UNIVERSITI PUTRA MALAYSIA

EXPRESSIONS OF TOLL-LIKE RECEPTORS AND CYTOKINE MODULATION OF ANTIVIRAL INNATE IMMUNE RESPONSES IN FELINE INFECTIOUS PERITONITIS VIRUS INFECTION

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

September 2020

This thesis is dedicated to my beloved mother and father, who have been the support of my life.

And to my sisters Putri Raihanah and Putri Safiyah, and my brother Megat Hasan for their constant encouragement for the completion of this thesis.

Without them and the Almighty, I would not have come this far in life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Feline infectious peritonitis (FIP) is a fatal immune-mediated disease of domestic cats caused by feline infectious peritonitis virus (FIPV), a member of coronavirus family. Previous studies have shown that the lack or absence of adaptive cellular immunity and aberrant antibody production may lead to cats succumbing to FIP. FIPV viral protein has been shown to antagonize the production of type I interferon (IFN), the key cytokine crucial during early stage of viral infection and downregulate the production of anti-inflammatory cytokine such as IL-10. Natural killer (NK) cells, another important innate immune cell has been shown to be dysregulated in FIPV infection, suggesting the importance of innate immunity in FIP pathogenesis. Despite these findings, the information on pattern recognition receptors (PRRs) that play a role in the detection of common molecules on pathogens, or also known as pathogen-associated molecular patterns (PAMPs) is still lacking. Therefore, this study attempts to investigate the role of the Toll-like receptors (TLRs), one of the PRRs in FIPV infection *in vitro* and *ex vivo*. Stimulation of TLR pathway activates the NF-κB and type I IFN-related pathways which in turn produce pro-inflammatory cytokines such as TNF-α, and type-I IFN (IFN- α, IFN-β). To achieve these objectives, Crandell-Rees Feline Kidney (CRFK) cells and feline CD14+ monocytes were infected with FIPV 79-1146 and harvested at 4, 12, and 24 hours post-infection (hpi). The infection of FIPV into these cells was confirmed by immunofluorescence (IF) assay. The mRNA expression of several TLRs (TLR3, TLR7, and TLR9) and some of downstream cytokines (TNF-α, IL-10, and IFN-β) were measured using real time PCR (qPCR). The results were then correlated with the viral load copy number. Results from the *in vitro* study revealed the involvement of TLR9 in TNF-α, and IFN-β cytokine modulation in CRFK cells. However, TLR3 was expressed at low level and remained stable throughout the *in vitro* infection while TLR7 was not detectable. In contrast, TLR7 expression was induced upon *ex vivo* infection of feline monocytes at earlier time point indicating the activation of TLR7 by binding to its ligand, single-stranded RNA. However, its expression was significantly reduced at later time points which could be due to the immune evasion strategy posed by FIPV. A similar trend was also observed

for TNF-α expression postulating the role of TLR7 in the regulation of TNF-α which has been implicated as the major pro-inflammatory cytokine seen in FIP cats. Furthermore, IFN-β gene was also expressed throughout the course of infection which could be mediated by TLR7 activation and independent of TLR3 and TLR9 signaling pathways as the level of expression of these two TLRs remained stable. In general, the viral replication kinetics in CRFK cells and feline monocytes were consistent with other studies where the virus increased from 4 hpi to 12 hpi and maintained or reduced from 12 hpi to 24 hpi. Interestingly, the expression of TLR7 in monocytes of one seronegative cat was sustained throughout the infection, and the expression of TNF-α, IL-10, and IFN-β were markedly upregulated suggesting a control of viral protein synthesis by the immune system which was confirmed by the absence of viral antigen by IF, although the viral RNA was present. Taken together, this study provides a new insight on the role of TLRs in modulating the immune responses in FIPV infection. Although different cells express a different set of TLRs as observed in this study, this report implicates the role of TLR7 and TLR9 in FIPV infection, therefore setting up an avenue for further investigation into their signaling pathway and possible formulation of new therapeutic strategies.

Keywords: Feline infectious peritonitis, Toll-like receptors, Cytokines, Gene expression, Monocytes

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

EKSPRESI RESEPTOR SEPERTI TOLL (TLR) DAN MODULASI SITOKIN DARI ANTIVIRUS IMUN SEMULAJADI DI DALAM JANGKITAN VIRUS PERITONITIS BERJANGKIT FELIN (FIPV)

Oleh

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Peritonitis berjangkit felin (FIP) adalah penyakit lantaran imun maut di dalam kucing domestik yang disebabkan oleh virus peritonitis berjangkit felin (FIPV), anggota keluarga coronavirus. Kajian terdahulu telah menunjukkan bahawa kekurangan atau ketiadaan keimunan sel penyesuaian dan penghasilan antibodi yang aberan boleh menyebabkan kucing tunduk kepada FIP. Protein yang dihasilkan oleh FIPV telah menunjukkan penentangan pengeluaran interferon jenis I, sitokin utama yang kritikal semasa peringkat awal jangkitan virus dan pengurangan kawal atur sitokin anti-radang seperti IL-10. Sel pembunuh semulajadi (NK) adalah sel keimunan semula jadi yang penting yang telah dinyahatur dalam jangkitan FIPV, mencadangkan kepentingan keimunan semula jadi dalam patogenesis FIP. Walaupun penemuan ini telah wujud, maklumat mengenai reseptor pengenalan pola (PRR) yang memainkan peranan dalam pengesanan molekul umum pada patogen, atau juga dikenali sebagai pola molekul yang berkaitan patogen (PAMP) masih kurang. Oleh itu, kajian ini cuba menyiasat peranan reseptor seperti toll (TLR), salah satu PRR dalam jangkitan FIPV *in vitro* dan *ex vivo*. Rangsangan laluan TLR mengaktifkan laluan yang berkaitan dengan NF-κB dan IFN jenis I yang seterusnya menghasilkan sitokin pro-radang seperti TNF-α, dan IFN jenis I (IFN-α, IFN-β). Untuk mencapai objektif ini, sel Crandell-Rees Feline Kidney (CRFK) dan monosit CD14+ felin telah dijangkiti dengan FIPV 79-1146 dan dituai pada 4, 12 dan 24 jam selepas jangkitan (hpi). Jangkitan FIPV ke dalam sel ini telah disahkan oleh ujian imunopendarfluor (IF). Ekspresi mRNA untuk beberapa TLR (TLR3, TLR7, dan TLR9) dan beberapa sitokin hilirannya (TNF-α, IL-10, dan IFN-β) telah diukur dengan menggunakan tindak balas berantai polimerase masa nyata (qPCR). Keputusannya kemudian dikaitkan dengan jumlah salinan beban virus. Keputusan daripada kajian *in vitro* menunjukkan penglibatan TLR9 dalam pemodulatan sitokin TNF-α, dan IFN-β dalam sel CRFK. Namun, TLR3 telah diekspresi pada paras yang rendah dan tetap stabil sepanjang jangkitan *in vitro* manakala TLR7 tidak dikesan. Sebaliknya, ekspresi TLR7 dalam monosit felin telah terinduksi pada masa yang lebih awal setelah dijangkiti secara *ex vivo*, menunjukkan pengaktifan TLR7 melalui ikatan dengan ligandnya, RNA bebenang tunggal. Namun, ekspresinya telah berkurang dengan

signifikan pada masa kemudian yang mungkin disebabkan oleh strategi pengelakan imun yang dipunyai FIPV. Trend yang sama juga telah diperhatikan pada ekspresi TNF-α yang mempostulatkan peranan TLR7 dalam mengawal atur TNF-α dimana ia telah terlibat sebagai sitokin pro-radang utama dalam kucing FIP. Tambahan pula, gen IFN-β juga telah diekspresi sepanjang jangkitan berlaku yang mungkin diperantara oleh pengaktifan TLR7 dan bebas daripada laluan isyarat TLR3 dan TLR9 yang mana paras ekspresi kedua-dua TLR tersebut tetap stabil. Secara umum, kinetik replikasi virus dalam sel CRFK dan monosit felin konsisten dengan kajian-kajian terdahulu dimana virus telah meningkat daripada 4 hpi ke 12 hpi dan tetap atau berkurang dari 12 hpi ke 24 hpi. Menariknya, ekspresi TLR7 dalam monosit seekor kucing seronegatif kekal sepanjang jangkitan, dan ekspresi TNF-α, IL-10, dan IFN-β telah meningkat dengan ketara yang mencadangkan bahawa kawalan protein sintesis virus oleh sistem imun yang telah disahkan dengan ketiadaan antigen virus menggunakan IF, walaupun terdapat kewujudan RNA virus. Secara kebersamaan, kajian ini menyediakan pemahaman baru tentang peranan TLR dalam modulasi tindak-balas imun dalam jangkitan FIPV. Walaupun sel-sel berlainan mengekspresi set TLR yang berlainan seperti yang diperhatikan dalam kajian ini, laporan ini telah membabitkan peranan TLR7 dan TLR9 dalam jangkitan FIPV, maka membuka sebuah jalan untuk penyiasatan lanjut ke dalam laluan isyaratnya dan formulasi termungkin untuk strategi terapeutik yang baru

Kata Kunci: Peritonitis berjangkit felin, reseptor seperti toll (TLR), sitokin, ekspresi gen, monosit

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LIST OF ABBREVIATIONS

Ab	Antibody
ADE	Antibody-Dependent Enhancement
Ag	Antigen
AP-1	Activator Protein 1
APN	Aminopeptidase N
BMDC	Bone Marrow-Derived DC
BSL2	Biosafety Level 2
CCL8	Chemokine (C-C Motif) Ligand 8
CCV	Canine Coronavirus
CD	Cluster of Differentiation
CD14	Cluster of Differentation 14
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
cDNA	Complementary DNA
CMI	Cell-Mediated Immunity
CPE	Cytopathic Effect
CpG	5'Cysteine-Phosphate-Guanine 3'
CRFK	Crandell Reese Feline Kidney
Ct	Threshold Cycle
CXCL10	C-X-C Motif Chemokine 10
DAPI	4′,6-Diamidino-2-Phenylindole
DC	Dendritic Cell
DD	Death Domain

Dif	Dorsal-Related Immune Factor
DMSO	Dimethylsufoxide
DSH	Domestic Short Hair
dsRNA	Double-Stranded RNA
E	Envelope
EDTA	Ethylenediaminetetraacetic Acid
EMCV	Encephalomyocarditis Virus
ER	Endoplasmic Reticulum
F	Forward
FBS	Fetal Bovine Serum
FCGS	Feline Chronic Gingivostomatitis
FCoV	Feline Coronavirus
fcwf-4	*Felis* Catus Whole Fetus
FECV	Feline Enteric Coronavirus
FeLV	Feline Leukemia Virus
FIP	Feline Infectious Peritonitis
FIPV	Feline Infectious Peritonitis Virus
FIV	Feline Immunodeficiency Virus
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
G-CSF	Granulocyte-Colony Stimulating Factor
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV-1	Human Immunodeficiency Virus 1
hpi	Hours Post Infection

HSV-1	Herpes Simplex Virus 1
HSV-2	Herpes Simplex Virus 2
hToll	Human Toll
IBV	Infectious Bronchitis Virus
ICAM-1	Intracellular Adhesion Molecule 1
ICC	Immunocytochemistry
IF	Immunofluorescence
IFAT	Immunofluorescent Antibody Test
IFN	Interferon
IFN-α	Interferon Alpha
IFN-β	Interferon Beta
IFN-γ	Interferon Gamma
IFN-ω	Interferon Omega
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IKK	IκB Kinase
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-17	Interleukin 17
IL-1RI	IL-1 Receptor Type I
IL-1β	Interleukin 1 Beta
IL-6	Interleukin 6
IL-8	Interleukin 8
ILC	Islet-Like Cluster

IRAK1	IL-1R-Associated Kinase 1
IRAK2	IL-1R-Associated Kinase 2
IRAK4	IL-1R-Associated Kinase 4
IRF	Interferon Regulatory Factor
IκB	Inhibitor of Kappa B
Jak-STAT	Janus Kinases Signal Transducer and Activator of Transcription Proteins
LPL	Lamina Propria Lymphocytes
LPS	Lipopolysaccharide
LRR	Leucine-Rich Repeat
LTA	Lipoteichoic Acid
LTC	Liposome-TLR Complex
M	Membrane
MAPK	Mitogen-Activated Protein Kinase
MAVS	Mitochondrial Antiviral-Signaling Protein
MCMV	Mouse Cytomegalovirus
MD-2	Myeloid Differentiation 2
MEM	Minimal Essential Media
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MHV	Mouse Hepatitis Virus
miRNA	Micro RNA
MLN	Mesenteric Lymph Node
MMP-9	Matrix Metalloproteinase-9
MOI	Multiplicity of Infection
mRNA	Messenger RNA

Mx	Myxovirus Resistance
MX1	MX Dynamin-Like Gtpase 1
MyD88	Myeloid Differentiation Primary Response 88
N	Nucleocapsid
NCBI	National Center for Biotechnology Information
NEMO	NF-Kappa-B Essential Modulator
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NK	Natural Killer
Nrdp1	Neuregulin Receptor Degradation Protein-1
NRT	No Reverse Transcriptase
NT	N Terminal
ODN-2216	Oligodeoxynucleotide-2216
ORF	Open Reading Frame
PAMP	Pathogen-Associated Molecular Pattern
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline 0.1% Tween
PC	Peritoneal Cells
pDC	Plasmacytoid Dendritic Cell
PEDV	Porcine Epidemic Diarrhea Virus
PFA	Paraformaldehyde
PI	Post Infection
PMN	Polymorphonuclear
Poly (I:C)	Polyinosinic:Polycytidylic Acid

PRR	Pathogen Recognition Receptors
qPCR	Real-Time PCR
R	Reverse
R-848	Resiquimod-848
RHD	Rel Homology Domain
RHIM	Receptor-Interacting Protein Homotypic Interaction Motif
RIG-1	Retinoic Acid-Inducible Gene 1
RIM	Rapid Immunochromatography
RIP1	Receptor-Interacting Protein 1
rpm	Rotation Per Minute
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RT-qPCR	Real-Time Reverse Transcriptase Polymerase Chain Reaction
S	Spike
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
ssRNA	Single-Stranded RNA
STAT2	Signal Transducer and Activator of Transcription 2
TAB2	TGF-Beta-Activated Kinase 1 and MAP3K7-Binding Protein 2
TAB3	TGF-Beta-Activated Kinase 1 and MAP3K7-Binding Protein 3
TAK1	Transforming Growth Factor-β (TGF-β)-Activated Kinase 1
TANK	TRAF Family Member-Associated NF-Kappa-B Activator
TBEV	Tick-Borne Encephalitis Virus
TBK1	TANK-Binding Kinase 1
$TCID_{50}$	Tissue Culture Infectious Dose 50
TGEV	Transmissible Gastroenteritis Virus

Th1	T Helper 1
Th17	T Helper 17
TICAM-2	TIR-Containing Adaptor Molecule 2
TIR	Toll-IL-1-Resistant
TIRAP	TIR Domain-Containing Adaptor Protein
TLR	Toll-Like Receptor
TNFR	Tumor Necrosis Factor Receptor
TNF-α	Tumor Necrosis Factor Alpha
TRADD	TNFR-Associated Death Domain
TRAF3	TNF Receptor-Associated Factor 3
TRAF6	TNF Receptor-Associated Factor 6
TRAM	TRIF-Related Adaptor Molecule
TRIF	TIR-Domain-Containing Adapter-Inducing Interferon-β
tRNA	Transfer RNA
Ubc13	Ubiquitin Conjugating Enzyme E2
Uev1A	Ubiquitin-Conjugating Enzyme E2 Variant 1A
Unc93b1	Unc-93 homolog B1
VCAM-1	Vascular Cell Adhesion Molecule 1
VEGF	Vascular Endothelial Growth Factor
VSV	Vesicular Stomatitis Virus
WNV	West Nile Virus
β2M	Beta-2-Microglobulin

CHAPTER 1

INTRODUCTION

Feline infectious peritonitis (FIP) is a severe inflammatory disease affecting young domestic cats caused by FIP virus (FIPV), one of the two biotypes of feline coronavirus (FCoV). The other biotype is called feline enteric coronavirus (FECV) that caused mild illnesses such as self-limiting enteritis in kittens (Pedersen, 2009). The FCoV is categorized under the Coronaviridae family that has the largest RNA genome among other families of RNA viruses, with size of about 32 kB of positive-strand RNA (Dye and Siddell, 2007). Other members of Coronaviridae species that caused significant disease in animals are infectious bronchitis virus (IBV) affecting chickens and transmissible gastroenteritis virus (TGEV) affecting pigs that caused huge economic losses in poultry and farm industry.

The frequency of cats infected with FECV is extremely common especially in catteries and shelters, yet the chance of them to develop FIP is very rare which could be due to the avirulent nature of most field strain (Addie, 2000). However, several key events can trigger the development of FIP. During an intense viral replication in the gut, there is a higher chance of internal mutation events to occur inside FECV that can lead to a biotype shift towards FIPV. Yet, this internal mutation is not the only determinant of FIP as other factors like host's genetics, geographical region, and immune status of the cat also play critical role on the disease outcome (Myrrha et al., 2011).

FIP has two forms which are the effusive ('wet') FIP which contributes largely in clinical cases and has higher percent mortality; and the non-effusive ('dry') FIP. Symptoms of both forms include fever, weight loss, and loss of appetite; but the distinguishing feature between the two is that wet FIP cats show distended abdomen that is filled with protein-rich fluid while dry FIP cats usually is presented with granulomatous lesions and phlebitis in multiple organs (Kipar and Meli, 2014). The underlying reason for these forms to arise is largely due to the immune strength of the host, where the cell-mediated immune (CMI) responses of wet FIP cats are generally abrogated while dry FIP cats can mount some undetermined level of CMI.

There are several indirect approaches used to diagnose FIP which includes examining the clinical history such as cat's previous exposure to multi-household environment, stress level, presence of fluctuating fever, and loss of appetite. The diagnosis can be followed by analysis of serum albumin to globulin (A:G) ratio, FCoV-specific serology test, and reverse transcriptase polymerase chain reaction (RT-PCR) detecting viral RNA in tissues or effusion. The use of one test is usually not sufficient to suggest for FIP because each test has its limitation, therefore, the combination of different tests is very important to provide definitive diagnosis of FIP (Tasker, 2018).

Various efforts have been established to develop safe and effective therapy for FIP. Many of the earliest treatment in the 70's to late 90's involved the use of

immunosuppressive drugs in combination with antibiotics such as prednisolone and penicillin. This strategy was proven to be ineffective as many cats suspected to have FIP succumb to the disease (Hartmann and Ritz, 2008). The approaches used today are essentially similar which mainly involved the combination of several therapeutics agents such as anti-viral drugs such as ribavirin and interferon-α. Nevertheless, the number of proper clinical trials and controls to test the safety and efficacy of such regime is still lacking, therefore, definitive treatment for FIP is still unavailable.

In an effort to answer the perplexing questions surrounding FIP and its treatment, many interests have drifted towards investigating the role of innate immunity in positively modulating the immune system to counteract with FIP. Although it was discovered in the 90's from experiments involving the drosophila Toll protein, significant progress has been made that further expands our knowledge of this system. Amongst the earliest component of innate immunity in mammalian cells discovered was the toll-like receptors (TLRs) family which functions as unique sensors for numerous types of microbial. Recognition of microbial by TLRs activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and interferon regulatory factor (IRF) pathways which are the molecular switches that lead to the production of various cytokines and type I interferons (IFN) for the clearance of pathogens (Akira and Takeda, 2004).

There are currently ten human TLRs and twelve mice TLRs being discovered and characterized. Feline tissues are also shown to express at least nine TLRs (Ignacio et al., 2005). TLR3, TLR7, and TLR8 specifically detects virus-associated nucleic acid such as single-stranded (ss) RNA or double-stranded (ds) RNA (Jiménez-Dalmaroni et al., 2015). A number of viruses that are being recognized by these TLRs do elicit protective immune response that ultimately results in virus clearance. However, some viruses also exploit the TLRs signaling in order to provide favorable conditions for their replication. Therefore, characterization of TLR-recognition pathways in virus infection is pertinent in order to design immunotherapies that can either stimulate or suppress the TLRs pathways so that specific viral infection can be controlled (Patel et al., 2014).

To provide better understanding on the role of TLRs on the pathogenesis of FIP, whether they provide protection to the host or use by the virus to evade immune responses, the gene expression of nucleic acid-sensing TLRs - TLR3, TLR7, and TLR9 were measured upon infection with FIPV in vitro using probe-based quantitative real-time PCR (qPCR). The expression of these TLRs to the production of pro-inflammatory cytokines and type I IFN were then correlated to the FIPV load at different periods of infection. The significance of our study is then verified by using cell types that are susceptible to FIPV infection both ex vivo and in vitro which are the peripheral blood monocytes and Crandell-Rees Feline Kidney (CRFK) cells.

The objectives of the study are summarized as follows:

1. To measure the gene expression of TLR3, TLR7, and TLR9, and the expression of pro-inflammatory cytokines and type I IFN upon infection with FIPV at different time points in vitro.

2. To measure the gene expression of TLR3, TLR7, and TLR9, and the expression of pro-inflammatory cytokines and type I IFN upon infection with FIPV ex vivo.

3. To determine the correlation between viral load, the TLR3, TLR7 and TLR9 and cytokines expression upon infection with FIPV.

REFERENCES

Acar, D. D., Olyslaegers, D. A. J., Dedeurwaerder, A., Roukaerts, I. D. M., Baetens, W., Van Bockstael, S., Nauwynck, H. J., et al. (2016). Upregulation of endothelial cell adhesion molecules characterizes veins close to granulomatous infiltrates in the renal cortex of cats with feline infectious peritonitis and is indirectly triggered by feline infectious peritonitis virus-infected monocytes. *The Journal of General Virology*, *97*(10), 2633–2642.

Addie, D. D. (2000). Clustering of feline coronaviruses in multicat households. *Veterinary Journal (London, England : 1997)*, *159*(1), 8–9.

Addie, D. D., le Poder, S., Burr, P., Decaro, N., Graham, E., Hofmann-Lehmann, R., Meli, M. L., et al. (2015). Utility of feline coronavirus antibody tests. *Journal of Feline Medicine and Surgery*, *17*(2), 152–162.

Addie, D. and Jarrett, O. (2001). Use of a reverse-transcriptase polymerase chain reaction for monitoring the shedding of feline coronavirus by healthy cats. *Veterinary Record*, 148(21), 649-653.

Akira, S., and Takeda, K. (2004). Toll-like receptor signalling. *Nature Reviews Immunology*, *4*(7), 499–511.

Alexopoulou, L., Holt, A. C., Medzhitov, R., and Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*, *413*(6857), 732–738.

Anderson, K. V., Bokla, L., and Nüsslein-Volhard, C. (1985). Establishment of dorsal-ventral polarity in the Drosophila embryo: the induction of polarity by the *Toll* gene product. *Cell*, *42*(3), 791–798.

Bank-Wolf, B. R., Stallkamp, I., Wiese, S., Moritz, A., Tekes, G., and Thiel, H.-J. (2014). Mutations of 3c and spike protein genes correlate with the occurrence of feline infectious peritonitis. *Veterinary Microbiology*, *173*(3–4), 177–188.

Barker, E. N., Stranieri, A., Helps, C. R., Porter, E. L., Davidson, A. D., Day, M. J., Tasker, S., et al. (2017). Limitations of using feline coronavirus spike protein gene mutations to diagnose feline infectious peritonitis. *Veterinary Research*, *48*(1), 60.

Beg, A. A., and Baldwin, A. S. (1993). The I kappa B proteins: multifunctional regulators of Rel/NF-kappa B transcription factors. *Genes & Development*, *7*(11), 2064–2070.

Bell, J. K., Mullen, G. E. D., Leifer, C. A., Mazzoni, A., Davies, D. R., and Segal, D. M. (2003). Leucine-rich repeats and pathogen recognition in Toll-like receptors. *Trends in Immunology*, *24*(10), 528–533.

Beutler, B. a. (2009). TLRs and innate immunity. *Blood*, *113*(7), 1399–1407.

Bhoj, V. G., and Chen, Z. J. (2009). Ubiquitylation in innate and adaptive immunity. *Nature*, *458*(7237), 430–437.

Botos, I., David M., S., and Davies, D. R. (2011). Structural biology of TLRs. *HHS Public Acces*, *19*(4), 447–459.

Brinkmann, M. M., Spooner, E., Hoebe, K., Beutler, B., Ploegh, H. L., and Kim, Y.-M. (2007). The interaction between the ER membrane protein UNC93B and TLR3, 7, and 9 is crucial for TLR signaling. *The Journal of Cell Biology*, *177*(2), 265–275.

Browne, E. P. (2012). Regulation of B-cell responses by Toll-like receptors. *Immunology*, *136*(4), 370–379.

Cambier, L. C., Heinen, M.-P. A.-L., Bagut, E. T., Antoine, N. A., and Mignon, B. R. (2016). Overexpression of TLR-2 and TLR-4 mRNA in feline polymorphonuclear neutrophils exposed to *Microsporum canis*. *Veterinary Dermatology*, *27*(2), 78-81e22.

Cao, L., Ge, X., Gao, Y., Ren, Y., Ren, X., and Li, G. (2015a). Porcine epidemic diarrhea virus infection induces NF-kB activation through the TLR2, TLR3 and TLR9 pathways in porcine intestinal epithelial cells. *Journal of General Virology*, *96*(7), 1757–1767.

Cao, L., Ge, X., Gao, Y., Herrler, G., Ren, Y., Ren, X., and Li, G. (2015b). Porcine epidemic diarrhea virus inhibits dsRNA-induced interferon-β production in porcine intestinal epithelial cells by blockade of the RIG-I-mediated pathway. *Virology Journal*, *12*(1), 127.

Chang, H.-W., Egberink, H. F., Halpin, R., Spiro, D. J., and Rottier, P. J. M. (2012). Spike protein fusion peptide and feline coronavirus virulence. *Emerging Infectious Diseases*, *18*(7), 1089–1095.

Channappanavar, R., Fehr, A. R., Zheng, J., Wohlford-Lenane, C., Abrahante, J. E., Mack, M., Perlman, S., et al. (2019). IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *The Journal of Clinical Investigation*, *130*(9), 3625–3639.

Choi, Y. J., Im, E., Chung, H. K., Pothoulakis, C., and Rhee, S. H. (2010). TRIF mediates Toll-like receptor 5-induced signaling in intestinal epithelial cells. *The Journal of Biological Chemistry*, *285*(48), 37570–37578.

Contreras, E. T., Olea-Popelka, F., Wheat, W., Dow, S., Hawley, J., and Lappin, M. R. (2019). Evaluation of liposome toll-like receptor ligand complexes for non-specific mucosal immunoprotection from feline herpesvirus-1 infection. *Journal of Veterinary Internal Medicine*, *33*(2), 831–837.

Conze, D. B., Wu, C.-J., Thomas, J. A., Landstrom, A., and Ashwell, J. D. (2008). Lys63-Linked Polyubiquitination of IRAK-1 Is Required for Interleukin-1 Receptor- and Toll-Like Receptor-Mediated NF- B Activation. *Molecular and Cellular Biology*, *28*(10), 3538–3547.

Cornelissen, E., Dewerchin, H. L., Van Hamme, E., and Nauwynck, H. J. (2007). Absence of surface expression of feline infectious peritonitis virus (FIPV) antigens on infected cells isolated from cats with FIP. *Veterinary Microbiology*, *121*(1–2), 131–137.

Coutinho, A., and Meo, T. (1978). Genetic basis for unresponsiveness to lipopolysaccharide in C57BL/10Cr mice. *Immunogenetics*, *7*(1), 17–24.

Dai, L., Aye Thu, C., Liu, X. Y., Xi, J., and Cheung, P. C. F. (2012). TAK1, more than just innate immunity. *IUBMB Life*, *64*(10), 825–834.

Dalpke, A., Frank, J., Peter, M., and Heeg, K. (2006). Activation of toll-like receptor 9 by DNA from different bacterial species. *Infection and Immunity*, *74*(2), 940–946.

Davis, N., Ghosh, S., Simmons, D. L., Tempst, P., Liou, H. C., Baltimore, D., and Bose, H. R. (1991). Rel-associated pp40: An inhibitor of the Rel family of transcription factors. *Science*, *253*(5025), 1268–1271.

De Groot-Mijnes, J. D. F., van Dun, J. M., van der Most, R. G., and de Groot, R. J. (2005). Natural History of a Recurrent Feline Coronavirus Infection and the Role of Cellular Immunity in Survival and Disease. *Journal of Virology*, *79*(2), 1036–1044.

De Nardo, D. (2015). Toll-like receptors: Activation, signalling and transcriptional modulation. *Cytokine*, *74*(2), 181–189.

Dean, G. A., Olivry, T., Stanton, C., and Pedersen, N. C. (2003). In vivo cytokine response to experimental feline infectious peritonitis virus infection. *Veterinary Microbiology*, *97*(1–2), 1–12.

Dewerchin, H. L., Cornelissen, E., and Nauwynck, H. J. (2005). Replication of feline coronaviruses in peripheral blood monocytes. *Archives of Virology*, *150*(12), 2483–2500.

Diaz, J. V., and Poma, R. (2009). Diagnosis and clinical signs of feline infectious peritonitis in the central nervous system. *The Canadian Veterinary Journal. La Revue Vétérinaire Canadienne*, *50*(10), 1091–1093.

Diebold, S. S., Kaisho, T., Hemmi, H., Akira, S., and Reis E Sousa, C. (2004). Innate Antiviral Responses by Means of TLR7-Mediated Recognition of Single-Stranded RNA. *Science*, *303*(5663), 1529–1531.

Doki, T., Yabe, M., Takano, T., and Hohdatsu, T. (2018). Differential induction of type I interferon by type I and type II feline coronaviruses in vitro. *Research in Veterinary Science*, *120*(August), 57–62.

Dolieslager, S. M. J., Lappin, D. F., Bennett, D., Graham, L., Johnston, N., and Riggio, M. P. (2013). The influence of oral bacteria on tissue levels of Toll-like receptor and cytokine mRNAs in feline chronic gingivostomatitis and oral health. *Veterinary Immunology and Immunopathology*, *151*(3–4), 263–274.

Dye, C., and Siddell, S. G. (2007). Genomic RNA sequence of feline coronavirus strain FCoV C1Je. *Journal of Feline Medicine and Surgery*, *9*(3), 202–213.

Ewald, S. E., Engel, A., Lee, J., Wang, M., Bogyo, M., and Barton, G. M. (2011). Nucleic acid recognition by Toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. *The Journal of Experimental*

Medicine, *208*(4), 643–651.

Felten, S., and Hartmann, K. (2019). Diagnosis of Feline Infectious Peritonitis: A Review of the Current Literature. *Viruses*, *11*(11), 1068. https://doi.org/10.3390/v11111068

Franchini, M., Zini, E., Osto, M., Jablonski, K., Kaufmann, K., Lutz, T. A., … Ackermann, M. (2010). Feline pancreatic islet-like clusters and insulin producing cells express functional Toll-like receptors (TLRs). *Veterinary Immunology and Immunopathology*, *138*(1–2), 70–78.

Freer, G., Matteucci, D., Mazzetti, P., Bozzacco, L., and Bendinelli, M. (2005). Generation of feline dendritic cells derived from peripheral blood monocytes for in vivo use. *Clinical and Diagnostic Laboratory Immunology*, *12*(10), 1202–1208.

Gay, N. J., and Keith, F. J. (1991). Drosophila Toll and IL-1 receptor. *Nature*, *351*(6325), 355–356.

Gelain, M. E., Meli, M., and Paltrinieri, S. (2006). Whole blood cytokine profiles in cats infected by feline coronavirus and healthy non-FCoV infected specific pathogen-free cats. *Journal of Feline Medicine and Surgery*, *8*(6), 389–399.

Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P., and Baltimore, D. (1990). Cloning of the p50 DNA binding subunit of NF-kappa B: homology to *rel* and *dorsal*. *Cell*, *62*(5), 1019–1029.

Giordano, A., and Paltrinieri, S. (2009). Interferon-gamma in the serum and effusions of cats with feline coronavirus infection. *Veterinary Journal (London, England : 1997)*, *180*(3), 396–398.

Gunn-Moore, D. A., Caney, S. M., Gruffydd-Jones, T. J., Helps, C. R., and Harbour, D. A. (1998). Antibody and cytokine responses in kittens during the development of feline infectious peritonitis (FIP). *Veterinary Immunology and Immunopathology*, *65*(2–4), 221–242.

Häcker, H., Redecke, V., Blagoev, B., Kratchmarova, I., Hsu, L. C., Wang, G. G., Karin, M., et al. (2006). Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. *Nature*, *439*(7073), 204–207.

Hartmann, K., and Ritz, S. (2008). Treatment of cats with feline infectious peritonitis. *Veterinary Immunology and Immunopathology*, *123*(1–2), 172–175.

Harun, M. S. R., Kuan, C. O., Selvarajah, G. T., Wei, T. S., Arshad, S. S., Hair Bejo, M., and Omar, A. R. (2013). Transcriptional profiling of feline infectious peritonitis virus infection in CRFK cells and in PBMCs from FIP diagnosed cats. *Virology Journal*, *10*(1), 329.

Hashimoto, C., Hudson, K. L., and Anderson, K. V. (1988). The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell*, *52*(2), 269–279.

Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Aderem, A., et al. (2001). The innate immune response to bacterial flagellin is

mediated by Toll-like receptor 5. *Nature*, *410*(6832), 1099–1103.

Hemmi, H., Kaisho, T., Takeuchi, O., Sato, S., Sanjo, H., Hoshino, K., Akira, S., et al. (2002). Small-antiviral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nature Immunology*, *3*(2), 196–200.

Henrick, B. M., Yao, X. D., Zahoor, M. A., Abimiku, A., Osawe, S., and Rosenthal, K. L. (2019). TLR10 senses HIV-1 proteins and significantly enhances HIV-1 infection. *Frontiers in Immunology*, *10*(MAR), 1–14. https://doi.org/10.3389/fimmu.2019.00482

Herrewegh, A. A., Smeenk, I., Horzinek, M. C., Rottier, P. J., and de Groot, R. J. (1998). Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. *Journal of Virology*, *72*(5), 4508–4514.

Hoebe, K., Du, X., Georgel, P., Janssen, E., Tabeta, K., Kim, S. O., Beutler, B., et al. (2003). Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. *Nature*, *424*(6950), 743–748.

Horng, T., Barton, G. M., and Medzhitov, R. (2001). TIRAP: an adapter molecule in the Toll signaling pathway. *Nature Immunology*, *2*(9), 835–841.

Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdörfer, B., Giese, T., Hartmann, G., et al. (2002). Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *Journal of Immunology (Baltimore, Md. : 1950)*, *168*(9), 4531–4537.

Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Akira, S., et al. (1999). Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *Journal of Immunology (Baltimore, Md. : 1950)*, *162*(7), 3749–3752.

Hutchens, M., Luker, K. E., Sottile, P., Sonstein, J., Lukacs, N. W., Núñez, G., Luker, G. D., et al. (2008). TLR3 Increases Disease Morbidity and Mortality from Vaccinia Infection. *The Journal of Immunology*, *180*(1), 483–491.

Ignacio, G., Nordone, S., Howard, K. E., and Dean, G. A. (2005). Toll-like receptor expression in feline lymphoid tissues. *Veterinary Immunology and Immunopathology*, *106*(3–4), 229–237.

Ip, Y. T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., González-Crespo, S., Levine, M., et al. (1993). *Dif*, a dorsal-related gene that mediates an immune response in Drosophila. *Cell*, *75*(4), 753–763.

Jacobse-Geels, H. E., Daha, M. R., and Horzinek, M. C. (1980). Isolation and characterization of feline C3 and evidence for the immune complex pathogenesis of feline infectious peritonitis. *Journal of Immunology (Baltimore, Md. : 1950)*, *125*(4), 1606–1610.

Jacobse-Geels, H. E., and Horzinek, M. C. (1983). Expression of feline infectious

peritonitis coronavirus antigens on the surface of feline macrophage-like cells. *The Journal of General Virology*, *64 (Pt 9)*(1983), 1859–1866.

Jaimes, J. A., and Whittaker, G. R. (2018). Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function. *Virology*, *517*(December 2017), 108–121.

Jiménez-Dalmaroni, M. J., Gerswhin, M. E., and Adamopoulos, I. E. (2015). The critical role of toll-like receptors--From microbial recognition to autoimmunity: A comprehensive review. *Autoimmunity Reviews*, *15*(1), 1–8.

Jin, M. S., Kim, S. E., Heo, J. Y., Lee, M. E., Kim, H. M., Paik, S., Lee, J.-O., et al. (2007). Crystal Structure of the TLR1-TLR2 Heterodimer Induced by Binding of a Tri-Acylated Lipopeptide. *Cell*, *130*(6), 1071–1082.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nature Immunology*, *11*(5), 373–384.

Kawai, T., and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*, *34*(5), 637–650.

Kessler, Y., Helfer-Hungerbuehler, A. K., Cattori, V., Meli, M. L., Zellweger, B., Ossent, P., … Hofmann-Lehmann, R. (2009). Quantitative TaqMan real-time PCR assays for gene expression normalisation in feline tissues. *BMC Molecular Biology*, *10*(1), 106.

Khan, J. A., Brint, E. K., O'Neill, L. A. J., and Tong, L. (2004). Crystal structure of the Toll/interleukin-1 receptor domain of human IL-1RAPL. *The Journal of Biological Chemistry*, *279*(30), 31664–31670.

Kim, H. M., Park, B. S., Kim, J.-I., Kim, S. E., Lee, J., Oh, S. C., Lee, J., et al. (2007). Crystal Structure of the TLR4-MD-2 Complex with Bound Endotoxin Antagonist Eritoran. *Cell*, *130*(5), 906–917.

Kim, Y.-M., Brinkmann, M. M., Paquet, M.-E., and Ploegh, H. L. (2008). UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes. *Nature*, *452*(7184), 234–238.

Kim, Y., Liu, H., Galasiti Kankanamalage, A. C., Weerasekara, S., Hua, D. H., Groutas, W. C., Pedersen, N. C., et al. (2016). Reversal of the Progression of Fatal Coronavirus Infection in Cats by a Broad-Spectrum Coronavirus Protease Inhibitor. *PLoS Pathogens*, *12*(3), e1005531. https://doi.org/10.1371/journal.ppat.1005531

Kipar, A., Bellmann, S., Gunn-Moore, D. A., Leukert, W., Köhler, K., Menger, S., and Reinacher, M. (1999). Histopathological alterations of lymphatic tissues in cats without feline infectious peritonitis after long-term exposure to FIP virus. *Veterinary Microbiology*, *69*(1–2), 131–137.

Kipar, A., Bellmann, S., Kremendahl, J., Köhler, K., and Reinacher, M. (1998). Cellular composition, coronavirus antigen expression and production of specific antibodies in lesions in feline infectious peritonitis. *Veterinary Immunology and*

Immunopathology, *65*(2–4), 243–257.

Kipar, A., Köhler, K., Leukert, W., and Reinacher, M. (2001). A comparison of lymphatic tissues from cats with spontaneous feline infectious peritonitis (FIP), cats with FIP virus infection but no FIP, and cats with no infection. *Journal of Comparative Pathology*, *125*(2–3), 182–191.

Kipar, A., May, H., Menger, S., Weber, M., Leukert, W., and Reinacher, M. (2005). Morphologic features and development of granulomatous vasculitis in feline infectious peritonitis. *Veterinary Pathology*, *42*(3), 321–330.

Kipar, A., and Meli, M. L. (2014). Feline infectious peritonitis: still an enigma? *Veterinary Pathology*, *51*(2), 505–526.

Kipar, Anja, Baptiste, K., Barth, A., and Reinacher, M. (2006a). Natural FCoV infection: Cats with FIP exhibit significantly higher viral loads than healthy infected cats. *Journal of Feline Medicine and Surgery*, *8*(1), 69–72.

Kipar, A., Meli, M. L., Failing, K., Euler, T., Gomes-Keller, M. A., Schwartz, D., Reinacher, M., et al. (2006b). Natural feline coronavirus infection: differences in cytokine patterns in association with the outcome of infection. *Veterinary Immunology and Immunopathology*, *112*(3–4), 141–155.

Kiss, I., Poland, A. M., and Pedersen, N. C. (2004). Disease outcome and cytokine responses in cats immunized with an avirulent feline infectious peritonitis virus (FIPV)-UCD1 and challenge-exposed with virulent FIPV-UCD8. *Journal of Feline Medicine and Surgery*, *6*(2), 89–97.

Klinman, D. M. (2004). Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nature Reviews Immunology*.

Krug, A., Luker, G. D., Barchet, W., Leib, D. A., Akira, S., and Colonna, M. (2004). Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. *Blood*, *103*(4), 1433–1437.

Kumar, A., Zhang, J., and Yu, F. S. X. (2005). Toll-like receptor 3 agonist poly(I:C)-induced antiviral response in human corneal epithelial cells. *Immunology*, *117*(1), 11–21.

Lamothe, B., Besse, A., Campos, A. D., Webster, W. K., Wu, H., and Darnay, B. G. (2007). Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of IκB kinase activation. *Journal of Biological Chemistry*, *282*(6), 4102–4112.

Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., Escriou, N., Flavell, R., Si-Tahar, M., et al. (2006). Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathogens*, *2*(6), e53. https://doi.org/10.1371/journal.ppat.0020053

Lee, B. L., Moon, J. E., Shu, J. H., Yuan, L., Newman, Z. R., Schekman, R., and Barton, G. M. (2013). UNC93B1 mediates differential trafficking of endosomal TLRs. *ELife*, *2*(2), e00291. https://doi.org/10.7554/eLife.00291

Lehman, T. L., O'Halloran, K. P., Fallon, S. A., Habermann, L. M., Campbell, J. A., Nordone, S., Avery, P. R., et al. (2009). Altered bone marrow dendritic cell cytokine production to toll-like receptor and CD40 ligation during chronic feline immunodeficiency virus infection. *Immunology*, *126*(3), 405–412.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M., and Hoffmann, J. A. (1996). The dorsoventral regulatory gene cassette *spätzle*/*Toll*/*cactus* controls the potent antifungal response in Drosophila adults. *Cell*, *86*(6), 973–983.

Leonard, J. N., Ghirlando, R., Askins, J., Bell, J. K., Margulies, D. H., Davies, D. R., and Segal, D. M. (2008). The TLR3 signaling complex forms by cooperative receptor dimerization. *Proceedings of the National Academy of Sciences*, *105*(1), 258–263.

Lester, S. N., and Li, K. (2014). Toll-like receptors in antiviral innate immunity. *Journal of Molecular Biology*, *426*(6), 1246–1264.

Li, S.-W., Wang, C.-Y., Jou, Y.-J., Huang, S.-H., Hsiao, L.-H., Wan, L., Lin, C.-W., et al. (2016). SARS Coronavirus Papain-Like Protease Inhibits the TLR7 Signaling Pathway through Removing Lys63-Linked Polyubiquitination of TRAF3 and TRAF6. *International Journal of Molecular Sciences*, *17*(5).

Lin, S. C., Lo, Y. C., and Wu, H. (2010). Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. *Nature*, *465*(7300), 885–890.

Liu, L., Botos, I., Wang, Y., Leonard, J. N., Shiloach, J., Segal, D. M., and Davies, D. R. (2008). Structural Basis of Toll-Like Receptor 3 Signaling with Double-Stranded RNA. *Science*, *320*(5874), 379–381.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402–408.

Longstaff, L., Porter, E., Crossley, V. J., Hayhow, S. E., Helps, C. R., and Tasker, S. (2017). Feline coronavirus quantitative reverse transcriptase polymerase chain reaction on effusion samples in cats with and without feline infectious peritonitis. *Journal of Feline Medicine and Surgery*, *19*(2), 240–245.

Lund, J., Sato, A., Akira, S., Medzhitov, R., and Iwasaki, A. (2003). Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *Journal of Experimental Medicine*, *198*(3), 513–520.

Majer, O., Liu, B., and Barton, G. M. (2017). Nucleic acid-sensing TLRs: trafficking and regulation. *Current Opinion in Immunology*, *44*(February 2017), 26–33.

Malbon, A. J., Meli, M. L., Barker, E. N., Davidson, A. D., Tasker, S., and Kipar, A. (2019). Inflammatory Mediators in the Mesenteric Lymph Nodes, Site of a Possible Intermediate Phase in the Immune Response to Feline Coronavirus and the Pathogenesis of Feline Infectious Peritonitis? *Journal of Comparative Pathology*, *166*, 69–86.

Matsushima, N., Tanaka, T., Enkhbayar, P., Mikami, T., Taga, M., Yamada, K., and Kuroki, Y. (2007). Comparative sequence analysis of leucine-rich repeats (LRRs) within vertebrate toll-like receptors. *BMC Genomics*, *8*(1), 124.

Mazaleuskaya, L., Veltrop, R., Ikpeze, N., Martin-Garcia, J., and Navas-Martin, S. (2012). Protective role of Toll-like Receptor 3-induced type I interferon in murine coronavirus infection of macrophages. *Viruses*, *4*(5), 901–923.

Medzhitov, R., Preston-Hurlburt, P., and Janeway, C. A. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*, *388*(6640), 394–397.

Meli, M., Kipar, A., Müller, C., Jenal, K., Gönczi, E., Borel, N., Lutz, H., et al. (2004). High viral loads despite absence of clinical and pathological findings in cats experimentally infected with feline coronavirus (FCoV) type I and in naturally FCoV-infected cats. *Journal of Feline Medicine and Surgery*, *6*(2), 69–81.

Moresco, E. M. Y., LaVine, D., and Beutler, B. (2011, July). Toll-like receptors. *Current Biology*. Elsevier.

Murphy, B. G., Perron, M., Murakami, E., Bauer, K., Park, Y., Eckstrand, C., Pedersen, N. C., et al. (2018). The nucleoside analog GS-441524 strongly inhibits feline infectious peritonitis (FIP) virus in tissue culture and experimental cat infection studies. *Veterinary Microbiology*, *219*(April), 226–233.

Mustaffa-Kamal, F., Liu, H., Pedersen, N. C., and Sparger, E. E. (2019). Characterization of antiviral T cell responses during primary and secondary challenge of laboratory cats with feline infectious peritonitis virus (FIPV). *BMC Veterinary Research*, *15*(1), 165.

Muzio, M., Bosisio, D., Polentarutti, N., D'amico, G., Stoppacciaro, A., Mancinelli, R., Mantovani, A., et al. (2000). Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *Journal of Immunology (Baltimore, Md. : 1950)*, *164*(11), 5998–6004.

Muzio, M., Ni, J., Feng, P., and Dixit, V. M. (1997). IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL- 1 signaling. *Science*, *278*(5343), 1612–1615.

Myrrha, L. W., Silva, F. M. F., Peternelli, E. F. D. O., Junior, A. S., Resende, M., and de Almeida, M. R. (2011). The paradox of feline coronavirus pathogenesis: a review. *Advances in Virology*, *2011*, 109849. https://doi.org/10.1155/2011/109849

Nomura, N., Nagase, T., Miyajima, N., Sazuka, T., Tanaka, A., Sato, S., Tabata, S., et al. (1994). Prediction of the coding sequences of unidentified human genes. II. The coding sequences of 40 new genes (KIAA0041-KIAA0080) deduced by analysis of cDNA clones from human cell line KG-1. *DNA Research : An International Journal for Rapid Publication of Reports on Genes and Genomes*, *1*(5), 223–229.

O'Neill, L. A. J., Golenbock, D., and Bowie, A. G. (2013). The history of Toll-like receptors - redefining innate immunity. *Nature Reviews. Immunology*, *13*(6), 453–460.

Ohto, U., Shibata, T., Tanji, H., Ishida, H., Krayukhina, E., Uchiyama, S., Shimizu, T.,

et al. (2015). Structural basis of CpG and inhibitory DNA recognition by Toll-like receptor 9. *Nature*, *520*(7549), 702–705.

Oliveira-Nascimento, L., Massari, P., and Wetzler, L. M. (2012). The role of TLR2 infection and immunity. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2012.00079

Olyslaegers, D. A. J., Dedeurwaerder, A., Desmarets, L. M. B., Vermeulen, B. L., Dewerchin, H. L., and Nauwynck, H. J. (2013). Altered expression of adhesion molecules on peripheral blood leukocytes in feline infectious peritonitis. *Veterinary Microbiology*, *166*(3–4), 438–449.

Oosting, M., Cheng, S. C., Bolscher, J. M., Vestering-Stenger, R., Plantinga, T. S., Verschueren, I. C., Joosten, L. A. B., et al. (2014). Human TLR10 is an anti-inflammatory pattern-recognition receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(42), E4478–E4484. https://doi.org/10.1073/pnas.1410293111

Oshiumi, H., Matsumoto, M., Funami, K., Akazawa, T., and Seya, T. (2003). TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-β induction. *Nature Immunology*, *4*(2), 161–167.

Oshiumi, H., Sasai, M., Shida, K., Fujita, T., Matsumoto, M., and Seya, T. (2003). TIR-containing Adapter Molecule (TICAM)-2, a Bridging Adapter Recruiting to Toll-like Receptor 4 TICAM-1 That Induces Interferon-β. *Journal of Biological Chemistry*, *278*(50), 49751–49762.

Pandey, S., Kawai, T., and Akira, S. (2014). Microbial sensing by Toll-like receptors and intracellular nucleic acid sensors. *Cold Spring Harbor Perspectives in Biology*, *7*(1), a016246. https://doi.org/10.1101/cshperspect.a016246

Patel, M. C., Shirey, K. A., Pletneva, L. M., Boukhvalova, M. S., Garzino-Demo, A., Vogel, S. N., and Blanco, J. C. (2014). Novel drugs targeting Toll-like receptors for antiviral therapy. *Future Virology*, *9*(9), 811–829.

Pedersen, N. C. (2009). A review of feline infectious peritonitis virus infection: 1963-2008. *Journal of Feline Medicine and Surgery*, *11*(4), 225–258.

Pedersen, N. C. (2014). An update on feline infectious peritonitis: diagnostics and therapeutics. *Veterinary Journal (London, England : 1997)*, *201*(2), 133–141.

Pedersen, N. C., Allen, C. E., and Lyons, L. A. (2008). Pathogenesis of feline enteric coronavirus infection. *Journal of Feline Medicine and Surgery*, *10*(6), 529–541.

Pedersen, N. C., Eckstrand, C., Liu, H., Leutenegger, C., and Murphy, B. (2015). Levels of feline infectious peritonitis virus in blood, effusions, and various tissues and the role of lymphopenia in disease outcome following experimental infection. *Veterinary Microbiology*, *175*(2–4), 157–166.

Pedersen, N. C., Kim, Y., Liu, H., Galasiti Kankanamalage, A. C., Eckstrand, C., Groutas, W. C., Chang, K. O., et al. (2018). Efficacy of a 3C-like protease inhibitor in treating various forms of acquired feline infectious peritonitis. *Journal of Feline Medicine and Surgery*, *20*(4), 378–392.

Pedersen, N. C., and Black, J. W. (1983). Attempted immunization of cats against feline infectious peritonitis, using avirulent live virus or sublethal amounts of virulent virus. *American Journal of Veterinary Research*, *44*(2), 229–234.

Perales-Linares, R., and Navas-Martin, S. (2013). Toll-like receptor 3 in viral pathogenesis: friend or foe? *Immunology*, *140*(2), 153–167.

Pestka, S., Krause, C. D., and Walter, M. R. (2004). Interferons, interferon-like cytokines, and their receptors. *Immunological Reviews*, *202*, 8–32.

Poland, A. M., Vennema, H., Foley, J. E., and Pedersen, N. C. (1996). Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *Journal of Clinical Microbiology*, *34*(12), 3180–3184.

Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Beutler, B., et al. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science (New York, N.Y.)*, *282*(5396), 2085–2088.

Porter, E., Tasker, S., Day, M. J., Harley, R., Kipar, A., Siddell, S. G., and Helps, C. R. (2014). Amino acid changes in the spike protein of feline coronavirus correlate with systemic spread of virus from the intestine and not with feline infectious peritonitis. *Veterinary Research*, *45*(1), 1–11.

Qureshi, S. T., Larivière, L., Leveque, G., Clermont, S., Moore, K. J., Gros, P., and Malo, D. (1999). Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *The Journal of Experimental Medicine*, *189*(4), 615–625.

Reed, L. J., and Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*, *27*(3), 493–497.

Rehli, M. (2002). Of mice and men: species variations of Toll-like receptor expression. *Trends in Immunology*, *23*(8), 375–378.

Roach, J. C., Glusman, G., Rowen, L., Kaur, A., Purcell, M. K., Smith, K. D., Aderem, A., et al. (2005). The evolution of vertebrate Toll-like receptors, *102*(27).

Robert-Tissot, C., Rüegger, V. L., Cattori, V., Meli, M. L., Riond, B., Gomes-Keller, M. A., … Lutz, H. (2011). The innate antiviral immune system of the cat: Molecular tools for the measurement of its state of activation. *Veterinary Immunology and Immunopathology*, *143*(3–4), 269–281.

Robert-Tissot, C., Rüegger, V. L., Cattori, V., Meli, M. L., Riond, B., Moore, P. F., Lutz, H., et al. (2012). Stimulation with a class A CpG oligonucleotide enhances resistance to infection with feline viruses from five different families. *Veterinary Research*, *43*(1), 60.

Robison, R. L., Holzworth, J., and Gilmore, C. E. (1971). Naturally occurring feline infectious peritonitis: signs and clinical diagnosis. *Journal of the American Veterinary Medical Association*, *158*(6), Suppl 2:981-6.

Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A., and Bazan, J. F. (1998). A family of human receptors structurally related to Drosophila Toll. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(2), 588–

593.

Safi, N., Haghani, A., Ng, S. W., Selvarajah, G. T., Mustaffa-Kamal, F., and Omar, A. R. (2017). Expression profiles of immune mediators in feline Coronavirus-infected cells and clinical samples of feline Coronavirus-positive cats. *BMC Veterinary Research*, *13*(1), 92.

Samuelsson, C., Hausmann, J., Lauterbach, H., Schmidt, M., Akira, S., Wagner, H., Hochrein, H., et al. (2008). Survival of lethal poxvirus infection in mice depends on TLR9, and therapeutic vaccination provides protection. *Journal of Clinical Investigation*, *118*(5), 1776–1784.

Satoh, T., and Akira, S. (2016). Toll-Like Receptor Signaling and Its Inducible Proteins. *Microbiology Spectrum*, *4*(6), 332–338.

Schromm, A. B., Lien, E., Henneke, P., Chow, J. C., Yoshimura, A., Heine, H., Golenbock, D. T., et al. (2001). Molecular genetic analysis of an endotoxin nonresponder mutant cell line: a point mutation in a conserved region of MD-2 abolishes endotoxin-induced signaling. *The Journal of Experimental Medicine*, *194*(1), 79–88.

Sheahan, T., Morrison, T. E., Funkhouser, W., Uematsu, S., Akira, S., Baric, R. S., and Heise, M. T. (2008). MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV. *PLoS Pathogens*, *4*(12), e1000240. https://doi.org/10.1371/journal.ppat.1000240

Shibata, T., Ohto, U., Nomura, S., Kibata, K., Motoi, Y., Zhang, Y., Miyake, K., et al. (2016). Guanosine and its modified derivatives are endogenous ligands for TLR7. *International Immunology*, *28*(5), 211–222.

Shimazu, R., Akashi, S., Ogata, H., Nagai, Y., Fukudome, K., Miyake, K., and Kimoto, M. (1999). MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *The Journal of Experimental Medicine*, *189*(11), 1777–1782.

Shimizu, T. (2017). Structural insights into ligand recognition and regulation of nucleic acid-sensing Toll-like receptors. *Current Opinion in Structural Biology*, *47*, 52–59.

Sinha, S. S., Cameron, J., Brooks, J. C., and Leifer, C. A. (2016). Complex Negative Regulation of TLR9 by Multiple Proteolytic Cleavage Events. *Journal of Immunology (Baltimore, Md. : 1950)*, *197*(4), 1343–1352.

Sisirak, V., Sally, B., D'Agati, V., Martinez-Ortiz, W., Özçakar, Z. B., David, J., Reizis, B., et al., (2016). Digestion of Chromatin in Apoptotic Cell Microparticles Prevents Autoimmunity. *Cell*, *166*(1), 88–101.

Skidmore, B. J., Chiller, J. M., and Weigle, W. O. (1977). Immunologic properties of bacterial lipopolysaccharide (LPS). IV. Cellular basis of the unresponsiveness of C3H/HeJ mouse spleen cells to LPS-induced mitogenesis. *Journal of Immunology (Baltimore, Md. : 1950)*, *118*(1), 274–281.

Tabeta, K., Georgel, P., Janssen, E., Du, X., Hoebe, K., Crozat, K., Beutler, B., et al. (2004). Toll-like receptors 9 and 3 as essential complonents of innate immune defense against mouse cytomegalovirus infection. *Proceedings of the National*

Academy of Sciences of the United States of America, *101*(10), 3516–3521.

Taguchi, T., Mitcham, J. L., Dower, S. K., Sims, J. E., and Testa, J. R. (1996). Chromosomal localization of TIL, a gene encoding a protein related to the Drosophila transmembrane receptor Toll, to human chromosome 4p14. *Genomics*, *32*(3), 486–488.

Takano, T., Akiyama, M., Doki, T., and Hohdatsu, T. (2019). Antiviral activity of itraconazole against type I feline coronavirus infection. *Veterinary Research*, *50*(1), 5.

Takano, T., Azuma, N., Hashida, Y., Satoh, R., and Hohdatsu, T. (2009). B-cell activation in cats with feline infectious peritonitis (FIP) by FIP-virus-induced B-cell differentiation/survival factors. *Archives of Virology*, *154*(1), 27–35.

Takano, T., Azuma, N., Satoh, M., Toda, A., Hashida, Y., Satoh, R., and Hohdatsu, T. (2009). Neutrophil survival factors (TNF-alpha, GM-CSF, and G-CSF) produced by macrophages in cats infected with feline infectious peritonitis virus contribute to the pathogenesis of granulomatous lesions. *Archives of Virology*, *154*(5), 775–781.

Takano, T., Hohdatsu, T., Hashida, Y., Kaneko, Y., Tanabe, M., and Koyama, H. (2007). A "possible" involvement of TNF-alpha in apoptosis induction in peripheral blood lymphocytes of cats with feline infectious peritonitis. *Veterinary Microbiology*, *119*(2–4), 121–131.

Takano, T., Hohdatsu, T., Toda, A., Tanabe, M., and Koyama, H. (2007). TNF-alpha, produced by feline infectious peritonitis virus (FIPV)-infected macrophages, upregulates expression of type II FIPV receptor feline aminopeptidase N in feline macrophages. *Virology*, *364*(1), 64–72.

Takano, T., Katoh, Y., Doki, T., and Hohdatsu, T. (2013). Effect of chloroquine on feline infectious peritonitis virus infection in vitro and in vivo. *Antiviral Research*, *99*(2), 100–107.

Takano, T., Ohyama, T., Kokumoto, A., Satoh, R., and Hohdatsu, T. (2011). Vascular endothelial growth factor (VEGF), produced by feline infectious peritonitis (FIP) virus-infected monocytes and macrophages, induces vascular permeability and effusion in cats with FIP. *Virus Research*, *158*(1–2), 161–168.

Takano, T., Tomiyama, Y., Katoh, Y., Nakamura, M., Satoh, R., and Hohdatsu, T. (2011). Mutation of neutralizing/antibody-dependent enhancing epitope on spike protein and 7b gene of feline infectious peritonitis virus: Influences of viral replication in monocytes/macrophages and virulence in cats. *Virus Research*, *156*(1–2), 72–80.

Takeda, K., Kaisho, T., and Akira, S. (2003). Toll-Like Receptors. *Annual Review of Immunology*, *21*(1), 335–376.

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell*, *140*(6), 805–820.

Takeuchi, O., Kawai, T., Mühlradt, P. F., Morr, M., Radolf, J. D., Zychlinsky, A., Akira, S., et al. (2001). Discrimination of bacterial lipoproteins by Toll-like

receptor 6. *International Immunology*, *13*(7), 933–940.

Takeuchi, O., Sato, S., Horiuchi, T., Hoshino, K., Takeda, K., Dong, Z., Akira, S., et al. (2002). Cutting Edge: Role of Toll-Like Receptor 1 in Mediating Immune Response to Microbial Lipoproteins. *The Journal of Immunology*, *169*(1), 10–14.

Tanji, H., Ohto, U., Shibata, T., Taoka, M., Yamauchi, Y., Isobe, T., Shimizu, T., et al. (2015). Toll-like receptor 8 senses degradation products of single-stranded RNA. *Nature Structural & Molecular Biology*, *22*(2), 109–115.

Tasker, S. (2018). Diagnosis of feline infectious peritonitis: Update on evidence supporting available tests. *Journal of Feline Medicine and Surgery*, *20*(3), 228–243.

Temeeyasen, G., Sinha, A., Gimenez-Lirola, L. G., Zhang, J. Q., and Piñeyro, P. E. (2018). Differential gene modulation of pattern-recognition receptor TLR and RIG-I-like and downstream mediators on intestinal mucosa of pigs infected with PEDV non S-INDEL and PEDV S-INDEL strains. *Virology*, *517*, 188–198.

Totura, A. L., Whitmore, A., Agnihothram, S., Schäfer, A., Katze, M. G., Heise, M. T., and Baric, R. S. (2015). Toll-Like Receptor 3 Signaling via TRIF Contributes to a Protective Innate Immune Response to Severe Acute Respiratory Syndrome Coronavirus Infection. *MBio*, *6*(3), e00638-15. https://doi.org/10.1128/mBio.00638-15

Tseng, P.-H., Matsuzawa, A., Zhang, W., Mino, T., Vignali, D. A. A., and Karin, M. (2010). Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. *Nature Immunology*, *11*(1), 70–75.

Uematsu, S., and Akira, S. (2007). Toll-like Receptors and Type I Interferons. *Journal of Biological Chemistry*, *282*(21), 15319–15323.

Uematsu, S., Fujimoto, K., Jang, M. H., Yang, B. G., Jung, Y. J., Nishiyama, M., … Akira, S. (2008). Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nature Immunology*, *9*(7), 769–776.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), RESEARCH0034. https://doi.org/10.1186/gb-2002-3-7-research0034

Ve, T., Gay, N. J., Mansell, A., Kobe, B., and Kellie, S. (2012). Adaptors in toll-like receptor signaling and their potential as therapeutic targets. *Current Drug Targets*, *13*(11), 1360–1374.

Vermeulen, B. L., Devriendt, B., Olyslaegers, D. A., Dedeurwaerder, A., Desmarets, L. M., Favoreel, H. W., … Nauwynck, H. J. (2013). Suppression of NK cells and regulatory T lymphocytes in cats naturally infected with feline infectious peritonitis virus. *Veterinary Microbiology*, *164*(1–2), 46–59.

Vennema, H., de Groot, R., Harbour, D., Dalderup, M., Gruffydd-Jones, T., Horzinek, M. and Spaan, W. (1990). Early death after feline infectious peritonitis virus

challenge due to recombinant vaccinia virus immunization. *Journal of Virology*, 64(3), 1407-1409.

Wang, C., Chen, T., Zhang, J., Yang, M., Li, N., Xu, X., and Cao, X. (2009). The E3 ubiquitin ligase Nrdp1 "preferentially" promotes TLR-mediated production of type I interferon. *Nature Immunology*, *10*(7), 744–752.

Wang, T., Town, T., Alexopoulou, L., Anderson, J. F., Fikrig, E., and Flavell, R. A. (2004). Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nature Medicine*, *10*(12), 1366–1373.

Wang, Y.-T., Hsieh, L.-E., Dai, Y.-R., and Chueh, L.-L. (2014). Polymorphisms in the feline TNFA and CD209 genes are associated with the outcome of feline coronavirus infection. *Veterinary Research*, *45*(123), 123.

Ward, J. M. (1970). Morphogenesis of a virus in cats with experimental feline infectious peritonitis. *Virology*, *41*(1), 191–194.

Watanabe, R., Eckstrand, C., Liu, H., and Pedersen, N. C. (2018). Characterization of peritoneal cells from cats with experimentally-induced feline infectious peritonitis (FIP) using RNA-seq. *Veterinary Research*, *49*(1), 81.

Weiss, R. C., Cox, N. R., and Martinez, M. L. (1993). Evaluation of free or liposome-encapsulated ribavirin for antiviral therapy of experimentally induced feline infectious peritonitis. *Research in Veterinary Science*, *55*(2), 162–172.

Weiss, Richard C., and Scott, F. W. (1981). Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. *Comparative Immunology, Microbiology and Infectious Diseases*, *4*(2), 175–189.

Wesche, H., Henzel, W. J., Shillinglaw, W., Li, S., and Cao, Z. (1997). MyD88: An adapter that recruits IRAK to the IL-1 receptor complex. *Immunity*, *7*(6), 837–847.

Wheat, W., Chow, L., Coy, J., Contreras, E., Lappin, M., and Dow, S. (2019). Activation of upper respiratory tract mucosal innate immune responses in cats by liposomal toll-like receptor ligand complexes delivered topically. *Journal of Veterinary Internal Medicine*, *33*(2), 838–845.

Whittaker, G. R., André, N. M., and Millet, J. K. (2018). Improving Virus Taxonomy by Recontextualizing Sequence-Based Classification with Biologically Relevant Data: the Case of the Alphacoronavirus 1 Species. *MSphere*, *3*(1).

Xu, Y., Tao, X., Shen, B., Horng, T., Medzhitov, R., Manley, J. L., and Tong, L. (2000). Structural basis for signal transduction by the toll/interleukin-1 receptor domains. *Nature*, *408*(6808), 111–115.

Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Akira, S., et al. (2003). Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science (New York, N.Y.)*, *301*(5633), 640–643.

Yamamoto, M., Sato, S., Mori, K., Hoshino, K., Takeuchi, O., Takeda, K., and Akira, S. (2002). Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter

that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *Journal of Immunology (Baltimore, Md. : 1950)*, *169*(12), 6668–6672.

Zhang, Z., Ohto, U., Shibata, T., Krayukhina, E., Taoka, M., Yamauchi, Y., Shimizu, T., et al. (2016). Structural Analysis Reveals that Toll-like Receptor 7 Is a Dual Receptor for Guanosine and Single-Stranded RNA. *Immunity*, *45*(4), 737–748.

Zhou, D., Kang, K. H., and Spector, S. A. (2012). Production of interferon α by human immunodeficiency virus type 1 in human plasmacytoid dendritic cells is dependent on induction of autophagy. *Journal of Infectious Diseases*, *205*(8), 1258–1267.

Zhou, Y., Wang, X., Liu, M., Hu, Q., Song, L., Ye, L., Ho, W., et al. (2010). A critical function of toll-like receptor-3 in the induction of anti-human immunodeficiency virus activities in macrophages. *Immunology*, *131*(1), 40–49. https://doi.org/10.1111/j.1365-2567.2010.03270.x

APPENDICES

Appendix A: Titration ($TCID_{50}$) and confirmation of FIPV 79-1146

Dilution	Dead	Alive	Cumulative			Percent Mortality
			Dead	Alive	Ratio	
10^{-1}	10	0	64	0	64/64	100
10^{-2}	10	0	54	0	54/54	100
10^{-3}	10	0	44	0	44/44	100
10^{-4}	10	0	34	0	34/34	100
10^{-5}	10	0	24	0	24/24	100
10^{-6}	10	0	14	0	14/14	100
10^{-7}	3	7	4	7	4/11	36.3636364
10^{-8}	1	9	1	16	1/17	5.88235294
10^{-9}	0	10	0	26	0/26	0
10^{-10}	0	10	0	36	0/36	0

$$\frac{\%\text{ Above 50\% - 50\%}}{\%\text{ Above 50\% - \% below 50\%}} = \frac{100 - 50}{100 - 36.363}$$

$$= 0.785714286$$

Dilution at which % above 50% $= 10^{-6} = 6$

$TCID_{50} = 6 + 0.785714286 = 6.786$

$TCID_{50}$/ml $= 10^{6.786}/0.1$ ml = $\mathbf{6.115 \times 10^{7}}$

A.1: Calculation of $TCID_{50}$ for FIPV 79-1146

Appendix B: *In vitro* type II FIPV infection in CRFK cells

Tabular results

	Normality and Lognormality Tests Tabular results	A CRFK TLR3	B CRFK TLR9	C CRFK IL-10	D CRFK IFNb	E CRFK TNFa	F CRFK Viral Load
1	**Test for normal distribution**						
2	**Anderson-Darling test**						
3	A2*	0.5847	0.4085	1.101	1.220	0.8900	0.9937
4	P value	0.0901	0.2701	0.0035	0.0016	0.0131	0.0068
5	Passed normality test (alpha=0.05)?	Yes	Yes	No	No	No	No
6	P value summary	ns	ns	**	**	*	**
7							
8	**D'Agostino & Pearson test**						
9	K2	2.445	1.379	3.444	2.421	2.665	3.299
10	P value	0.2945	0.5019	0.1787	0.2981	0.2638	0.1922
11	Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
12	P value summary	ns	ns	ns	ns	ns	ns
13							
14	**Shapiro-Wilk test**						
15	W	0.8691	0.8944	0.7375	0.7231	0.7871	0.7673
16	P value	0.1203	0.2211	0.0039	0.0026	0.0145	0.0086
17	Passed normality test (alpha=0.05)?	Yes	Yes	No	No	No	No
18	P value summary	ns	ns	**	**	*	**
19							
20	**Kolmogorov-Smirnov test**						
21	KS distance	0.2839	0.2130	0.3198	0.3826	0.3207	0.3019
22	P value	0.0351	>0.1000	0.0085	0.0004	0.0082	0.0177
23	Passed normality test (alpha=0.05)?	No	Yes	No	No	No	No
24	P value summary	*	ns	**	***	**	*
25							
26	**Number of values**	9	9	9	9	9	9

B.1: Normality test for viral load quantification and gene expression in CRFK cells.

ANOVA results | Multiple comparisons

	Kruskal-Wallis test Multiple comparisons						
1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	0.000	No	ns	>0.9999	A-B	
7	4 vs. 24	4.000	No	ns	0.2209	A-C	
8	12 vs. 24	4.000	No	ns	0.2209	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	6.333	6.333	0.000	3	3	0.000
12	4 vs. 24	6.333	2.333	4.000	3	3	1.789
13	12 vs. 24	6.333	2.333	4.000	3	3	1.789

B.2: Kruskal-Wallis test comparing TLR3 expression between different time points in CRFK cells.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-3.333	No	ns	0.4081	A-B	
7	4 vs. 24	-5.667	Yes	*	0.0338	A-C	
8	12 vs. 24	-2.333	No	ns	0.8902	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	2.000	5.333	-3.333	3	3	1.491
12	4 vs. 24	2.000	7.667	-5.667	3	3	2.534
13	12 vs. 24	5.333	7.667	-2.333	3	3	1.043

B.3: Kruskal-Wallis test comparing TLR9 expression between different time points in CRFK cells.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-0.3333	No	ns	>0.9999	A-B	
7	4 vs. 24	-4.667	No	ns	0.1107	A-C	
8	12 vs. 24	-4.333	No	ns	0.1579	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	3.333	3.667	-0.3333	3	3	0.1491
12	4 vs. 24	3.333	8.000	-4.667	3	3	2.087
13	12 vs. 24	3.667	8.000	-4.333	3	3	1.938

B.4: Kruskal-Wallis test comparing IL-10 expression between different time points in CRFK cells.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-3.000	No	ns	0.5391	A-B	
7	4 vs. 24	-6.000	Yes	*	0.0219	A-C	
8	12 vs. 24	-3.000	No	ns	0.5391	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	2.000	5.000	-3.000	3	3	1.342
12	4 vs. 24	2.000	8.000	-6.000	3	3	2.683
13	12 vs. 24	5.000	8.000	-3.000	3	3	1.342

B.5: Kruskal-Wallis test comparing IFN-β expression between different time points in CRFK cells.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-3.000	No	ns	0.5391	A-B	
7	4 vs. 24	-6.000	Yes	*	0.0219	A-C	
8	12 vs. 24	-3.000	No	ns	0.5391	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	2.000	5.000	-3.000	3	3	1.342
12	4 vs. 24	2.000	8.000	-6.000	3	3	2.683
13	12 vs. 24	5.000	8.000	-3.000	3	3	1.342

B.6: Kruskal-Wallis test comparing TNF-α expression between different time points in CRFK cells.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-4.000	No	ns	0.2209	A-B	
7	4 vs. 24	-5.000	No	ns	0.0760	A-C	
8	12 vs. 24	-1.000	No	ns	>0.9999	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	2.000	6.000	-4.000	3	3	1.789
12	4 vs. 24	2.000	7.000	-5.000	3	3	2.236
13	12 vs. 24	6.000	7.000	-1.000	3	3	0.4472

B.7: Kruskal-Wallis test comparing viral load between different time points in CRFK cells.

Appendix C: *Ex vivo* type II FIPV infection in feline monocytes

Tabular results

Normality and Lognormality Tests Tabular results	A TLR7	B TLR3	C TLR9	D IL-10	E IFNb	F TNFa	G Viral Load
Test for normal distribution							
Anderson-Darling test							
A2*	2.125	1.886	0.7767	2.723	4.143	0.3034	0.2882
P value	<0.0001	<0.0001	0.0375	<0.0001	<0.0001	0.5459	0.5873
Passed normality test (alpha=0.05)?	No	No	No	No	No	Yes	Yes
P value summary	****	****	*	****	****	ns	ns
D'Agostino & Pearson test							
K2	34.24	34.88	4.418	45.08	21.25	4.993	2.331
P value	<0.0001	<0.0001	0.1098	<0.0001	<0.0001	0.0824	0.3118
Passed normality test (alpha=0.05)?	No	No	Yes	No	No	Yes	Yes
P value summary	****	****	ns	****	****	ns	ns
Shapiro-Wilk test							
W	0.6946	0.7193	0.9239	0.5965	0.5939	0.9444	0.9660
P value	<0.0001	<0.0001	0.0712	<0.0001	<0.0001	0.2040	0.5695
Passed normality test (alpha=0.05)?	No	No	Yes	No	No	Yes	Yes
P value summary	****	****	ns	****	****	ns	ns
Kolmogorov-Smirnov test							
KS distance	0.2412	0.2449	0.1731	0.2502	0.3247	0.09552	0.09852
P value	0.0009	0.0007	0.0610	0.0004	<0.0001	>0.1000	>0.1000
Passed normality test (alpha=0.05)?	No	No	Yes	No	No	Yes	Yes
P value summary	***	***	ns	***	****	ns	ns
Number of values	24	24	24	24	24	24	24

C.1: Normality test for viral load quantification and gene expression in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test Multiple comparisons						
Number of families	1					
Number of comparisons per family	3					
Alpha	0.05					
Dunn's multiple comparisons test	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
4 vs. 12	7.875	No	ns	0.0778	A-B	
4 vs. 24	12.75	Yes	***	0.0009	A-C	
12 vs. 24	4.875	No	ns	0.5038	B-C	
Test details	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
4 vs. 12	19.38	11.50	7.875	8	8	2.227
4 vs. 24	19.38	6.625	12.75	8	8	3.606
12 vs. 24	11.50	6.625	4.875	8	8	1.379

C.2: Kruskal-Wallis test comparing TLR7 expression between different time points in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	2.125	No	ns	>0.9999	A-B	
7	4 vs. 24	0.5000	No	ns	>0.9999	A-C	
8	12 vs. 24	-1.625	No	ns	>0.9999	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	13.38	11.25	2.125	8	8	0.6010
12	4 vs. 24	13.38	12.88	0.5000	8	8	0.1414
13	12 vs. 24	11.25	12.88	-1.625	8	8	0.4596

C.3: Kruskal-Wallis test comparing TLR3 expression between different time points in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-2.000	No	ns	>0.9999	A-B	
7	4 vs. 24	-2.125	No	ns	>0.9999	A-C	
8	12 vs. 24	-0.1250	No	ns	>0.9999	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	11.13	13.13	-2.000	8	8	0.5657
12	4 vs. 24	11.13	13.25	-2.125	8	8	0.6010
13	12 vs. 24	13.13	13.25	-0.1250	8	8	0.03536

C.4: Kruskal-Wallis test comparing TLR9 expression between different time points in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-1.000	No	ns	>0.9999	A-B	
7	4 vs. 24	-2.000	No	ns	>0.9999	A-C	
8	12 vs. 24	-1.000	No	ns	>0.9999	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	11.50	12.50	-1.000	8	8	0.2828
12	4 vs. 24	11.50	13.50	-2.000	8	8	0.5657
13	12 vs. 24	12.50	13.50	-1.000	8	8	0.2828

C.5: Kruskal-Wallis test comparing IL-10 expression between different time points in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-6.750	No	ns	0.1687	A-B	
7	4 vs. 24	-3.375	No	ns	>0.9999	A-C	
8	12 vs. 24	3.375	No	ns	>0.9999	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	9.125	15.88	-6.750	8	8	1.909
12	4 vs. 24	9.125	12.50	-3.375	8	8	0.9546
13	12 vs. 24	15.88	12.50	3.375	8	8	0.9546

C.6: Kruskal-Wallis test comparing IFN-β expression between different time points in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	9.750	Yes	*	0.0175	A-B	
7	4 vs. 24	5.625	No	ns	0.3348	A-C	
8	12 vs. 24	-4.125	No	ns	0.7300	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	17.63	7.875	9.750	8	8	2.758
12	4 vs. 24	17.63	12.00	5.625	8	8	1.591
13	12 vs. 24	7.875	12.00	-4.125	8	8	1.167

C.7: Kruskal-Wallis test comparing TNF-α expression between different time points in feline monocytes.

ANOVA results | Multiple comparisons

Kruskal-Wallis test
Multiple comparisons

1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	**Dunn's multiple comparisons test**	**Mean rank diff.**	**Significant?**	**Summary**	**Adjusted P Value**		
6	4 vs. 12	-6.125	No	ns	0.2496	A-B	
7	4 vs. 24	-1.375	No	ns	>0.9999	A-C	
8	12 vs. 24	4.750	No	ns	0.5373	B-C	
9							
10	**Test details**	**Mean rank 1**	**Mean rank 2**	**Mean rank diff.**	**n1**	**n2**	**Z**
11	4 vs. 12	10.00	16.13	-6.125	8	8	1.732
12	4 vs. 24	10.00	11.38	-1.375	8	8	0.3889
13	12 vs. 24	16.13	11.38	4.750	8	8	1.344

C.8: Kruskal-Wallis test comparing viral load between different time points in feline monocytes.

BIODATA OF STUDENT

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