoub HA. An overview of infectious bursal disease. Archives of Virology. 2012 Nov 1;157(11):2047-57.

Mandal A, Viswanathan C. Natural killer cells: in health and disease. Hematology/Oncology and Stem Cell Therapy. 2015 Jun 1;8(2):47-55.

McFerran JB, McNulty MS, McKillop ER, Connor TJ, McCracken RM, Collins DS, Allan GM. Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys and ducks: demonstration of a second serotype. Avian Pathology. 1980 Jul 1;9(3):395-404.

Melanson VR, Kalina WV, Williams P. Ebola virus infection induces irregular dendritic cell gene expression. Viral Immunology. 2015 Feb 1;28(1):42-50.

Metzker ML. Sequencing technologies—the next generation. Nature Reviews Genetics. 2010 Jan;11(1):31.

Meyer L, Leymarie O, Chevalier C, Esnault E, Moroldo M, Da Costa B, Georgeault S, Roingeard P, Delmas B, Quéré P, Le Goffic R. Transcriptomic profiling of a chicken lung epithelial cell line (CLEC213) reveals a mitochondrial respiratory chain activity boost during influenza virus infection. PloS One. 2017 Apr 25;12(4):e0176355.

Mitra RD, Shendure J, Olejnik J, Church GM. Fluorescent in situ sequencing on polymerase colonies. Analytical Biochemistry. 2003 Sep 1;320(1):55-65.

Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clinical Microbiology Reviews. 2009 Apr 1;22(2):240-73.

Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. Cell. 2007 May 18;129(4):665-79.

Moretta A, Sivori S, Ponte M, Mingari MC, Moretta L. Stimulatory receptors in NK and T cells. InSpecificity, Function, and Development of NK Cells 1998 (pp. 15-23). Springer, Berlin, Heidelberg.

Müller H, Scholtissek CH, Becht HE. The genome of infectious bursal disease virus consists of two segments of double-stranded RNA. Journal of Virology. 1979a Sep 1;31(3):584-9.

Müller R, Käufer I, Reinacher M, Weiss E. Immunofluorescent studies of early virus propagation after oral infection with infectious bursal disease virus (IBDV). Zentralblatt für Veterinärmedizin Reihe B. 1979b Jun;26(5):345-52.

Mundt E, Beyer J, Müller H. Identification of a novel viral protein in infectious bursal disease virus-infected cells. Journal of General Virology. 1995 Feb 1;76(2):437-43.

Mundt E, Köllner B, Kretzschmar DI. VP5 of infectious bursal disease virus is not essential for viral replication in cell culture. Journal of Virology. 1997 Jul 1;71(7):5647-51.

Nagata K, Kawaguchi A, Naito T. Host factors for replication and transcription of the influenza virus genome. Reviews in Medical Virology. 2008 Jul;18(4):247-60.

Ndashe K, Simulundu E, Hang'ombe BM, Moonga L, Ogawa H, Takada A, Mweene AS. Molecular characterization of infectious bursal disease viruses detected in vaccinated commercial broiler flocks in Lusaka, Zambia. Archives of Virology. 2016 Mar 1;161(3):513-9.

Neulen ML, Göbel TW. Identification of a chicken CLEC-2 homologue, an activating C-type lectin expressed by thrombocytes. Immunogenetics. 2012 May 1;64(5):389-97.

Ng P, Wei CL, Sung WK, Chiu KP, Lipovich L, Ang CC, Gupta S, Shahab A, Ridwan A, Wong CH, Liu ET. Gene identification signature (GIS) analysis for transcriptome characterization and genome annotation. Nature Methods. 2005 Feb;2(2):105.

Noda M, Omatsu Y, Sugiyama T, Oishi S, Fujii N, Nagasawa T. CXCL12-CXCR4 chemokine signaling is essential for NK-cell development in adult mice. Blood. 2011 Jan 13;117(2):451-8.

Öppling V, Müller H, Becht H. The structural polypeptide VP3 of infectious bursal disease virus carries group-and serotype-specific epitopes. Journal of General Virology. 1991 Sep 1;72(9):2275-8.

O'rourke JP, Ness SA. Alternative RNA splicing produces multiple forms of c-Myb with unique transcriptional activities. Molecular and Cellular Biology. 2008 Mar 15;28(6):2091-101.

Ou C, Wang Q, Zhang Y, Kong W, Zhang S, Yu Y, Ma J, Liu X, Kong X. Transcription profiles of the responses of chicken bursae of Fabricius to IBDV in different timing phases. Virology Journal. 2017 Dec;14(1):93.

Ouyang W, Wang YS, Meng K, Pan QX, Wang XL, Xia XX, Zhu YM, Bi ZW, Zhang HB, Luo K. gga-miR-2127 downregulates the translation of chicken p53 and attenuates chp53-mediated innate immune response against IBDV infection. Veterinary Microbiology. 2017 Jan 1;198:34-42.

Ouyang W, Zhu X, Wang Y, Wang Y, Pan Q, Bi Z, Wang X. gga-miR-21 regulates infectious bursal disease virus (IBDV) replication by targeting VP1 gene of IBDV. Zhongguo Yufang Shouyi Xuebao/Chinese Journal of Preventive Veterinary Medicine. 2012;34(11):847-53.

Panigrahy B, Misra LK, Adams LG. Humoral and cell-mediated immune responses in chickens with infectious bursal disease. Veterinary Microbiology. 1982 Sep 1;7(4):383-7.

Peng X, Gao QS, Zhou L, Chen ZH, Lu S, Huang HJ, Zhan CY, Xiang M. MicroRNAs in avian influenza virus H9N2-infected and non-infected chicken embryo fibroblasts. Genetics Molecular Resources. 2015 Jan 1;14(3):9081-91.

Pertile TL, Sharma JM, Walser MM. Reovirus infection in chickens primes splenic adherent macrophages to produce nitric oxide in response to T cell-produced factors. Cellular Immunology. 1995 Sep 1;164(2):207-16.

Pollmann J, Götz JJ, Rupp D, Strauss O, Granzin M, Grünvogel O, Mutz P, Kramer C, Lasitschka F, Lohmann V, Björkström NK. Hepatitis C virus-induced natural killer cell proliferation involves monocyte-derived cells and the OX40/OX40L axis. Journal of Hepatology. 2018 Mar 1;68(3):421-30.

Portou MJ, Baker D, Abraham D, Tsui J. The innate immune system, toll-like receptors and dermal wound healing: a review. Vascular Pharmacology. 2015 Aug 1;71:31-6.

Pradhan SN, Prince PR, Madhumathi J, Roy P, Narayanan RB, Antony U. Protective immune responses of recombinant VP2 subunit antigen of infectious bursal disease virus in chickens. Veterinary Immunology and Immunopathology. 2012 Aug 15;148(3-4):293-301.

Quan R, Zhu S, Wei L, Wang J, Yan X, Li Z, Liu J. Transcriptional profiles in bursal B-lymphoid DT40 cells infected with very virulent infectious bursal disease virus. Virology Journal. 2017 Dec;14(1):7.

Ranson T, Vosshenrich CA, Corcuff E, Richard O, Müller W, Di Santo JP. IL-15 is an essential mediator of peripheral NK-cell homeostasis. Blood. 2003 Jun 15;101(12):4887-93.

Rasoli M, Yeap SK, Tan SW, Roohani K, Kristeen-Teo YW, Alitheen NB, Rahaman YA, Aini I, Bejo MH, Kaiser P, Omar AR. Differential modulation of immune response and cytokine profiles in the bursae and spleen of chickens infected with very virulent infectious bursal disease virus. BMC Veterinary Research. 2015 Dec;11(1):75.

Rathjen T, Pais H, Sweetman D, Moulton V, Munsterberg A, Dalmay T. High throughput sequencing of microRNAs in chicken somites. FEBS Letters. 2009 May 6;583(9):1422-6.

Rauf A, Khatri M, Murgia MV, Saif YM. Expression of perforin–granzyme pathway genes in the bursa of infectious bursal disease virus-infected chickens. Developmental & Comparative Immunology. 2011 May 1;35(5):620-7.

Reddy SK, Silim A, Ratcliffe MJ. Biological roles of the major capsid proteins and relationships between the two existing serotypes of infectious bursal disease virus. Archives of Virology. 1992 Mar 1;127(1-4):209-22.

Reeves RK, Gillis J, Wong FE, Yu Y, Connole M, Johnson RP. CD16− natural killer cells: enrichment in mucosal and secondary lymphoid tissues and altered function during chronic SIV infection. Blood. 2010 Jun 3;115(22):4439-46.

Rehman ZU, Meng C, Umar S, Munir M, Ding C. Interaction of infectious bursal disease virus with the immune system of poultry. World's Poultry Science Journal. 2016 Dec;72(4):805-20.

Repp H, Nieper H, Draheim HJ, Koschinski A, Müller H, Dreyer F. Infectious bursal disease virus changes the potassium current properties of chicken embryo fibroblasts. Virology. 1998 Jul 5;246(2):362-9.

Ritz JE, Schmidt RE, Michon J, Hercend TH, Schlossman SF. Characterization of functional surface structures on human natural killer cells. Advance Immunology. 1988 Jan 1;42(181):32.

Rodenberg J, Sharma JM, Belzer SW, Nordgren RM, Naqi S. Flow cytometric analysis of B cell and T cell subpopulations in specific-pathogen-free chickens infected with infectious bursal disease virus. Avian Diseases. 1994 Jan 1:16-21.

Rogers SL, Göbel TW, Viertlboeck BC, Milne S, Beck S, Kaufman J. Characterization of the chicken C-type lectin-like receptors B-NK and B-lec suggests that the NK complex and the MHC share a common ancestral region. The Journal of Immunology. 2005 Mar 15;174(6):3475-83.

Rogers SL, Kaufman J. High allelic polymorphism, moderate sequence diversity and diversifying selection for B-NK but not B-lec, the pair of lectin-like receptor genes in the chicken MHC. Immunogenetics. 2008 Aug 1;60(8):461.

Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annual Review of Immunology. 2000 Apr;18(1):217-42.

Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C T method. Nature Protocols. 2008 Jun;3(6):1101.

Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. Current Opinion in Virology. 2011 Dec 1;1(6):519-25.

Schulte LN, Eulalio A, Mollenkopf HJ, Reinhardt R, Vogel J. Analysis of the host microRNA response to Salmonella uncovers the control of major cytokines by the let-7 family. The EMBO Journal. 2011 May 18;30(10):1977-89.

Sharma JM, Dohms JE, Metz AL. Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and their effect on humoral and cellular immune competence of specific-pathogen-free chickens. Avian Diseases. 1989 Jan 1:112-24.

Sharma JM, Kim IJ, Rautenschlein S, Yeh HY. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Developmental & Comparative Immunology. 2000 Mar 1;24(2-3):223-35.

Sharma JM, Lee LF. Effect of infectious bursal disease on natural killer cell activity and mitogenic response of chicken lymphoid cells: role of adherent cells in cellular immune suppression. Infection and Immunity. 1983 Nov 1;42(2):747-54.

Shendure J, Porreca GJ, Reppas NB, Lin X, McCutcheon JP, Rosenbaum AM, Wang MD, Zhang K, Mitra RD, Church GM. Accurate multiplex polony sequencing of an evolved bacterial genome. Science. 2005 Sep 9;309(5741):1728-32.

Sivanandan V, Maheswaran SK. Immune profile of infectious bursal disease: I. Effect of infectious bursal disease virus on peripheral blood T and B lymphocytes of chickens. Avian Diseases. 1980 Jul 1:715-25.

Smith J, Sadeyen JR, Butter C, Kaiser P, Burt DW. Analysis of the early immune response to infection by infectious bursal disease virus in chickens differing in their resistance to the disease. Journal of Virology. 2015 Mar 1;89(5):2469-82.

Song H, Josleyn N, Janosko K, Skinner J, Reeves RK, Cohen M, Jett C, Johnson R, Blaney JE, Bollinger L, Jennings G. Monkeypox virus infection of rhesus macaques induces massive expansion of natural killer cells but suppresses natural killer cell functions. PloS One. 2013 Oct 17;8(10):e77804.

Straub C, Neulen ML, Sperling B, Windau K, Zechmann M, Jansen CA, Viertlboeck BC, Göbel TW. Chicken NK cell receptors. Developmental & Comparative Immunology. 2013 Nov 1;41(3):324-33.

Sullivan R, Leong J, Fehniger T. MicroRNA regulation of natural killer cells. Frontiers in Immunology. 2013 Feb 28;4:44.

Sun S, Yu JP, Chen F, Zhao TJ, Fang XH, Li YQ, Sui SF. TINY, a dehydration-responsive element (DRE)-binding protein-like transcription factor connecting the DRE-and ethylene-responsive element-mediated signaling pathways in Arabidopsis. Journal of Biological Chemistry. 2008 Mar 7;283(10):6261-71.

Suzuki Y, Nakabayashi Y, Takahashi R. Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. Proceedings of the National Academy of Sciences. 2001 Jul 17;98(15):8662-7.

Takaoka, A., Hayakawa, S., Yanai, H., Stoiber, D., Negishi, H., Kikuchi, H., Sasaki, S., Imai, K., Shibue, T., Honda, K. and Taniguchi, T. (2003). Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. Nature. 424(6948), pp.516-523.

Takeda K, Tsutsui H, Yoshimoto T, Adachi O, Yoshida N, Kishimoto T, Okamura H, Nakanishi K, Akira S. Defective NK cell activity and Th1 response in IL-18–deficient mice. Immunity. 1998 Mar 1;8(3):383-90.

Testi R, D'Ambrosio D, De Maria R, Santoni A. The CD69 receptor: a multipurpose cell-surface trigger for hematopoietic cells. Immunology Today. 1994 Oct 1;15(10):479-83.

Tham KM, Moon CD. Apoptosis in cell cultures induced by infectious bursal disease virus following in vitro infection. Avian Diseases. 1996 Jan 1:109-13.

Thornberry NA, Lazebnik Y. Caspases: enemies within. Science. 1998 Aug 28;281(5381):1312-6.

Trinchieri G. Biology of natural killer cells. InAdvances in immunology 1989 Jan 1 (Vol. 47, pp. 187-376). Academic Press.

Tsang HF, Xue VW, Koh SP, Chiu YM, Ng LP, Wong SC. NanoString, a novel digital color-coded barcode technology: current and future applications in molecular diagnostics. Expert Review of Molecular Diagnostics. 2017 Jan 2;17(1):95-103.

Tsukamoto K, Tanimura N, Mase M, Imai K. Comparison of virus replication efficiency in lymphoid tissues among three infectious bursal disease virus strains. Avian Diseases. 1995 Oct 1:844-52.

Turcatti G, Romieu A, Fedurco M, Tairi AP. A new class of cleavable fluorescent nucleotides: synthesis and optimization as reversible terminators for DNA sequencing by synthesis. Nucleic Acids Research. 2008 Feb 7;36(4):e25-.

Vaday GG, Peehl DM, Kadam PA, Lawrence DM. Expression of CCL5 (RANTES) and CCR5 in prostate cancer. The Prostate. 2006 Feb 1;66(2):124-34.

Vance RE, Kraft JR, Altman JD, Jensen PE, Raulet DH. Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1b. Journal of Experimental Medicine. 1998 Nov 16;188(10):1841-8.

Vasconcelos AC, Lam KM. Apoptosis in chicken embryos induced by the infectious bursal disease virus. Journal of Comparative Pathology. 1995 May 1;112(4):327-38.

Vasconcelos AC, Lam KM. Apoptosis induced by infectious bursal disease virus. Journal of General Virology. 1994 Jul 1;75(7):1803-6.

Veldman-Jones MH, Brant R, Rooney C, Geh C, Emery H, Harbron CG, Wappett M, Sharpe A, Dymond M, Barrett JC, Harrington EA. Evaluating robustness and sensitivity of the NanoString technologies nCounter platform to enable multiplexed gene expression analysis of clinical samples. Cancer Research. 2015 Jul 1;75(13):2587-93.

Verhelst J, Parthoens E, Schepens B, Fiers W, Saelens X. Interferon-inducible protein Mx1 inhibits influenza virus by interfering with functional viral ribonucleoprotein complex assembly. Journal of Virology. 2012 Dec 15;86(24):13445-55.

Vervelde L, Davison TF. Comparison of the in situ changes in lymphoid cells during infection with infectious bursal disease virus in chickens of different ages. Avian Pathology. 1997 Dec 1;26(4):803-21.

Viertboeck BC, Wortmann A, Schmitt R, Plachý J, Göbel TW. Chicken C-type lectin-like receptor B-NK, expressed on NK and T cell subsets, binds to a ligand on activated splenocytes. Molecular Immunology. 2007 Mar 1;45(5):1398-404.

Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nature Immunology. 2008 May;9(5):503.

Vladimer GI, Górna MW, Superti-Furga G. IFITs: emerging roles as key anti-viral proteins. Frontiers in Immunology. 2014 Mar 10;5:94.

Walliser I, Göbel TW. Chicken IL-17A is expressed in αβ and γδ T cell subsets and binds to a receptor present on macrophages, and T cells. Developmental & Comparative Immunology. 2018 Apr 1;81:44-53.

Wang C, Zhang Y, Luo J, Ding H, Liu S, Amer S, Xie L, Lyv W, Su W, Li M, Sun Q. Identification of miRNomes reveals ssc-miR-30d-R_1 as a potential therapeutic target for PRRS viral infection. Scientific Reports. 2016 Apr 27;6:24854.

Wang D, Zhou X, She R, Xiong J, Sun Q, Peng K, Liu L, Liu Y. Impaired intestinal mucosal immunity in specific-pathogen-free chickens after infection with very virulent infectious bursal disease virus. Poultry Science. 2009 Aug 1;88(8):1623-8.

Wang F, Tian Z, Wei H. Genomic expression profiling of NK cells in health and disease. European Journal of Immunology. 2015 Mar;45(3):661-78.

Wang P, Gu Y, Zhang Q, Han Y, Hou J, Lin L, Wu C, Bao Y, Su X, Jiang M, Wang Q. Identification of resting and type I IFN-activated human NK cell miRNomes reveals microRNA-378 and microRNA-30e as negative regulators of NK cell cytotoxicity. The Journal of Immunology. 2012 Jul 1;189(1):211-21.

Wang Q, Gao Y, Ji X, Qi X, Qin L, Gao H, Wang Y, Wang X. Differential expression of microRNAs in avian leukosis virus subgroup J-induced tumors. Veterinary Microbiology. 2013b Feb 22;162(1):232-8.

Wang Q, Ou C, Wei X, Yu Y, Jiang J, Zhang Y, Ma J, Liu X, Zhang G. CC chemokine ligand 19 might act as the main bursal T cell chemoattractant factor during IBDV infection. Poultry Science. 2018 Sep 17;98(2):688-94.

Wang WC, Lin FM, Chang WC, Lin KY, Huang HD, Lin NS. miRExpress: analyzing high-throughput sequencing data for profiling microRNA expression. BMC Bioinformatics. 2009 Dec;10(1):328.

Wang Y, Brahmakshatriya V, Lupiani B, Reddy SM, Soibam B, Benham AL, Gunaratne P, Liu HC, Trakooljul N, Ing N, Okimoto R. Integrated analysis of microRNA expression and mRNA transcriptome in lungs of avian influenza virus infected broilers. BMC Genomics. 2012 Dec;13(1):278.

Wang Y, Brahmakshatriya V, Zhu H, Lupiani B, Reddy SM, Yoon BJ, Gunaratne PH, Kim JH, Chen R, Wang J, Zhou H. Identification of differentially expressed miRNAs in chicken lung and trachea with avian influenza virus infection by a deep sequencing approach. BMC Genomics. 2009 Dec;10(1):512.

Wang YS, Ouyang W, Pan QX, Wang XL, Xia XX, Bi ZW, Wang YQ, Wang XM. Overexpression of microRNA gga-miR-21 in chicken fibroblasts suppresses replication of infectious bursal disease virus through inhibiting VP1 translation. Antiviral Research. 2013a Oct 1;100(1):196-201.

Whitfill CE, Haddad EE, Ricks CA, Skeeles JK, Newberry LA, Beasley JN, Andrews PD, Thoma JA, Wakenell PS. Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antibody with IBDV. Avian Diseases. 1995 Oct 1:687-99.

Yang X, Gao W, Liu H, Li J, Chen D, Yuan F, Zhang Z, Wang H. MicroRNA transcriptome analysis in chicken kidneys in response to differing virulent infectious bronchitis virus infections. Archives of Virology. 2017 Nov 1;162(11):3397-405.

Yao Y, Zhao Y, Smith LP, Lawrie CH, Saunders NJ, Watson M, Nair V. Differential expression of microRNAs in Marek's disease virus-transformed T-lymphoma cell lines. Journal of General Virology. 2009 Jul 1;90(7):1551-9.

Yasmin AR, Yeap SK, Tan SW, Hair-Bejo M, Fakurazi S, Kaiser P, Omar AR. In vitro characterization of chicken bone marrow-derived dendritic cells following infection with very virulent infectious bursal disease virus. Avian Pathology. 2015 Nov 2;44(6):452-62.

Yokoyama WM. The role of natural killer cells in innate immunity to infection. InInnate immunity 2003 (pp. 321-339). Humana Press, Totowa, NJ.

Yu Z, Gao X, Liu C, Lv X, Zheng S. Analysis of microRNA expression profile in specific pathogen-free chickens in response to reticuloendotheliosis virus infection. Applied Microbiology and Biotechnology. 2017 Apr 1;101(7):2767-77.

Zhang B, Liu X, Chen W, Chen L. IFIT5 potentiates anti-viral response through enhancing innate immune signaling pathways. Acta Biochim Biophys Sin. 2013 Aug 13;45(10):867-74.

Zhang N, Lu C, Chen L. miR-217 regulates tumor growth and apoptosis by targeting the MAPK signaling pathway in colorectal cancer. Oncology Letters. 2016 Dec 1;12(6):4589-97.

Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, De Sauvage FJ, Ouyang W. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nature Medicine. 2008 Mar;14(3):282.

Zorman Rojs O, Krapež U, Slavec B, Juršič-Cizerl R, Poljanec T. Field efficacy of different vaccines against infectious bursal disease in broiler flocks. Acta Veterinaria Hungarica. 2011 Jul 4;59(3):385-98.