VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable 7.2

Novel reservoir host infection models for BSL3 zoonotic pathogens

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1 Summary

This deliverable summarizes work done to analyse the susceptibility and pathogenesis in potential or defined reservoir hosts after challenge with zoonotic agents.

Objectives:

1) Infection studies with RVFV (Rift Valley Fever Virus) and Lyssavirus in two different fruit bat host species (Rousettus aegyptiacus, Eidolon helvum) (FLI):

1.1.- Evaluation of the general susceptibility of Egyptian fruit bats (*Rousettus aegyptiacus*) for Rift Valley Fever Virus (RVFV) and their suitability as a possible reservoir host (viremia, persistence, determination of virus shedding).

1.2.- The second aim of this objective addresses the pathogenesis during infections with a dog-related rabies strain and Lagos Bat Lyssavirus, which has been transmitted at several occasions from bats to domestic animals and wildlife in Africa. Although no human cases have been reported so far, this agent is of concern, since the commercial rabies vaccine is not expected to offer full protection against this virus. We therefore initiated comparative challenge experiments using intramuscular and interdermal (footpad) inoculation with both strains, to decipher the neuropathogenesis of these infections in mice and bats.

2) Infections studies with MERS-CoV *(Middle East Respiratory Syndrome corona virus) (IRTA-CRESA)*:

The objective of this study was to challenge experimentally alpacas with different MERS-CoV strains, including the prototypic strain EMC/2012, in order to perform an exhaustive study on early events of infection encompassing histopathological examination of the respiratory tract and detection of mucosal innate immune responses. Since a proper local innate immune response is a prerequisite condition for the establishment of an adaptive memory immune response, studies performed under this task provide valuable information on the type of cytokines required to mount an effective mucosal innate immune response against MERS-CoV. This information will be valuable for the development of new antiviral drugs (none so far is effective in curing MERS) and vaccines delivered by the mucosal route (ie. Intranasal delivery).

Rationale:

Although the main reservoir host of the RVFV has not yet been identified, small mammals such as rodents and bats may act as amplifying hosts, which would explain the re-occurrence of the

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virus in situations which can hardly be explained by mosquito-borne transmission alone. *Rousettus aegyptiacus* (Egyptian fruit bat) are abundant in Northern Africa, Egypt, Cyprus and Turkey. We therefore inoculated *R. aegyptiacus* individuals first with the vaccine strain MP-12, in order to elucidate the general competence of this species for virus propagation and transmission, and in a second step with a highly virulent RVFV strain 35/74.

A recent experiment revealed the general susceptibility of the straw-colored fruit bat *Eidolon helvum* for an experimental infection with Lagos Bat Lyssavirus, a bat-borne highly pathogenic rabies virus occurring in southern and central Africa [Suu-Ire et al.,: Pathogenesis of bat rabies in a natural reservoir: Comparative susceptibility of the straw-colored fruit bat (Eidolon helvum) to three strains of Lagos bat virus. PLoS Negl Trop Dis;12(3):e0006311]. As a follow-up of this study, we aim at performing a comparative infection experiment in *Eidolon helvum* bats with dog-related rabies and with Lagos Bat Lyssavirus.

In regards MERS-CoV objective, three strains of this virus were selected; all were isolated from human cases. The prototype strain EMC-2012 (clade A) was previously tested in dromedary camels by the IRTA-CReSA team and by others in alpacas. The MERS-CoV Qatar15/2015 strain (clade B) was tested in llamas by our team. Alpacas were intranasally infected and sacrificed by groups of 3 animals from day 1 to day 4 post inoculation for the Qatar15/2015 strain and from day 1 to day 3 for the EMC/2012 and Jordan-1/2015 strains (clade B). Tissues from the respiratory tract were fixed and processed for immunohistochemistry (IHC) with antibodies specific for the MERS-CoV N protein. These fixed tissues were also used for RNA extraction and nasal samples were processed by laser micro-dissection, prior RNA extraction, to isolate the epithelial layer from the adjacent sub-mucosa. Tissues from non-infected alpacas were collected and processed as above to serve as controls. After conversion of RNA into cDNA, a panel of 51 pair of primers were used to assess cytokine gene expression by the Fluidigm technology.

Teams involved:

Objective 1: Infection studies with RVFV and Lyssavirus in fruit bat host species were conducted by FLI in collaboration with Erasmus University Rotterdam, Netherlands

Objective 2: MERS-CoV work was accomplished with funds of the VetBioNet and the IMI-ZAPI (grant 115760). Beside the IRTA-CReSA team, the Coronavirus team from the department of Viroscience of the Erasmus Medical Center (Netherlands) was involved.

2 Introduction

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Middle East respiratory syndrome coronavirus (MERS-CoV) is the cause of a respiratory disease provoking high mortality in humans. While the MERS-CoV infection symptomatology ranges from asymptomatic to fatal multiorgan failure in humans[1], dromedary camels, the natural reservoir host[2], suffer a mild subclinical infection, rapidly clear the virus and mount a robust acquired immunity[3,4,5]. Furthermore, all camelids are susceptible to the MERS-CoV infection, as naturally and experimentally determined[3–9].

Innate immune responses upon MERS-CoV infection are thought to play a key role in the pathology of the disease and could determine its outcome. However, studies correlating innate immune responses elicited after MERS-CoV infection and the severity of the disease in humans are limited. *In vivo* studies described a downregulation of cellular immune responses, together with a massive inflammatory cytokine response (IL-1α, IL-1β and IL-8) at the lower respiratory tract[10]. Remarkably, the levels of inflammatory responses correlated with MERS-CoV case fatality rate in this study[10]. Also, specific *in vitro* studies, using human macrophages isolated from healthy donors or infected patients, confirmed that macrophages overexpress pro-inflammatory cytokines upon MERS-CoV infection, such as TNF-α, IL-6, MIP1-α and CXCL10 [11,12]. There are no *in vivo* studies on the innate immune response elicited at the respiratory mucosa of humans, but primary human airway epithelial cells can be readily infected *in vitro* by MERS-CoV[13] as are human immortalized lung epithelial cells[14,15] or respiratory explants[16–18]. Although some studies reported contradictory results, there is a consensus that MERS-CoV infections on these cells or tissues did not trigger proper type I and III interferon (IFN) responses, since they were moderately induced or delayed.

Overall, impaired type I and III IFN responses at the mucosal level, high secretions and persistent increase of pro-inflammatory cytokines in lung macrophages and the inhibition of cellular immune responses are likely to contribute in humans to a more severe infection, characterized by lung inflammation, and immunopathology. The receptor for MERS-CoV is abundant in the upper respiratory tract of dromedaries and absent in humans. A few cells of the dromedary lower respiratory tract express the virus receptor. This could explain the preferential tropism of the virus in the upper and lower respiratory tract for camelids and humans respectively and therefore the severity of the disease[19]. However, innate immune responses in camelids towards MERS-CoV infection remain unexplored. In our studies, we used an alpaca model infection as a surrogate to dromedary camel, in order to investigate the innate immune responses upon MERS-CoV infection in mucosa of the respiratory tract.

3 Results

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3.1 Results obtained from infection studies in fruit bat host species

3.1.1. RVFV infection studies

First of all, we immunized three *R. aegyptiacus* bats with the live-attenuated RVFV vaccine strain MP-12, and kept them together with one non-immunized animal. The three immunized bats were sacrificed at days 3, 7 and 31, while the non-immunized bat was kept for 31 days. None of the animals developed any clinical symptoms throughout the study.

Oral and rectal swabs as well as serum samples were collected every three days. At necropsy, all relevant tissues were collected as fresh material (for molecular analysis and virus re-isolation) and as formalin-fixed tissues for histology. Molecular analysis by qRT-PCR revealed low levels of RVFV RNA in one serum sample collected 3 days post inoculation with a Ct-value of 38.25 and an estimated copy number of 9.5 x 10^1, while RVFV RNA was also found in the spleen tissue of all three bats which had been inoculated with the MP-12 vaccine strain, as indicated by Ct-values ranging between 31.11 and 33.94. Furthermore, we also recovered RVFV derived RNA in the liver of the animal sacrificed at 7 dpi with a Ct-value of 36.02.

The serum samples collected throughout the experiment as well as during the necropsies of the animals were tested using a commercial multi-species competition ELISA based on the detection of antibodies raised against the RVFV nucleoprotein. One serum collected from the animal sacrificed at 7 days post infection (dpi) gave a positive result. Moreover, all serum samples turned positive for all subsequent sampling dates until day 31. For the animal sacrificed at 31 dpi, we also determined positive SNT titers between 1:160 and 1:2560 between day 7 and day 31 of the experiment.

Histopathology revealed a mild and moderate hepatitis in the animals sacrificed at 7 and 31 dpi respectively, as well as RVFV Gc-protein-positive granula within the necrotizing liver lesions in the animal sacrificed at 7 dpi, interpreted as debris remaining after virus-induced hepatocellular death.

The results of this study have been published: Balkema-Buschmann et al. Viruses 2018;10(12). pii: E681. doi: 10.3390/v10120681.

In the following experiment, 12 *R. aegyptiacus* bats were infected with the highly virulent RVFV strain 35/74. These animals carried an intraperitoneal temperature transponder to monitor the body core temperature throughout the experiment. The animals were again sacrificed after 3 dpi (4 animals), 7 dpi (4 animals) and 31 dpi (4 animals). As in the experiment with the live-attenuated vaccine strain MP-12, none of the animals developed any clinical signs.

The determined body core temperature data were equivalent to those determined on an earlier study using animals from the FLI breeding colony. Interestingly, the animals of both groups

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display a circadian temperature oscillation between 34°C and 41°C. These data are a prerequisite for the interpretation of temperature data generated during an infection study in bats.

The compilation and interpretation of the detailed virological, serological and histological results of this study is currently ongoing and will be summarized in the next report.

3.1.2. Lyssavirus infection studies

In order to analyse the neuroinvasion pathways of dog-related rabies as compared to Lagos Bat Lyssavirus (LBV), Balb/C mice and *E. helvum* bats, we have planned to comparably inoculate mice with dog related rabies and LBV through intramuscular and intradermal (foot pad) inoculation. In the next step and after interpretation of the results of this pilot study in mice, an equivalent study will be performed in *E. helvum* bats. The ethical approval to perform this experiment has been submitted and is currently under review by the relevant authority. As soon as the approval has been released, we will initiate the pilot study in mice, and then continue with the bat study.

3.2 Outcome of the MERS-CoV infections in alpacas.

Specific reagents to study expression of immune genes in camelids are scarce. Mediating analysis of published genome drafts of camelids, 48 pairs of primers were designed (see the table of primers in annexes) to amplify genes coding for interferons (IFNs), interferon stimulated genes (ISGs), pattern recognition receptors (PRRs), pro-inflammatory cytokines or chemokines and other interleukins, enzymes and transcription factors involved in innate immunity. Primers for 3 normalizer genes (called also housekeeping genes or endogenous controls) were also designed. Primers were validated in stimulated mononuclear lymphocytes from blood of camelids (dromedary camels, llamas and alpacas) and nasal tissues from MERS-CoV-infected alpacas. The methods of validation were first by conventional and quantitative PCR, followed by the Fluidigm technology, which allows multiple amplifications of genes from multiple samples in a single plate. The technique of IHC for detection in formalin fixed tissues of the MERS-CoV nucleoprotein was already set in previous published works. Fixation of tissues in methacarn (reagent to preserve RNA integrity), laser micro-dissection (LMD) on nasal tissues and subsequent extraction and purification of RNA was implemented during the VetBioNet project.

Experimental intranasal infection with the 3 different MERS-CoV strains in alpacas were successful. Presence of the replicating virus was assessed by IHC. In all cases, the higher number of infected cells was found in the nasal epithelia at 2 days post inoculation (dpi). Epithelia of trachea and large bronchus was affected but to a much lesser extent. Some infection was also noticed in small bronchus of alpacas infected with the Qatar15/2015 strain

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only, this being the first report in camelids of presence of MERs-CoV in the lower respiratory tract. At 1 dpi, infected nasal epithelial cells were observed in alpacas inoculated with the Qatar15/2015 and the Jordan-1/2015 strains. These results were corroborated with data obtained by viral titration of nasal swabs collected during the course of infection. Indeed, alpacas inoculated with the EMC/2012 strain shed less virus than those infected with the Qatar and Jordan-1/2015 strains. In addition, lower viral RNA loads were found in different tissues of the respiratory tract from alpacas infected with the EMC/2012 strain than for those challenged with the 2 other strains. Therefore, the MERS-CoV Qatar15/2015 and Jordan-1/2015 strains provoke a more severe infection than the EMC/2012 strain. Nonetheless, animals were asymptomatic during the course of the experiment.

Expression of genes involved in innate immunity were monitored in the respiratory tract from alpacas infected during 4 days with the Qatar15/2015 strain (see figure in annexes). The Fluidigm Biomark technology was applied on tissues from infected and control non-infected alpacas. Levels of expression of a certain gene, in a given tissue, are quantified and compared between non-infected (n=3) and infected (n=3 for each day of infection) animals; therefore results are expressed in fold changes (Fc). Hence, a gene in relation to controls can be upregulated, downregulated or invariable. Infected and control nasal epithelia and submucosa were subjected to LMD and processed for gene expression analysis. At the epithelial level most of the transcription alterations occurred and peaked at 2 dpi. Genes affected coded for IFN-beta (mean of 200 Fc) and IFN-lambda3 (mean of 350 Fc) and to a lesser extent IFN-alpha (mean of 6 Fc) and IFN-lambda1 (mean of 11 Fc). Expression of Type I INF (alpha and beta) and type III INF (lambda 1 and 3) decreased progressively at 3 and 4 dpi. The same patterns of gene transcription were observed for interferon stimulated genes with antiviral activity (ISG15, MX1, CXCL10 and OAS1), the interferon regulatory transcription factor IRF7, cytoplasmic viral RNA sensors (RIG-1 and MDA-5) and, although moderately upregulated, the endosome viral RNA sensors (TLR-3 and 7). In addition, the pro-inflammatory antagonist interleukin IL-10 was upregulated at 2 dpi (mean of 10Fc) and returned to nearly steady states levels at 3 and 4 dpi. Only two genes involved in inflammation, Casp-1 and IL-6, were found moderately upregulated, while the levels of expression of other important pro-inflammatory factors, such as the cytokines IL-8, IL-1-beta and TNF-alpha or the pattern recognition receptor NLRP3, were not affected by the infection. On line with histological observations that mononuclear and polymorphonuclear cells infiltrate the submucosa and lamina propia at sites of infection, the chemo-attractant chemokines CCL2 and 3 were moderately upregulated (mean of 5 Fc). At the submucosa level, genes for type I and III INFs were not induced but ISGs, IRF7 and TLR-7 were upregulated. Also, moderate increases of expression were noticed for TNF-alpha and Casp-1 but no other pro-inflammatory cytokine genes were affected. Similar

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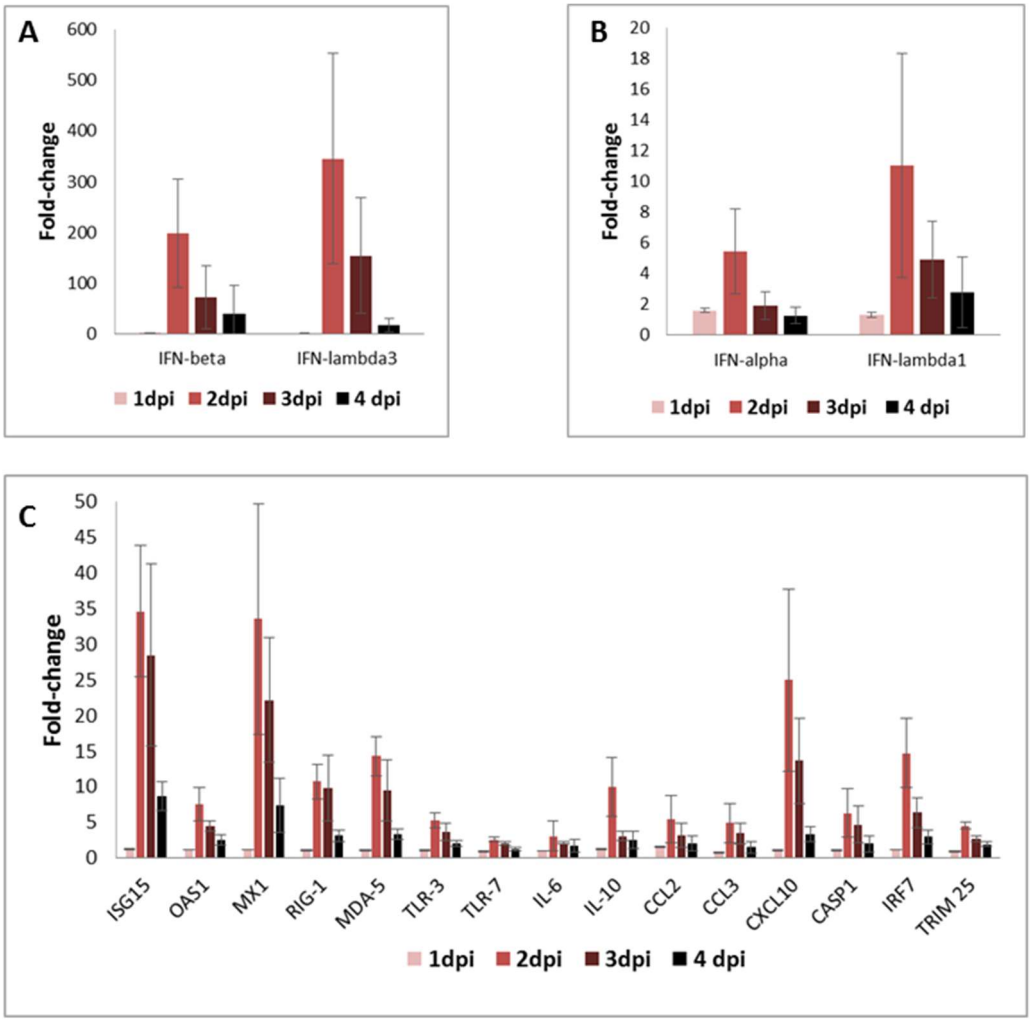
results with those obtained in the nasal submucosa were found in trachea and lung, providing evidence that type I and III INFs are expressed at the protein level in the nasal epithelium and that their antiviral biological action affects the entire respiratory tract. Expression of IFN-gamma, IL-2, -4 and -15, important for the development of adaptive immunity, were not altered during the 4 days of the experiment.

Figure: Kinetics of alpaca innate immune responses at the nasal epithelia upon MERS-CoV Qatar15/2015 infection. Alpacas were intranasally infected and euthanized (n=3 each day) after 1, 2, 3 and 4 days post-inoculation (dpi). Control animals (n=3) were euthanized prior infection. The nasal epithelium of each alpaca was microdissected and infected areas were selected and isolated for further RNA extraction. After RNA convertion to cDNA, Fluidigm Biomark technology was used to establish cytokine profiles. A) IFN-beta and lambda3 kinetic profiles. B) IFN-alpha and lambda1 kinetic profiles. C) Kinetic profiles of ISGs (ISG15, OAS1, MX1, CXCL10), PRRs (RIG-1, MDA-5, TLR-3, TLR-7), inflammatory cytokines and chemokines (IL-6, IL-10, CCL2, CCL3), transcription factors (IRF7), caspase-1 (CASP1) and ubiquitination enzyme (TRIM25).



4 Conclusions

Objective 1: The RVFV MP-12 immunization experiment revealed clear evidence of virus replication. The here described study indicates that *R. aegyptiacus* fruit bats can be

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productively infected, even when only the attenuated RVFV vaccine strain MP-12 is used, since we found clear evidence of virus replication in all three inoculated animals, as RVFV-associated RNA was detected in the spleens. In addition, one individual was viraemic at 3 dpi and viral RNA was also detected in the liver at 7 dpi. The presence of RVFV Gc antigen was also demonstrated by immunohistochemistry within the subacute necrotizing liver lesions, which is a clear indication of virus propagation in this tissue. However, no virus shedding via excretions such as saliva, feces or urine was observed using this vaccine strain. Taken together, this pilot experiment clearly proved the general susceptibility of *R. aegyptiacus* fruit bats for the MP-12 RVFV vaccine strain.

The challenge study using the highly virulent RVFV strain 35/74 did not reveal any clinical symptoms in the infected animals. The molecular, serological and histological analyses are currently ongoing and will be summarized in the next report.

The LBV study in mice and bats has been prepared and the ethical application for the animal study is currently ongoing. As soon as the approval has been released, the mouse experiment can be initiated without further delay. After completion of this experiment, the histological analysis will be performed and interpreted immediately to enable to completion of the experiment also in bats.

Objective 2: Different strains of MERS-CoV can provoke mild (EMC/2012 strain) to more severe infections affecting the upper and lower respiratory tract (strain Qatar-15/2015) and this from the first day (Jordan-1/2015 strain) after experimental intranasal inoculation. Alpacas did not show clinical signs with any strain upon infection. Discrete microscopical lesions characterized by some infiltrations of macrophages, neutrophils, lymphocytes and plasma cells were observed in the lamina propria and submucosa of the infected epithelia. Although slightly delayed (2 dpi), innate immune responses involving type I and III INFs and antiviral effectors (ISGs) are induced essentially at the nasal epithelial level, where viral replication is most active. Concomitant to the peak of cytokines observed at 2 dpi, viral loads in tissues decrease during the 2 following days. Importantly, although INFs are not expressed, antiviral cytokines are induced in trachea and lungs. Few pro-inflammatory factors were very mildly upregulated and returned quickly to steady state levels in all tissues examined. High upregulation of genes coding for cytoplasmic sensors of viral RNA and the transcription factor IRF7 highlight on signaling pathways and defense mechanisms used by host cells to clear infection. This is the first monitoring of an effective mucosal innate immune response *in vivo* in a MERS-CoV natural host, which clearly indicates targets for development of antiviral drugs and the requisites needed for the development of effective mucosal vaccines. In addition, the newly developed

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panel of primers meant to amplify cytokines of all camelids will be a useful tool for determining immune response profiles occurring in other diseases.

5 Annexes

References Objective 2 Introduction

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Publications

Balkema-Buschmann A, Rissmann M, Kley N, Ulrich R, Eiden M, Groschup MH. Productive Propagation of Rift Valley Fever Phlebovirus Vaccine Strain MP-12 in Rousettus aegyptiacus Fruit Bats. Viruses. 2018 Nov 30;10(12). pii: E681. doi: 10.3390/v10120681.

Primers

Excel file including a Table with the primers specific for all camelids used to study innate and cell mediated immunity.

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Table: Primers specific for all camelids used to study innate and cell mediated immunity

Cytokine/Protein	Cytokine/Protein type and function	Primer Name	Product size (bp)	Primers (5' - 3')
GAPDH	Normalizer gene	GAPDH F	108	GGTCGGAGTGAACGGATTTGG
		GAPDH R		TTGAGGTCAATGAAGGGGTCG
UbC	Normalizer gene	Ubc F	129	AGGCGAAGATCCAAGACAAGG
		Ubc R		CCAAGTGCAGAGTGGATTCCT
HPRT1	Normalizer gene	HPRT1 F	82	CAAAGATGGTCAAGGTCGCAA
		HPRT1 R		TCAAATCCAACAAAGTCTGGTCT
IFN-alpha	Type I IFN, antiviral	IFN-alpha F	87	TCTTCAGCGAGACACTTGCAA
		IFN-alpha R		GTTGGTCAGTGAGAATCATTTCCA
IFN-β	Type I interferon, antiviral	IFN-β F2	99	GCATCCTCCAAATCGCTCTCC
		IFN-β R2		ATGCCAAGTTGCTGCTCCTTT
IFN-γ	Type II interferon, Antiviral activity and mediator of cell immunity	IFN-γ F	199	ACTGGAAAGAGGAGAGTGACAAAA
		IFN-γ R		CAACCGGAATTTGAATCAGCT
IFN-lambda1	Type III IFN	IFN-lambda1 F	82	CTGCCACATGGGCTGGTT
		IFN-lambda1 R		CGATTCTTCCAAGGCATCCTT
IFN-lambda3	Type III IFN	IFN-lambda3 F	81	CCACCTGGCCCAATTCAA
		IFN-lambda3 R		AGTGACTCTTCAAAGGCGTCCTT
RIG-1 (DDX58)	PRRs and ISG, recognises dsRNA and ssRNA; induce IFN production and ISG	RIG-1 F	199	ACAAGTCAGAACACAGGAATGA
		RIG-1 R		CTCTTCCTCTGCCTCTGGTTT
MDA5 (IFIH1)	PRRs and ISG, recognises dsRNA and ssRNA; induce IFN production and ISG	MDA5 F	129	ACACCAGAGTTCAAGAGACTGTAT
		MDA5 R		CACCATCATCGTTCCCCAAGA
MAVS	PRRs, interract with RIG-1	MAVS F	106	CAGCCTCCACAACTGCTACAGA
		MAVS R		CTGTGGGACTTTCTTTGAACTCTCT
TLR3	PRRs and ISG, recognises dsRNA; induce IFN production and ISG	TLR3 F	197	AGAAATAGACAGACAGCCAGAG
		TLR3 R		TGCTCCTTTTGATGCTATTAACGA
TLR7	PRRs and ISG,Recognises ssRNA induce IFN production and ISG	TLR7 F	104	AGAGAGGAGTCACCAGCGTAT
		TLR7 R		GACACAAATGCAAATGGAGAC
NLRP3	PRRs, increased expression of pro-inflammatory cytokines	NLRP3 F	91	ATGGCCACATGGATTTTTGC
		NLRP3 R		AAACATTGGCATTGTCCCATTC
STAT1	Transcription factor activated by IFNs; Increased expression of ISG	STAT1 F	191	TCTCTGTGTCTGAAGTTCACCCT
		STAT1 R		GGGAATCACAGGTGGGAAGGA
IRF3	Transcription factor and ISG, activated by IFNs; Increased expression of ISG	IRF3 F	102	TCACCACGCTACACCCTCTGGT
		IRF3 R		GAGGCACATGGGCACAACCTTGA
IRF5	Transcription factor and ISG, activated by IFNs; Increased expression of ISG	IRF5 F	121	TCAGAAGGGCCAGACCAACACC
		IRF5 R		TGCTACGGGCACCACCTGTA
IRF7	Transcription factor and ISG, activated by IFNs; Increased expression of ISG	IRF7 F	96	CGTGATGTTGCAAGACAACTCA
		IRF7 R		TGGTTAACGCCTGGGTCTCT
NFKB1	Transcription factor activated by IFNs; Increased expression of pro-inflammatory cytokines	NFKB1 F	90	GGGACAGTGTCTTACACTTAGCAATC
		NFKB1 R		CATCAGAAATCAAGCCAGATGTG
RELA	Transcription factor, binds to NF-kappa-B	RELA F	81	AGAGTCCTTTCAATGGCCCCACCG
		RELA R		GGATGGAAGTTGAGCTGCGGGA
IKBKB	Transcription factor activated by IFNs; Increased expression of pro-inflammatory cytokines	IKBKB F	91	TAATGAACGAAGACGAGAAGATGGT
		IKBKB R		ACCTTGCTACACGCAATCTTCAG
CXCL10	ISG, activation and migration of immune cells to the infected sites	CXCL10 F	184	CGTGTTGAGATTATTGCCACAATG

		CXCL10 R		GAGGTAGCTTCTCTCTGGTCCT
MX1	ISG, GTPase with antiviral activity	MX1 F	146	GAAGATGGTTTATTCTGACTCG
		MX1 R		TTCTCCTCGTACTGGCTGT
OAS1	ISG, antiviral enzyme; degradates viral RNA	OAS1 F	198	TGAAGAAGCAGCTCGGGAAAC
		OAS1 R		AGTAACTGTCTTTTCTGGGCAGC
ISG15	ISG, antiviral activity	ISG15 F	91	CACAGCCATGGGTGGAATC
		ISG15 R		CAGCTCCGATAACAGCATGGA
IL-10	Interleukin, Inflammatory antagonist	IL-10 F	187	CTGCTGGAGGACTTTAAGGGT
		IL-10 R		AGGGGAGAAATCGATGACAGC
IL1-beta	Interleukin, pro-Inflammatory response	IL1-beta F	125	AGGATATGAGCCGAGAAGTGGT
		IL1-beta R		CCCTTTCATCACACAAGACAGGT
IL-6	Interleukin, pro-Inflammatory response	IL-6 F	192	TCTGGGTTCAATCAGGAGACCT
		IL-6 R		AGGGGTGCTTACTTCTTCTGGT
IL-8 (CXCL8)	Interleukin, pro-inflammatory response	IL-8 F	176	TGTGTGAAGCTGCAGTTCTGT
		IL-8 R		GCAGACCTCTCTTCCATTGGC
IL-15	Interleukin, induce proliferation of antiviral natural killer cells	IL-15 F	93	CAGCCTACAGAAGGTCATGAAGTACTC
		IL-15 R		GGGTAACTCCTTAAGTATCGAAGAAGAG
IL-2	Interleukin, cell-mediated immunity	IL-2 F	202	AAACTCTCCAGGATGCTCAC
		IL-2 R		TTTCAGATCCCTTCAGTTCC
IL-4	Interleukin, humoral immunity mediator	IL-4 F	168	CCCTGGTCTGCTTACTGGTTT
		IL-4 R		TCTCAGTCGTGTTCTTTGGGG
IL-12p35	Interleukin, cell-mediated immunity	IL-12p35 F2	140	AATCACCTGGACCACCTCAGT
		IL-12p35 R2		TCTAGGGTTTGTCTGGCCTTC
TNF-α	Cytokine, pro-Inflammatory response	TNF-α F	143	TGGCCCAGACCCTCAGATCA
		TNF-α R		TTCCAGCTTCACACCATTGGC
MIP-1α (CCL3)	Inflammatory chemokine, attract monocytes, macrophages and neutrophils	CCL3 F	113	GCTCAGCGTCATGCAGGTGCC
		CCL3 R		AGCAGGCGGTTGGGGTGTCAG
MIF	Inflammatory cytokine, Macrophage migration inhibitory factor	MIF F	176	GCGAGTTGGTCGGTTCCTGTGTT
		MIF R		ACCACGTGCACTGCGATGTACT
CCL2	Chemokine, recruite monocytes and dendritic cells at the sites of inflammation	CCL2 F	93	CCAGTAAGAAGATCCCCATGCA
		CCL2 R		GTGTGGTCTTGAAGATCACAGCTT
CXCL1	Chemokine, attract neutrophils	CXCL1 F	91	CGTGCAGGGAATTCACTTCAA
		CXCL1 R		GAGAGTGGCTACGACTTCCGTTT
CASP1	Enzyme, Caspase-1 initiate inflammatory responses	CASP1 F	164	ACTCCACCAAGACCTCAACCAGT
		CASP1 R		GGGTAAATCTCCGCTGACTTCTCG
CASP10	Enzyme, Caspase 10 is involved in apoptosis and inflammation	CASP10 F	107	CGGTAGCCACGGGAACTGAGTCAT
		CASP10 R		ATCTTGCCAGGACCCCTCCGAT
CYLD	Enzyme, involved in transcription factor NF-kappa-B activation	CYLD F	135	TCGGGATGGTGGTCAGAATGGC
		CYLD R		AGTCTTCGTGCACAGCCCTGGAT
AZI2	Enzyme, NF-Kappa-B-Activating Kinase-Associated Protein 1	AZI2 F	86	TGAGCGTCTCCAGCGCTAA
		AZI2 R		CTGCACTTGCGTCACCAGAT
PACT (PRKRA)	Enzyme, protein kinase activated by double-stranded RNA	PACT F	92	TGCAGTTCCTGACCCCTTAATG
		PACT R		GATGAATAGCCAGTTCCTGTAGTGAA
TBK1	Enzyme, activates the transcription factor IRF3	TBK1 F	81	GTACAGAAAGCAGAAAATGGACCAA
		TBK1 R		AACTTGAAGGCCCCGAGAAA
TRIM25	Enzyme and ISG, ubiquitination of RIG-1	TRIM25 F	93	GCCCGAGCTCCTACAGTATGC
		TRIM25 R		GAAGCGACGGTGTAGGTCTTG

NFKBIA	NF-Kappa-B inhibitor	NFKBIA F	138	TCCCTCTTTTCCCCGCAGGTT
		NFKBIA R		TGGAGTGGAGTCTGCTGCAGGT
TRADD	Adaptor, mediates NF-Kappa-B activation and apoptosis	TRADD F	81	CGGCCAGGAAGCAAGATG
		TRADD R		TGAAGACTCCACAAACAGGTATGC
CARD9	Adaptor, activates pro-inflammatory and anti-inflammatory cytokines through NF-Kappa-B	CARD9 F	92	GGCAGTGCAAGGTCCTGAAC
		CARD9 R		CAGGAGCACACCCACTTTCC
PYCARD	Adaptor, activates caspases and inflammasome	PYCARD F	105	CAAGCCAGCACCGCACTT
		PYCARD R		TCTGTCAGGACCTTCCCATACA
IFNLR1	Receptor for type III IFNs	IFNLR1 F	90	CAGGGTGTGTGATCTGGAAGAG
		IFNLR1 R		GTCTGTGTCCAGAGAAATCCAGG
IFNAR1	Receptor of type I IFNs	IFNAR1 F	83	TGCGAGGAAACCAAACCAGGAAAT
		IFNAR1 R		ACGACGACGATACAAAACACCGC