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WHO IS KILLING WHOM? HANTAVIRUSES VS PROGRAMMED CELL DEATH

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Who is Killing Whom?
Hantaviruses VS Programmed Cell Death
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To my family

Deep into that darkness peering, long I stood there wondering, fearing…

From The Raven by Edgar Allan Poe 1845

ABSTRACT

Hantaviruses belong to the *Bunyaviridae* family of negative stranded RNA viruses. They carry a tri-segmented genome and consist of four structural proteins. The four structural proteins are two glycoproteins Gn and Gc, a nucleocapsid (N) protein and an RNA-dependent RNA-polymerase. An additional nonstructural protein can be expressed by some hantaviruses. Hantavirus-infection cause two severe diseases in humans with potential deadly outcome, namely hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardio-pulmonary syndrome (HCPS). One main target for hantavirus-infection is the endothelial cells and vascular leakage is a hallmark for both HFRS and HCPS. In patients strong cytotoxic lymphocyte responses are seen. Cytotoxic lymphocytes, such as natural killer (NK) cells and cytotoxic T lymphocytes, cause apoptosis in virus-infected cells via the cytotoxic granule pathway or the death receptor pathway. The cytotoxic granule pathway uses granzyme B to facilitate programmed cell death (PCD) in the target. The death receptor pathway uses death ligands, among them tumor necrosis factor related apoptosis-inducing ligand (TRAIL) binds to death receptor (DR) 4 or 5, to induce PCD.

One potential mechanism regarding hantavirus pathogenesis might be killing of infected endothelial cells by cytotoxic lymphocytes, thus causing leakage of the endothelium. This is contradicted by findings showing that in patient autopsy, hantavirus-infected cells are intact. The aim of this PhD thesis is to give a possible explanation of this dichotomy and to better understand hantavirus pathogenesis.

The first part of this thesis (**paper I and II**) shows that hantavirus-infection protects cells from cytotoxic lymphocytes via inhibiting granzyme B activity and by down-regulating DR5 from the cell surface. Granzyme B and caspase 3, enzymes needed for apoptosis (a type of PCD), both interacts with hantavirus N protein, and they are both inhibited by the N protein. Further, hantavirus-infection of primary endothelial cells causes miss-localization of DR5. In infected cells DR5 is found in the nucleus instead of on the cell surface. Taken together, hantavirus-infection blocks the two major pathways used by cytotoxic lymphocytes to induce cell death, suggesting that hantavirus pathogenesis is not due to killing of infected cells by cytotoxic lymphocytes. The last part of this thesis (**paper III**) focuses on hantavirus activated NK cell mediated killing of uninfected endothelial cells. NK cells co-incubated with hantavirus-infected endothelial cells are activated. This activation is contact dependent and was attributed to IL-15 and IL-15Rα expression on hantavirus-infected cells' surface. Interestingly, these activated NK cells induce cell death in uninfected cells with normal HLA class I levels, indicating that hantavirus might cause NK cell mediated killing of uninfected bystander cells.

Taken together, the **papers I, II** and **III** included in this thesis shows that hantavirus-infection protects cells from cytotoxic lymphocyte mediated killing, while infected cells can cause NK cell activation and possibly subsequent NK cell killing of uninfected cells.

LIST OF SCIENTIFIC PAPERS

I. **Gupta S**, Braun M, Tischler ND, Stoltz M, Sundström KB, Björkström NK, Ljunggren HG, Klingström J. Hantavirus-infection Confers Resistance to Lymphocyte-Mediated Apoptosis. *PLoS Pathogens, 2013 9(3):e1003272.*

II. **Gupta S**, Solà Riera C, Braun M, Björkström NK, Ljunggren HG, Klingström J. Hantavirus Causes Nuclear Translocation of Death Receptor 5 and Protects Infected Cells from TRAIL-Induced Apoptosis. *Manuscript*.

III. Braun M, Björkström NK, **Gupta S**, Sundström K, Ahlm C, Klingström J, Ljunggren HG. NK Cell Activation in Human Hantavirus Infection Explained by Virus-Induced IL-15/IL-15Rα Expression. *PLoS Pathogens, 2014 20;10(11):e1004521.*

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LIST OF ABBREVIATIONS

ACD	Accidental cell death
ANDV	Andes virus
Apaf-1	Apoptotic protease activating factor 1
Bcl-2	B-cell leukemia/lymphoma-2
Bid	BH3 interacting-domain death agonist
CD	Cluster of differentiation
CMV	Cytomegalovirus
CrmA	Cytokine response modifier A protein
CTL	Cytotoxic T lymphocyte
DAXX	Death-domain associated protein-6
DC	Dendritic cell
DcR1	Decoy receptor 1 (also known as TRAIL-receptor 3)
DcR2	Decoy receptor 2 (also known as TRAIL-receptor 4)
DISC	Death inducing signaling complex
DOBV	Dobrava virus
DR4	Death receptor 4 (also known as TRAIL-receptor 1)
DR5	Death receptor 5 (also known as TRAIL-receptor 2)
ER	Endoplasmic reticulum
FADD	Fas-associated death domain
HCPS	Hantavirus cardio-pulmonary syndrome
HFRS	Hemorrhagic fever with renal syndrome
HLA	Human Leukocyte Antigen
HTNV	Hantaan virus
IFN	Interferon
IL	Interleukin
MDA-5	Melanoma differentiation-associated gene 5
MOMP	Mitochondrial outer membrane permeability
mRNA	Messenger ribonucleic acid
N	Nucleocapsid

NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cell
NK	Natural Killer
NLR	Nucleotide-binding oligomerization domain receptors
PAMP	Pathogen-associated molecular pattern
PCD	Programmed cell death
PHV	Prospect Hill virus
PRR	Pattern recognition receptor
PUUV	Puumala virus
RCD	Regulated cell death
RIG-I	Retinoic acid inducible gene-I
RLR	RIG-I-like receptor
RNA	Ribonucleic acid
STAT	Signal transducer and activator of transcription
SNV	Sin Nombre virus
STS	Staurosporine
tBid	Truncated Bid
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAF	TNF-receptor-associated factor
TRAIL	TNF-related apoptosis inducing ligand
TULV	Tula virus
VEGF	Vascular endothelial growth factor

1 INTRODUCTION

ABOUT HANTAVIRUSES

Hantavirus, *Orthobunyavirus*, *Phlebovirus*, *Nairovirus* and *Tospovirus* are five genera of viruses belonging to the *Bunyaviridae* family. There are human pathogens among four of these genera and one, *Tospovirus*, is only pathogenic in plants. The pathogenic hantaviruses may cause hemorrhagic fever in humans, and in most cases humans are considered as dead-end host for these viruses. Pathogenic hantaviruses are rodent borne in contrast to the other genera of bunyaviruses, which are arthropod borne.

The history

In 1913 clinical records from Russia describes a disease which is now designated as hemorrhagic fever with renal syndrome (HFRS) (Johnson, 2001). Although, already around A.D. 960, in Chinese medical archives, a similar disease was described. This illness, HFRS, have occasionally been referred as a disease of wars. "Field nephritis", which befell soldiers from both the allied and the German troops during World War I, might also have been a similar disease. Japanese military faced a similar disease in the1930s after their invasion of Manchuria. In World War II Finish and German soldiers encountered similar disease (Johnson, 2001). United Nations troop stationed in Korea during 1950s was challenged by Korean hemorrhagic fever (Smadel, 1953), now known as HFRS (Johnson, 2001), which now is known to be caused by the prototypical hantavirus, Hantaan Virus (HTNV) (Johnson 2001).

Efforts to isolate the agents of one of these diseases yielded results in 1976, published 1978, when the pathogen causing HFRS was characterized (Lee et al., 1978; Johnson, 2001). HTNV was discovered in the lungs of a field mouse, captured near the river Hantaan in South Korea. Next, in 1980, the HFRS causing Puumala virus (PUUV) was isolated from a bank vole caught in Puumala, a municipality in Finland (Brummer-Korvenkontio, et al., 1980). Within a couple of years, in 1982, a new hantavirus, the HFRS causing Seoul virus (SEOV) was isolated in rats from Seoul, South Korea (Lee et al., 1982). In 1992, yet another hantavirus that cause HFRS was characterized, Dobrava virus (DOBV), in former Yugoslavia (Avsic-Zupanc et al., 1992).

Hantaviruses are known to be the causative agent of both HFRS and hantavirus cardio-pulmonary syndrome (HCPS; also known as hantavirus pulmonary syndrome, HPS). The story of HCPS leads us to the Americas. An outbreak of a disease with respiratory distress, in 1993 in the four corner region (Arizona, Colorado, New Mexico and Utah) in USA, led to the finding of Sin Nombre virus (SNV) (Nichol et al., 1993). In 1995, another HCPS causing

virus was discovered, Andes virus (ANDV), in Argentina (López et al., 1996), which like SNV cause high case fatality rate.

It has to be mentioned, a non-pathogenic hantavirus, Thottampalayam virus (TPMV) was already described in the early 70s (Carey et al., 1971), therefor probably the first characterized hantavirus.

Host and distribution

Having animal reservoirs as their hosts, hantaviruses are zoonotic viruses (Jonsson et al., 2010). Pathogenic hantaviruses are carried by rodents, however, hantaviruses can be found in insectivores and also, as recently described by Gou and colleagues, in bats (Guo et al., 2013). In nature hantavirus-infection of rodents is persistent, and it is thought that the virus has coevolved with its host (Meyer and Schmaljohn, 2000). This persistent infection cause continuous virus replication during the entire life of the rodent (Jonsson et al., 2010). Humans are believed to be dead-end hosts and are accidently infected by inhaling virus contaminated rodent excreta (Vaheri et al., 2013). ANDV is the only hantavirus so far known to cause human-to-human transmission (Wells et al., 1997). Pathogenic hantaviruses can be found worldwide, HFRS causing hantaviruses are mainly found in Eurasia and HCPS causing hantaviruses are found in the Americas (Manigold and Vial, 2014).

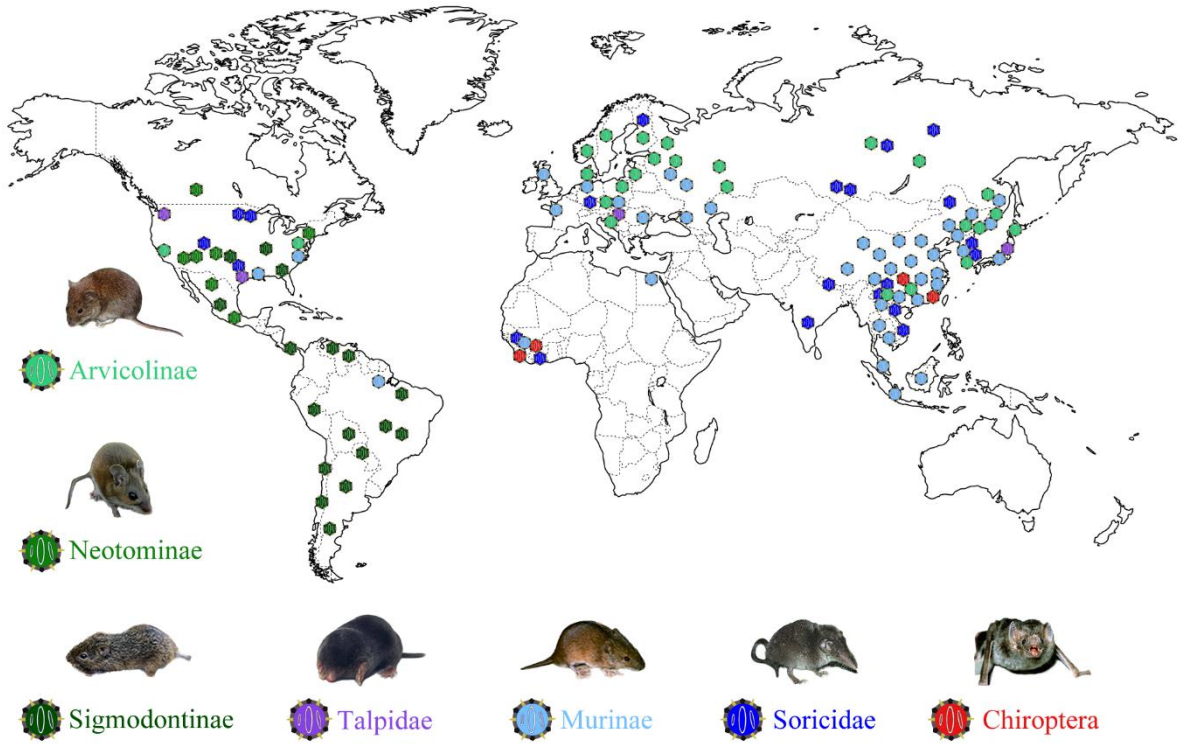

Figure 1. Hantavirus hosts and distribution of the hosts. Reprinted from Gou et al., 2013. PLoS Pathog. 2013;9(2):e1003159 (Guo et al., 2013) under the terms of the Creative Commons Attribution License.

Diseases

HFRS and HCPS have distinct clinical manifestation with similarities and dissimilarities. One of the common hallmarks is vascular leakage. Another shared feature is thrombocytopenia, which may add to symptoms involving bleeding (Zaki et al., 1995; Kanerva et al., 1998; Vapalahti et al., 2003; Rasche et al., 2004; Latus et al., 2015). Both of the diseases can have lethal outcome. HFRS causes up to 15% case fatality (around 0.5-15% depending on the virus), and HCPS causes up to 40% case fatality (Vaheri et al., 2013).

The incubation time for HFRS is normally between one to six weeks, which is followed by a febrile phase with flu like symptoms, such as myalgia, headache, abdominal pain and malaise, and occasionally also neurological, cardiovascular and gastrointestinal symptoms (Hautala et al., 2012). Hemorrhage may occur, afterward the patients enter a hypotensive phase with symptoms of vascular leakage, associated with thrombocytopenia, and sometimes shock. The resulting oliguric phase can last up to five days with risk for hypertension, pulmonary edema and renal failure. After the subsequent diuretic phase, the convalescent phase starts. Unfortunately for some HFRS patients the outcome may be fatal (Nichol 2001; Vaheri et al., 2013; Manigold and Vial 2014).

Incubation time for HCPS ranges from two to six weeks (Vial et al., 2006; Jonsson et al., 2008; MacNeil et al., 2011). Comparable to HFRS, the HCPS patients exhibits fever, myalgia, headache, and malaise. Sometime gastrointestinal, and neurological signs might be seen, additionally, thrombocytopenia, leukocytosis and leucopenia have been reported, and increased creatinine levels, hyponatremia and proteinuria (Hallin et al., 1996; Duchin et al., 1994; Castillo et al., 2001; Riquelme et al., 2003). The cardiopulmonary phase consist of dyspnea, cough, tachycardia and hypotension, which reflect rapidly progressive pulmonary edema caused by capillary leakage and low cardiac output, which can lead to cardiac failure (Talamonti et al., 2011). Rapid progression of HCPS might lead to death within 48 hours (Vial et al., 2013; Manigold and Vial, 2014). Currently, there is no worldwide-approved vaccine or clinically approved drugs for HCPS or HFRS.

Structure

Hantavirus, as all bunyaviruses are tri-segmented negative sense RNA virus.The three segments are designated large, medium and small segment (Schmaljohn et al., 1983a, Schmaljohn and Dalrymple, 1983b). For hantaviruses these segments are around 6500, 3600, and 1700-2000 nucleotides long respectively. Each RNA segment contains an open reading frame, and is flanked by non-coding regions (Plyusnin et al., 1996; Vaheri et al., 2013.). The both end termini of the non-coding regions are complementary to each other and form a panhandle structure. Four structural proteins are encoded by these RNA segments, the large segment encodes a RNA-dependent RNA polymerase (RdRp), the medium segment encodes a precursor glycoprotein which later is processed into two glycoproteins (Gn and Gc), and the

small segment encodes a nucleocapsid (N) protein. These four proteins together with the virus RNA segments and cellular membrane forms an enveloped virus which is around 120-160 nm, with a pleomorphic shape (Figure 2) (Vaheri et al., 2013).

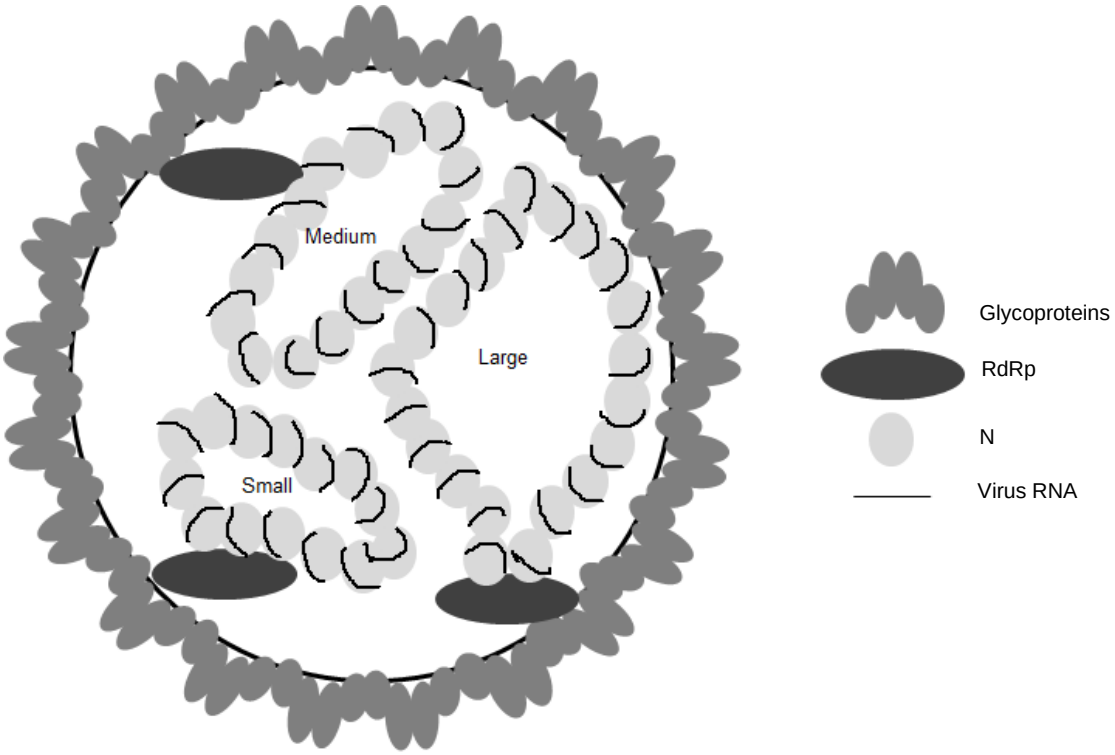

Figure 2. Schematic illustration of a hantavirus particle.

Hantavirus proteins

Expressing only few proteins mean that some or all of the virus proteins should have more than one function for successful virus propagation. The RdRp, a 250 KDa protein, have RNA transcriptase and replicase activity, and it was also demonstrated that RdRp has a perinuclear localization (Kukkonen et al., 2004; Kukkonen et al., 2005). Since hantaviruses are negative strand viruses the RdRp must be carried by the virus in order for it to replicate.

The hantavirus glycoproteins seem to have several functions, though most of them are linked to host receptor recognition (Klingström and Ahlm, 2011). Initially, the Gn and Gc are translated as one single glycoprotein precursor, which is later cleaved after a conserved WAASA motif (Löber et al., 2001). The glycoproteins have key roles in virus attachment and binds to several receptors (Vaheri et al., 2013). Besides surface receptors, the glycoproteins also interact with intracellular host proteins (Klingström and Ahlm 2011). Trafficking of

arenavirus, hantavirus, coronavirus, orthomyxovirus, and filovirus glycoproteins requires endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) pathway (Klaus et al., 2013). New York 1 virus (NY-1) Gn protein inhibits interferon (IFN) responses by interaction with retinoic acid-inducible gene I (RIG-I) and tumor necrosis factor (TNF) receptor-associated factor 3 (TRAF3) (Alff et al., 2006; Allf et al., 2008). IFN responses can also be down-regulated by inhibition of STAT1 phosphorylation by hantavirus glycoproteins (Spiropoulou et al., 2007). These data indicates that hantavirus glycoproteins are not only essential for attachment to the cell surface, they also have important roles in the regulation of host immune responses.

Probably the most studied hantavirus protein is the N protein, and it has been shown that this specific protein can interact with different host proteins and it harbors various functions (Klingström and Ahlm, 2011; Vaheri et al., 2013). Black Creek Canal virus N protein interacts with actin microfilament and actin is important for Black Creek Canal virus progeny release (Ravkov et al., 1998). HTNV N protein is distributed along microtubule and is colocalized with the ERGIC (Ramanathan et al., 2007). It has also been described that ANDV N, Black Creek Canal virus N, SEOV N also use microtubule (Ramanathan and Jonsson, 2008). Together, showing that hantavirus N protein interact with host cytoskeleton, and the host proteins are essential for virus propagation, as disrupting the host cytoskeleton impaired virus propagation. HTNV can cause expression of heat shock protein 70 (Hsp70) and HTNV N protein forms a complex with Hsp70 (Ye et al., 2001), but overexpression of Hsp70 was suggested to be disadvantageous for virus propagation (Yu et al., 2009). Pull down assay showed that PUUV N protein interacts with death-domain associated protein-6 (DAXX) (Li et al., 2002), indicating that hantavirus may have the ability to interact with cell death pathways. Further, it has been proposed that SNV N protein replaces eukaryotic initiation factor 4F, suggesting that N protein might be able to directly facilitate translation initiation (Mir and Panganiban, 2008). Binding of HTNV N to importin-α proteins has been reported and this binding caused inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signals by obstructing importin-α-mediated transport of NF-κB to the nucleus (Taylor et al., 2009a). HTNV N protein also directly interacts with NF-κB (Ontiveros et al., 2010). ANDV N protein is an inhibitor of both granzyme B and caspase 3, thus also able to inhibit apoptosis, and both theses enzymes can cleave the N protein (**paper I**). These are some examples of the host proteins that hantavirus N protein interact with, showing how one single virus protein can be used in different ways by the virus during its lifecycle.

Certain hantaviruses, such as Tula virus (TULV), PUUV and ANDV, may in the infected cells express a nonstructural protein (NSs) (Jääskeläinen et al., 2007; Jääskeläinen et al., 2008; Vera-Otarola et al., 2012). The role for ANDV NSs has yet to be defined. However, TULV and PUUV NSs have been suggested to interfere with IFN responses (Jääskeläinen et al., 2007; Jääskeläinen et al., 2008). The localization of TULV NSs is in the pernuclear region of the infected cell (Virtanen et al., 2010).

Life cycle

The life cycle of hantavirus starts with attachment and binding of the virus to its cellular receptors. There are several known receptors that hantaviruses binds to; αVβ3 integrin (Gavrilovskaya et al., 1998; Gavrilovskaya et al., 1999), complement decay-accelerating factor (Krautkrämer and Zeier, 2008), a glycosylphosphatidylinositol-anchored protein of the complement system, GC1QR (globular heads of complement C1q receptor; also known as C1QBP) (Choi et al., 2008), and an unidentified 70 KDa protein (Mou et al., 2006).

Entry into the cells differs between HFRS and HCPS causing hantaviruses, HFRS causing viruses use clathrin dependent endocytosis, while the exact entry mechanism of HCPS causing hantaviruses remains unknown (Jin et al., 2002; Ramnathan and Jonsson 2008). Other ways of entry than clathrin dependend endocytosis has been shown for other bunyaviruses. For example, phleboviruses enters the cell through penetration of host cell by acid-activated membrane fusion (Lozach et al., 2010). Inside the cell the hantaviruses are transported via the early and late endosomes, where lowering of pH leads to detachment of virus particles (Jin et al., 2002).

As there is a lack of reverse genetics for hantaviruses, studies of virus transcription and replication have been limited. Attempt at making reverse genetics for hantaviruses was made (Flick et al., 2003), but this has not been successfully repeated again.

The replication and transcription of hantaviruses are thought to be similar to other bunyaviruses. mRNA and negative stranded genomic virus RNA is produced by the RdRp (Kukkonen et al., 2005). The negative strand virus RNA is replicated to positive strand complementary RNA which is then replicated to negative strand virus RNA. In order to make new virus proteins, negative strand virus RNA is transcribed to mRNA leading to subsequent translation of virus proteins (Jonsson and Schmaljohn, 2001; McAllister and Jonsson, 2014; Vaheri et al., 2013).

When replication is performed, the virus genomes are encapsidated by N proteins (Hepojoki et al., 2012). It is not exactly known where the assembly of the virus particle takes place, the HFRS causing hantaviruses have been suggested to be assembled and matured in the Golgi where budding of the virus particle occurs, and the virion exit the cell through exocytosis. Whereas, for HCPS causing hantaviruses it has been proposed that assembly might occur at the host plasma membrane (Jonsson and Schmaljohn, 2001; McAllister and Jonsson, 2014; Vaheri et al., 2013).

IMMUNOLOGY AND HANTAVIRUSES

Classically, our immune system is divided in two parts: the innate immunity (also known as natural or native immunity) and the adaptive immunity (sometime referred as acquired or specific immunity). These two parts of the immune system is critical for host defense against pathogenic agents. The innate immune system uses pattern recognition receptors (PRRs) to sense infection and mount an immune response. This will lead to induction of interferons (IFN) and recruitment of immune cells such as natural killer (NK) cells and dendritic cells (DC). DCs bridges the innate immune system with the adaptive immune system. When the adaptive immune system is active, cytotoxic T lymphocytes (CTL) are activated. Both NK cells and CTLs can kill infected target cells.

Pattern recognition receptors

Virus RNA can be recognized as pathogen-associated molecular patterns (PAMPs) by PRRs in infected cells, and by that activate innate immune responses (Kato et al., 2006; Kato et al., 2008). There are different types of PRRs: toll like receptors (TLRs), nucleotide-binding oligomerization domain receptors (NLRs), and RIG-I-like receptors (RLRs) are some example of PRRs used by cells to sense virus-infection.

Nucleic acids can be recognized by TLRs, which play a pivotal role in recognizing virus infection (Chow et al., 2015). Different TLRs recognizes different types of PAMPs: double-stranded virus RNA is recognized by TLR3 (Alexopoulou et al., 2001), single stranded virus RNA is recognized by TLR7 and TLR8 (Diebold et al., 2004; Heil et al., 2004), DNA containing unmethylated CpG can be recognized by TLR9 (Lund et al., 2003). There have been some reports regarding TLRs during hantavirus-infection. HTNV triggers TLR3 and causes innate immune responses and inflammatory responses (Handke et al., 2009; Zhang et al., 2014). Hantavirus triggers TLR4 and antiviral immunity through MyD88 pathway (Yu et al., 2012), and it has also been shown that HTNV-infection induces TLR4 expression that may enhance the host immune responses (Jiang et al., 2008).

The RLR family consist of RIG-I, melanoma differentiation-associated gene 5 (MDA-5), and laboratory of genetics and physiology-2 (Nan et al., 2014). RLRs are cytoplasmic receptors that detects virus RNA in the cytosol (Schlee, 2013). RIG-I recognizes double stranded RNA, then recruits caspases and activates NFκB and by that inducing IFN responses (Yoneyama et al., 2004). Several studies have shown that RIG-I and MDA-5 can detect RNA viruses (Nan et al., 2014). For example, phlebovirus and dengue virus are recognized by RIG-I (Habjan et al., 2008; Nasirudeen et al., 2011; Ning et at., 2014). Evidently there are viruses that can evade RLRs. For example, arenaviruses use decoys to avoid detection by RIG-I, by using a single unpaired 5' ppp-nucleotide that render RIG-I incapable of inducing IFN responses (Marq et al., 2010; Marq et al., 2011). It has been suggested that HTNV-infection

might be recognized by RIG-I and MDA-5 (Zhang et al., 2014; Lalwani et al., 2013; Lee et al., 2011), although hantavirus genomic RNA by itself is not sufficient to trigger recognition by RIG-I (Habjan et al., 2008). Additional complexity is added to hantavirus-infection and RIG-I interaction as several reports show that hantavirus Gn protein inhibits RIG-I (Alff et al., 2006; Matthys et al., 2014).

The NLR family of PRRs consist of nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR) and PYD domains-containing protein (NLRPs; also known as NALPs) (Schroder and Tschopp, 2010). Of the NLR family NLRP3 is the only PRR that has been shown to recognize viruses. Activated NLRP3 triggers the formation of the inflammasome. Inflammasomes contain caspase 1, and actived caspase 1 in turn cleaves IL-1β. The cleaved processed IL-1β is secreted and causes inflammation (Tschopp and Schroder 2010). Several viruses have been described to interact with NLRP3: influenza A virus (McAuley et al., 2013; Pothlichet et al., 2013), respiratory syncytial virus (Segovia et al., 2012), Rift Valley fever virus (Ermler et al., 2014) and many more (Jacobs and Damania, 2012). It has recently been described that HTNV cause IL-1β secretion in human monocytes through NLRP3 inflammasome activated caspase 1 (Ye et al., 2015).

Nuclear factor kappa-light-chain-enhancer of activated B cells

NFκB is activated by PRRs upon virus infection and this activation causes IFN responses (Zhao et al., 2015). Adapter molecules of PRRs recruits TRAF (Nan et al., 2014), which subsequently in a cascade of events lead to degradation of inhibitor of κB (Zandi et al., 1997; Zhao et al., 2015). NFκB is activated following the degradation of inhibitor of κB, and the active NFκB is transported to the nucleus, where it induce transcription and subsequent translation of various cytokines and other proteins to mount a strong innate immune response (Zandi et al., 1997). As a consequence, some viruses have evolved strategies to evade NFκB signaling pathways. For instance, Epstein–Barr virus deubiquitinates TRAF6 (Saito et al., 2013), and HSV-1 blocks activation of NFκB (Xing et al., 2013; Wang et al., 2014) and varizella zoster virus blocks translocation of NFκB to the nucleus (Sloan et al., 2012). HTNV N protein blocks NFκB translocation to the nucleus via interaction with importin alpha proteins, as well as inhibiting TNF-induced activation of NFκB (Taylor et al., 2009a; Taylor et al., 2009b). The N protein of HTNV can directly bind to NFκB, but the effect of this finding remains to be explored (Ontiveros et al., 2010).

Interferons

IFNs play an essential role during virus infection. They are used by infected cells to signal the neighboring cells to enter an antiviral state, thus limiting spread of virus. IFNs also have direct antiviral effect on virus-infected cells including by inducing apoptosis (Clemens, 2003; McInerney and Karlsson Hedestam, 2009). There are three types of IFNs, type I

(among them, IFN-α and IFN-β are most studied), II (includes only IFN-γ) and III (several type of IFN-λ) (Chelbi-Alix and Wietzerbin, 2007). Any nucleated cell can produce IFN-α/β (Chelbi-Alix and Wietzerbin, 2007) but plasmacytoid dendritic cells are the main producers of IFN-α (Barchet et al., 2002). Type III IFNs, IFN-λ, do have antiviral activity similar to the type I IFNs, although with weaker effects (Kotenko et al., 2003; Sheppard et al., 2003). Studies of IFN induction by dsRNA viruses led to the discovery of IFN regulatory factors (IRFs) during the late 80's (Miyamoto et al., 1988). IRFs are required for both IFN-α/β production, as IRFs promotes induction of IFNs (Randall and Goodbourn, 2008). Upon virus recognition by RIG-I or MDA5, these PRRs signal via the mitochondrial adaptor protein (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005), and recruits and activates IRFs and NFκB. Activated IRF and NFκB are translocated to the nucleus where they attach to the IFN promoter and type I IFNs are expressed (Zandi et al., 1997; McInerney and Karlsson Hedestam, 2009; Zhao et al., 2015). After being secreted IFN binds to interferon-α/β receptor and type I IFN signals through the JAK-STAT (JAK; janus kinase, STAT; signal transducer and activator of transcription) pathway, to activate STAT1/2 by phosphorylation. Phosphorylated STAT1/2 are transported to the nucleus, where more IFNs and IFN stimulated genes are transcribed.

Due to the strong antiviral capacity of IFNs some viruses have evolved strategies to inhibit induction of IFNs. For example, cytomegalovirus (CMV), Hepatitis B and C virus, and Hendra virus targets STAT1 and/or STAT2 to block induction of IFNs (Rodriguez et al., 2003; Paulus et al., 2006; Lin et al., 2006; Wu et al., 2007).

Both HCPS and HFRS causing hantaviruses inhibit STAT1/2 phosphorylation and thereby inhibit the antiviral ability of IFNs (Spiropoulou et al., 2007; Stoltz et al., 2007). Other ways that hantaviruses inhibit induction of type I IFNs is by inhibition of RIG-I (Matthys et al., 2014). It has also been shown that hantavirus N protein and glycoproteins antagonize type I IFNs (Alff et al., 2006; Alff et al., 2008; Levine et al., 2010; Matthys et al., 2014). Very recently, it has been suggested that PUUV-infection cause IFN type I-induced STAT1-dependent expression of tissue plasminogen activator (Strandin et al., 2015). Pretreatment of cells with IFN-λ alone have antiviral effect on HTNV replication (Stoltz et al., 2007). Later it was also demonstrated that HTNV can cause type I IFN independent induction of IFN-λ (Stoltz and Klingström, 2010), and that HCPS causing hantaviruses induce high levels of IFN-λ secretion (Prescott et al., 2010). Altogether, hantaviruses induce type III IFN production and have mechanisms to inhibit the induction of type I IFNs and the antiviral effect of IFNs.

Dendritic cells

DCs were discovered 1973 by Steinman (Steinman and Cohn, 1973) and this finding was awarded the Nobel Prize 2011. DCs are professional antigen presenting cells that are considered to be the initiator and modulator of the adaptive immune response and they

bridges the innate immune system with the adaptive immune system (Banchereau and Steinman, 1999).There are different sets of DCs, classical DCs (cDCs), plasmacytoid DCs (pDCs), Langerhans cells (LCs), and monocyte-derived DCs (moDCs) (Murphy et al., 2015). DCs can experimentally be infected by both HFRS and HCPS causing hantaviruses, and a result of this is that proinflammatory cytokines, TNF and IFN-α, are released (Raftery et al., 2002; Markotic et al., 2007; Marsac et al., 2011). Further, DCs infected with hantavirus might stimulate T cells (Raftery et al., 2002).

Natural killer cells

NK cells play important roles in early host responses against virus-infection. NK cells can be activated by various cytokines, such as IFN-α, IFN-β, interleukin-2 (IL-2), IL-12, IL-15 and IL-18 (Jost and Altfelt, 2013). The antiviral effect of NK cells are to directly destroy target infected-cells or produce antiviral, immune stimulatory and proinflammatory cytokines, such as IFN-γ and TNF, which might act on the virus-infected cells and surrounding cells (Jost and Altfelt, 2013). The way NK cells recognizes infected cells is by a manner referred to as the "missing self-recognition". NK cells upon detecting cells that lack, or have reduced, major histocompatibility complex class I, also known as human leukocyte antigen (HLA) class I, on their cell surface, kill the target infected-cell (Kärre et al., 1986; Ljunggren et al., 1990). NK cells uses different ways to kill virus-infected cells, either through cytotoxic granule-mediated apoptosis or via death receptor-mediated apoptosis (Shresta et al.; 1998; Zamai et al., 1998; Sato et al., 2001; Lieberman 2003; Chowdhury and Lieberman, 2008). Cytotoxic granule contents, such as granzyme B and perforin, are delivered into the target cell through the immunological synapse formed between the NK cell and the target infected cell. NK cells may also express death ligands on its surface and by binding of death ligand to death receptor, expressed on the target cell, kill the target cell.

NK cells can be divided into two subgroups, designated $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells. $CD56^{bright}$ NK cells mainly have immunomodulatory effects, specifically by producing and releasing IFN-γ and TNF, whereas $CD56^{dim}$ NK cells mainly are involved in cellular cytotoxicity. However in specific conditions, both of the NK subgroups can produce IFN-γ and TNF, and be cytotoxic (Cooper et al., 2001; Lanier 2008; Björkström et al., 2010; Fauriat et al., 2010; Stegmann et al., 2010).

In order to recognize and react towards target cells, NK cells express different activating and inhibitory receptors. The function of a NK cell is determined by the balance between stimulated activating and inhibitory receptors (Lanier 2003; Bryceson et al., 2006; Lanier 2008; Bryceson and Long 2008).

The role of NK cells during hantavirus-infection is not well known. Increased levels of NK cells during hantavirus-infection has been reported (Linderholm et al., 1993; Björkström et al., 2011). In the article by Björkström and colleagues in 2011, it was also shown that NK

cells are rapidly elevated during HFRS, and strikingly this NK cell elevation persisted up to 60 days after disease onset. Analysis of HCPS patients also showed elevated levels of cytokines that promotes tissue migration of NK cells (Morzunov et al., 2015). *In vitro* NK cells in contact with hantavirus-infected cells get activated, and these activated NK cells might be able to kill uninfected cells (**paper III**).

Cytotoxic T lymphocytes

CTLs, $CD8^+$ T cells, are an important part of the adaptive immune system during virus infection, as they are equipped with various means to eradicate infected cells. Just as NK cells, CTLs also uses granzyme B-mediated apoptosis or death receptor mediated apoptosis to eradicated virus-infected cell (Jeremias et al., 1998; Shresta et al., 1998; Kayagaki et al., 1999; Lieberman 2003; Chowdhury and Lieberman, 2008). Antigens, for example viral peptides, presented by HLA class I on the surface of the antigen presenting cell are recognized by CTLs. This recognition activates CTLs, with the aid of T helper cells. Proliferation of CTLs is stimulated by IL-2 (Chaplin, 2010).

Studies have reported that both HFRS and HCPS patients display increased levels of $CD8^+$ T cells (Linderholm et al., 1993; Kilpatrick et al., 2004; Lindgren et al., 2011; Xie et al., 2013). Several CTL epitopes, for hantaviruses have been identified and they seem to be restricted mainly to the N protein (Asada et al., 1988; Van Epps et al., 1999; Van Epps et al., 2002; Wang et al., 2011). CTLs have been suggested to increase vascular permeability by killing hantavirus-infected cells (Terajima et al., 2007; Schönrich et al., 2008). CTLs are also capable of lysing cells expressing hantavirus N protein, in these studies viral vectors was used instead of hantavirus-infection (Van Epps et al., 1999; Van Epps et al., 2002; Safronetz et al., 2009). Hantavirus-infections protects cells from cytotoxic granule-mediated and TRAIL-induced apoptosis, indicating that hantavirus-infected cells should be protected from CTLs (**paper I** and **II**).

PROGRAMMED CELL DEATH

There are various type of cell deaths. Classically cell death used to be divided into necrosis or programmed cell death (PCD). The main difference between PCD and necrosis is that PCD is controlled and do not cause any adverse effect on the surrounding (D'Amours et al., 2001) While necrosis is uncontrolled and leads to tissue inflammation (Kelly et al., 2001). However, recently some forms of PCD have been described to cause inflammation (Kroemer et al., 2009). These two types of cell death have also recently been suggested to be renamed to accidental cell death (ACD) and regulated cell death (RCD), by the Nomenclature Committee on Cell Death (Galluzzi et al., 2015). The Nomenclature Committee on Cell Death also suggest the use of the term PCD only during tissue/organism development or for tissue homeostasis. There are many types of RCDs, some examples are apoptosis, necroptosis, autophagy, and pyroptosis (Kroemer et al., 2009). Necroptosis and pyroptosis are RCDs which in contrast to apoptosis and autophagy induce inflammation (Kroemer et al., 2009). Thus both ACD and RCD can induce inflammation (Galluzi et al., 2012; Galluzi et al., 2015). If cell death can be prevented therapeutically, then it is considered to be RCD, but if it is impossible to hinder the cell death then it counts as ACD (Lettre and Hengartner, 2006; Taylor et al., 2008; Fuchs and Steller, 2011; Delbridge et al., 2012). In this thesis I will use the term PCD instead of RCD, since the term RCD is not commonly used yet.

Among the different type of PCDs, apoptosis is the one that is most studied and various studies have been performed on the subject of hantaviruses and apoptosis (Kang et al., 1999; Markotic et al., 2003; Li et al., 2004; Li et al., 2005; Hardestam et al., 2005; Ontiveros et al., 2010; Khaiboullina et al., 2013; **paper I** and **II**).

Apoptosis

The term apoptosis was created in 1972 in order to describe detailed morphological feature of cell death (Kerr et al., 1972). These morphological aspects are rounding up of the cell, retraction of the pseudopodia, reduced cellular volume, nuclear fragmentation, plasma membrane blebbing, and *in vivo* also engulfment by phagocytes. Biochemically, apoptosis is defined as caspase-dependent cell death (Taylor et al., 2008; Galluzi et al., 2012). Two types of apoptosis can be initiated by either intracellular or extracellular stimuli, designated intrinsic apoptosis or extrinsic apoptosis respectively (Galluzi et al., 2015). One important feature of apoptosis is that it is energy dependent (D'Amours et al., 2001), as is the other forms of PCDs (Galluzi et al., 2012).

Intrinsic apoptosis

Intrinsic apoptosis can be triggered by many things, for example UV-irradiation, starvation, cellular stress etc. These triggers cause release of cytochrome C due to mitochondrial outer membrane permeability (MOMP). MOMP is essential for intrinsic apoptosis. B-cell leukemia/lymphoma-2 (Bcl-2) family of proteins are important players in mitochondrial pathway of apoptosis, and they consist of both anti-apoptotic, such as Bcl-XL, Bcl-w, and pro-apoptotic proteins, such as Bax, Bak, Bh3-only family (Cory and Adams, 2002; Wang and Youle, 2009). Upon stress signal BH3 interacting-domain death agonist (Bid) is cleaved to truncated-Bid (tBid), tBid activates Bax and Bak via oligomerization causing MOMP and this leads to the release of cytochrome C. Bcl-2 blocks Bax, thus hindering cytochrome C release, and Bcl-2 can be inhibited by BH3 domain-containing proteins (Cory and Adams, 2002). During MOMP not only cytochrome C is released but also second mitochondria-derived activator of caspase, which can inhibit inhibitor of apoptosis protein. The release of cytochrome C leads to the formation of apoptosome by recruitment of apoptotic protease activating factor 1 (APAF-1) and caspase 9, then subsequent activation of caspase 9, which activates caspase 3 (Liu et al., 1996; Yang et al., 1997; Zou et al., 1997; Li et al., 1997; Ow et al., 2008). Hantaviruses can inhibit chemically-induced intrinsic apoptosis (**paper I**).

Extrinsic apoptosis

The term extrinsic apoptosis is used to define apoptosis caused by extracellular stimuli, such as binding of death inducing ligands, including Fas, TNF, TNF-related apoptosis inducing ligand (TRAIL), to their cognate death receptors (DR), such as Fas-receptor, TNF-receptor 1, and death receptor 4 and 5 (DR4, DR5). Extrinsic apoptosis relies on activation of caspase 8 and caspase 3 or the MOMP pathway (Scaffidi et al., 1998; Suliman et al., 2001; Galluzzi et al., 2012; Galluzzi et al., 2015). TRAIL-induced extrinsic apoptosis is inhibited by hantavirus-infection (**paper II**).

Caspases

Caspases are cysteine proteases that can cleave their target proteins after aspartic acid residues (Riedl and Shi, 2004). All caspases in a cell are zymogens that needs proteolytic processing and activation in order to be functional. There are different type of caspases, initiator caspases, execution caspases, and inflammatory caspases (Cohen 1997). Caspases contain death effector domain, caspase recruitment-domain, a large and a small catalytic subunit (Riedl and Shi, 2004; Lavrik et al., 2005). The most important executioner is caspase 3, and caspase 3 is activated by the initiator caspases 8, 9 and 10. Caspase 3 cleaves inhibitors of caspase activated DNase, thus releasing caspase activated DNase, leading to DNA degradation and chromatin condensation (Sakahira et al., 1998). There are certain viruses, for

example Junin virus, that inhibits caspase 3 activity (Wolff et al., 2013). ANDV N protein is also a caspase 3 inhibitor (**paper I**).

Viruses and apoptosis

Virus-infection may also cause intrinsic induction of PCD. One such PCD, apoptosis, during virus-infection has been well studied. Some examples are; adenovirus that uses several mechanism to induce apoptosis (Rao et al., 1992; Marcellus et al., 1996; Marcellus et al., 1998; Lavoie et al., 1998; Shtrichman and Kleinberger, 1998, Hart et al., 2007), Parvovirus B19 causes DNA strand breaks (Op De Beeck and Caillet-Fauquet, 1997), human immunodeficiency virus-1 induces apoptosis in various ways (Banda et al., 1992; Westendorp et al., 1995; Shi et al., 1996a; Moutouh et al., 1998; Hesselgesser et al., 1998; Sasaki et al., 2002), respiratory syncytial virus cause apoptosis via induction of IFNs (Wang et al., 1998), the arenavirus Tacaribe virus induces apoptosis in a yet unknown way (Wolff et al., 2016), and there are countless other viruses that causes apoptosis (Roulston et al., 1999; Galluzzi et al., 2008). On the other hand, viruses also have evolved strategies to either avoid apoptosis or even inhibit apoptosis. As there are numerous ways for viruses to cause apoptosis there are also various ways used by viruses to evade apoptosis (Galluzzi et al., 2008). To mention some; cowpox virus and vaccinia virus inhibits caspases (Tewari et al., 1995; Dobbelstein and Shenk, 1996), adenovirus inhibits apoptosis using different mechanisms (Moore et al., 1996; Teodoro and Brenton, 1997; Nevels et al., 1999), and there are of course many other apoptotic pathways blocked by several other viruses (Roulston et al., 1999, Galluzzi et al., 2008).

The studies regarding hantaviruses and apoptosis have been conflicting. Some studies show that hantaviruses might induce apoptosis (Kang et al., 1999; Markotic et al., 2003; Li et al., 2004; Li et al., 2005), but later it was shown that hantaviruses normally do not cause apoptosis (Hardestam et al., 2005). It has also been demonstrated that hantaviruses inhibit apoptosis (Ontiveros et al., 2010; Khaiboullina et al., 2013; **paper I** and **II**).

Early studies indicated that HTNV and Prospect Hill virus (PHV) cause apoptosis in cultured African green monkey kidney epithelial (Vero E6) cells (Kang et al., 1999). Studies also showed that HTNV-infection of human embryonic kidney (HEK293) cells render neighboring bystander cells prone to apoptosis (Markotic et al., 2003). These differences might be due to use of different cell-lines for these studies. Li and colleagues showed Tula virus (TULV) caused apoptosis in Vero E6 cells, and this induction of apoptosis was attributed to ER stress, and subsequent activation of caspase 8 and caspase 3 (Li et al., 2004; Li et al., 2005). Here, the question rises, whether if this apoptosis is specific for TULV or could this be due to the cell-line used in these studies. TULV is considered to be a non-pathogenic hantavirus. Could non-pathogenic hantaviruses could cause apoptosis *in vitro*? There has been reports that TULV cause illness in an immunocompromised patient (Zelená et al., 2013), and more interestingly, a very recent report showed that TULV-infection led to

hospitalization of a patient without any former medical conditions (Reynes et al., 2015). Thus, maybe TULV might be considered a pathogenic hantavirus, which should mean that apoptosis caused by hantaviruses may not be attributed only to the non-pathogenic hantaviruses. HFRS causing hantaviruses, such as HTNV, DOBV, and PUUV does not induce apoptosis in confluent Vero E6 or adenocarcinomic human alveolar basal epithelial cells (Hardestam et al., 2005).

Lately, it has also been revealed that hantaviruses harbor specific strategies to block apoptosis, for example, hantavirus-infection might interfere with DAXX mediated apoptosis with the aid of the N protein (Khaiboullina et al., 2013). Both HFRS and HCPS causing hantaviruses are able to inhibit staurosporine (STS is a broad kinase-inhibitor that induces apoptosis (Karaman et al 2008; Manns et al., 2011)) mediated apoptosis (**paper I**).

Granzyme B-mediated cell death

Granzyme B is a serine protease and is unique amongst the granzymes, since it is the only granzyme that cleaves its target protein after aspartic acid residue. The delivery of granzyme B into a target cell is mediated by perforin in a manner not fully understood (Caputo et al., 1994; Lord et al., 2003; Trapani and Sutton, 2003; Chowdhury and Lieberman, 2008). Granzyme B induces apoptosis through directly cleaving and thereby activating caspase 3. *In vitro*, several caspases have been shown to be cleaved by granzyme B, this includes caspase 1, 2, 3, 6, 7, 8, 9 and 10, but *in vivo* only caspase 3 and 8 are substrates for granzyme B (Darmon et al., 1995; Darmon et al., 1996; Martin et al., 1996; Shi et al., 1996b; Orth et al., 1996; Chinnaiyan et al., 1996; Duan et al., 1996; Medema et al., 1997; Talanian et al., 1997; Yang et al., 1998; Atkinson et al., 1998; Metkar et al., 2003). It has been suggested that caspase 3 activation by granzyme B involves both direct activation of caspase 3 and liberation from caspase inhibition via the release of inhibitor of apoptosis protein from the mitochondria (Sutton et al., 1997; Goping et al., 2003). Granzyme B also cleaves the proapoptotic protein Bid into t-Bid directly or via caspase 8. The t-Bid is translocated to mitochondria, and activates Bax and Bak, leading to cytochrome C release and subsequent induction of apoptosis (Figure 3) (Martinou et al., 1998; Heibein et al., 1999; Sutton et al., 2000; Alimonti et al., 2001; Zamzami and Kroemer, 2003; Andrade et al., 2004). Caspase independent granzyme B mediated cytochrome C release has been proposed as a non-apoptotic way of cell death (Figure 3) (Heibein et al., 1999).

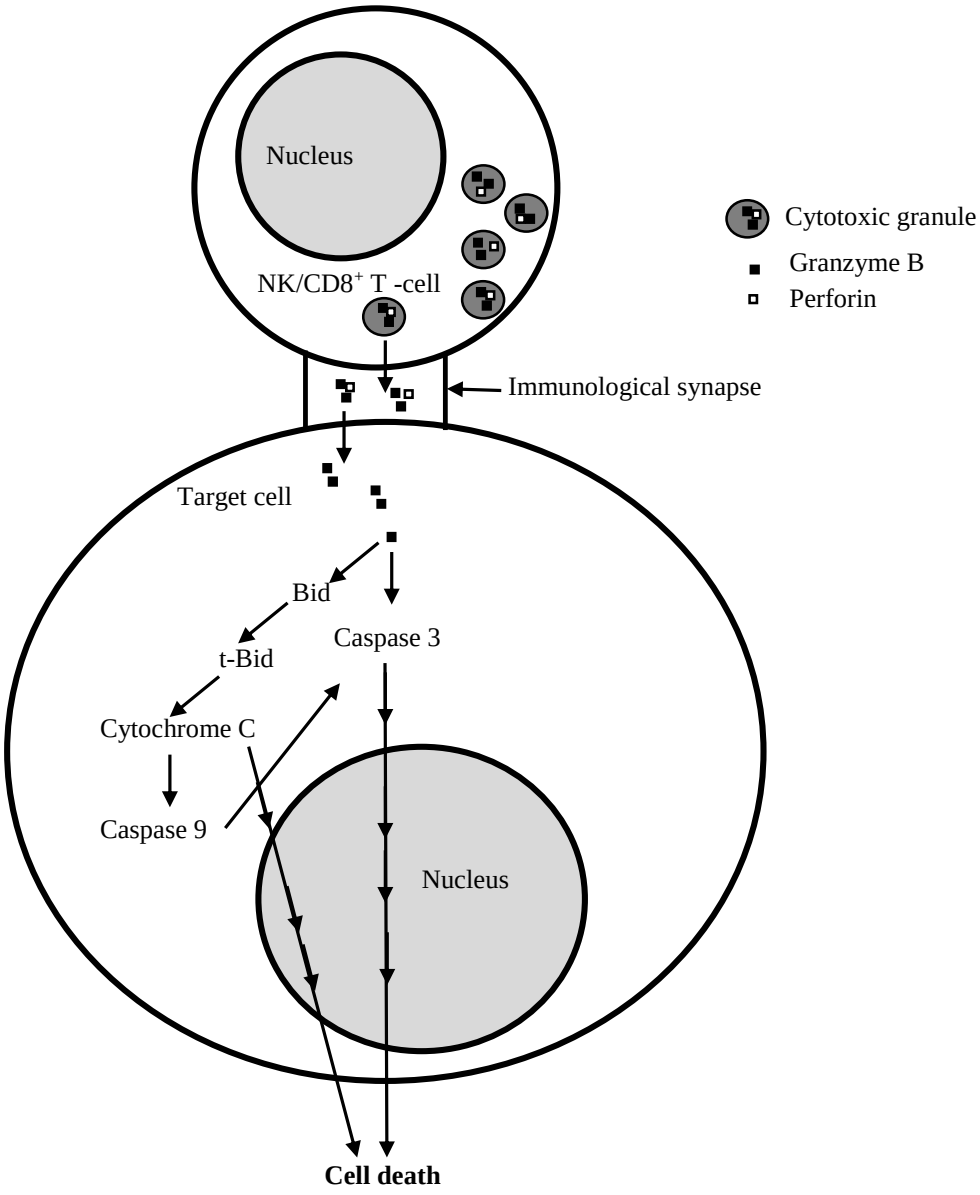

Figure 3. Illustration of how granzyme b cause cell death.

Many inhibitors of granzyme B have been described (Chowdhury and Lieberman, 2008), but for viruses only three inhibitors are known, adenovirus L4-100K assembly protein, cowpox virus cytokine response modifier A protein (CrmA) (Tewari et al., 1995; Quan et al., 1995; Andrade et al., 2001), and hantavirus N protein (**paper I**).

TRAIL-induced cell death

TRAIL belongs to the TNF superfamily and it induces cell death (summarized in figure 4) via binding to its cognate receptors, DR4 and/or DR5, both of which contain cytoplasmic death domains (Schneider et al., 1997a; Schneider et al., 1997b; Falschlehner et al., 2007; Falschlehner et al., 2009; Collison et al., 2009). When TRAIL binds to DR4 or DR5,

recruitment of Fas-associated death domain protein (FADD) and pro-caspase 8 leads to the formation of death inducing signaling complex (DISC) (Bodmer et al., 2000). Subsequently, caspase 3 is directly activated by caspase 8 or indirectly through the MOMP pathway leading to cell death (Scaffidi et al., 1998; Suliman et al., 2001). It has been suggested that TRAIL might use the MOMP pathway independent of caspase 8 to induce cell death (Petak et al., 2003). Recently it was suggested that TRAIL binding to DR5 on the cell surface is not necessary to induce apoptosis. Increased amount of DR5 in the cytosol during ER stress due to unfolded protein response may cause apoptosis without the aid of TRAIL (Lu et al., 2014). Other receptors that TRAIL binds to are the decoy receptors DcR1, which lacks a cytoplasmic domain, and DcR2, which has a truncated cytoplasmic death domain. TRAIL binding to these receptors does not induce apoptosis (Zauli and Secchiero, 2006).

TRAIL has recently emerged as an important player for inducing apoptosis in virus-infected cells (Clarke et al., 2000; Kotelkin et al., 2003). For instance, CMV-infection of fibroblasts and colonic epithelial cells induces expression of TRAIL, DR4 and DR5, rendering the infected cells sensitive to TRAIL mediated apoptosis (Sedger et al., 1999; Sträter et al., 2002). Consequently, there are also viruses that have evolved strategies to evade TRAIL-induced apoptosis, for example adenovirus downregulates DR4 and DR5 and hepatitis B virus blocks DR5 expression (Benedict et al., 2001; Du et al., 2009). In addition, hantavirus-infected cells are protected from TRAIL-induced apoptosis through down-regulation of cell surface DR5 (**paper II**).

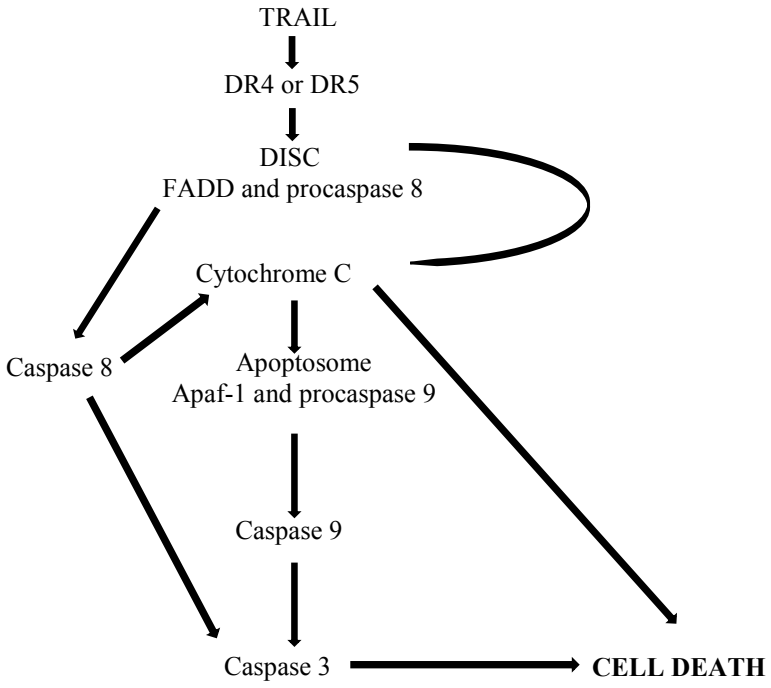

Figure 4. Schematic picture of TRAIL mediated cell death.

DR4 and DR5 can be translocated to the nucleus, thus protecting cancer cells from TRAIL-induced cell death (Leithner et al., 2009; Chen et al., 2012). Hantavirus-infection of endothelial cells leads to nuclear localization of DR5 (**paper II**). In some cells, protein mislocalisation can alter the known function of the protein. One such example for DR5 was shown recently, as nuclear localization of DR5 may promote proliferation of tumor cells by inhibition of Drosha-mediated maturation of let-7 microRNA precursor (Haselmann et al., 2014). Inhibition of TRAIL and DR4/DR5 pathway can be important for cancer development during virus-infection with hepatitis B virus, as it has been suggested that hepatitis B virus can cause hepatocellular carcinoma via blocking TRAIL through downregulation of the death receptors DR4 and/or DR5 (Yano et al., 2003). Another oncogenic virus, human papilloma virus uses its E5 protein to block TRAIL-mediated DISC formation (Kabsch and Alonso, 2002). Resistance to TRAIL mediated killing has also been demonstrated for Epstein Barr virus (Kawanishi et al., 2002), presenting an additional oncogenic virus capable of inhibiting TRAIL-induced killing of infected cells.

HANTAVIRUS PATHOGENESIS

Pathogenesis caused by hantaviruses have been studied frequently but we are still far from explaining the cause of HFRS or HCPS. Hantavirus pathogenesis is mostly considered to be immune-mediated but direct effects of the virus-infection may also contribute to pathogenesis. One important aspect of hantavirus related pathogenesis might be their relationship to cell death. One of the greatest challenges to explain hantavirus pathogenesis is due to the lack of good *in vivo* models. The best model so far that recapitulates that which is observed in humans is the macaque, nonhuman primate, model (Yanagihara et al., 1988; Groen et al., 1995; Klingström et al., 2002; McElroy et al., 2002; Klingström et al., 2005; Sironen et al., 2008; Klingström et al., 2008; Safronetz et al., 2014). Another model used, is the Syrian hamster model (Hooper et al., 2001, Safronetz et al., 2011; Safronetz et al., 2012; Brocato et al., 2014). Most of the studies done to explore hantavirus pathogenesis are *in vitro* experiments. In many cases clinical samples from hantavirus-infected patients have been analyzed and associations to pathogenesis have been suggested.

The release of cytokines in the vascular system likely plays an important role during hantavirus-infection. Numerous studies have been done in patient samples to evaluate cytokine levels during HFRS and HCPS (Borges et al., 2008; Saksida et al., 2011; Li et al., 2012; Kyriakidis and Papa 2013; Morzunov et al., 2015; Bondu et al., 2015; Baigildina et al., 2015). The results in these studies vary but the major coherent finding is that there are changes in cytokine levels during HFRS and HCPS. Thus, this imbalance of cytokine levels can be considered to be a part of the pathogenesis. Elevated levels of IL-2, IL-6, IL-8 and TNF have been recorded in acute PUUV-infection (Sadhegi et al., 2011), and elevated levels of IL-6 has been suggested as a marker for disease severity during PUUV-infection (Outinen et al., 2010). Very recently it was showed, using an *in vitro* 3-dimensional air-exposed organotypic human lung tissue model, that ANDV-infection cause a late and prolonged effect on IL-6, IL-8, INF-γ-induced protein-10, and vascular endothelial growth factor A (VEGF-A) responses (Sundström et al., 2016). In fatal case of HCPS, high levels of proinflammatory cytokine producing immune cells have been found (Mori et al., 1999).

VEGF has been implicated to have important roles in hantavirus pathogenesis. VEGF causes vascular permeabilization through degrading the adherens junction protein vascular endothelial-cadherin (Vestweber, 2007). Increased levels of VEGF have been observed in HFRS and HCPS patients, and *in vitro* studies show that hantavirus-infection of endothelial cells renders the endothelial cells sensitive to permeabilization by VEGF (Gavrilovskaya et al., 2008; Shrivastava-Ranjan et al., 2010; Ma et al., 2012; Li et al., 2012; Tsergouli and Papa, 2013). It was also suggested that high levels of VEGF during convalescence phase of HFRS may contribute to renal recovery in patients (Ma et al., 2012). In the ANDV-infected 3D air-exposed organotypic human lung tissue model, no obvious adverse effects on the model were observed, even though prolonged elevated levels of extracellular VEGF-A was

detected (Sundström et al., 2016). In a recent study, using an *in vitro* capillary blood vessel-like system, it was suggested that in contrast to monolayer cells, vascular endothelial-cadherin is not degraded during hantavirus-infection (Taylor et al., 2013). Further, in the same study it was shown that activation of kallikrein-kinin system and bradykinin, causing increased permeabilization of the capillary blood vessel-like model, during hantavirus-infection could have an impact on pathogenesis. This permeabilization was decreased by inhibition of kallikrein-kinin system and bradykinin (Taylor et al., 2013). Severe cases of PUUV-infection have been successfully treated with bradykinin receptor antagonist (Antonen et al., 2013; Vaheri et al., 2014). However, it is difficult to yet draw any clear conclusion about these successful treatments since PUUV-infection compared to other hantavirus-infection is consider to be mild, due to its low case fatality rate.

Hantavirus might directly cause increased cellular permeability, by increased expression of the cellular microRNA miR-126 and the miR-126 associated proteins sprouty-related EVH1 domain containing protein 1 (SPRED1) and phosphoinositide-3-kinase, regulatory subunit 2 during ANDV-infection (Pepini et al., 2010). Knocking down SPRED1 in ANDV-infected cells decreased cellular permeability (Pepini et al., 2010). In HTNV- and PHV-infected macrophages and endothelial cells, subsets of microRNA were expressed differently. These microRNAs were associated with inflammatory and innate immune signaling pathways (Shin et al., 2013).

Cells expressing hantavirus N protein, introduced with viral vectors rather than hantavirus-infection, can be killed by CTLs (Van Epps et al., 1999; Van Epps et al., 2002; Safronetz et al., 2009), and it has been suggested that CTL may cause vascular permeability by killing infected cells (Terajima et al., 2007; Schönrich et al., 2008). Corroborating this, strong T cell responses have been reported during hantavirus-infection in patients (Linderholm et al., 1993; Kilpatrick et al., 2004; Lindgren et al., 2011; Xie et al., 2013). In contrast, autopsies from patients does not seem to display any obvious demise of infected cells (Duchin et al., 1994; Zaki et al., 1995; Nolte et al., 1995). An explanation for this dichotomy could be that hantavirus-infection protects cells from cytotoxic lymphocyte mediated apoptosis (**paper I**). Additionally, depletion of T cells in a Syrian golden hamster HCPS-model did not have any effect on the disease outcome (Hammerbeck and Hooper, 2011; Prescott et al., 2013), indicating that T cells may not be involved in hantavirus pathogenesis.

Long-term consequences of hantavirus-infection have been demonstrated. For instance, following HFRS the risk for lymphoma is increased (Klingström et al., 2014). Cardiovascular causes of death in HFRS patients early after recovery from HFRS, have been reported (Connolly-Andersen et al., 2013). Long-term hormonal deficiencies with outcome such as chronic subclinical testicular failure have been reported (Mäkelä et al., 2010). It can be speculated that hantavirus-pathogenesis might be extended long after recovery from HFRS, and with consequences that are virtually impossible to predict.

2 AIM

Different pathogenesis models for hantavirus-infection have been proposed. One of the potential mechanism might be that hantavirus-infected endothelial cells are killed by cytotoxic lymphocytes, thus causing vascular leakage. This proposal is conflicting with several published work, and not clearly evaluated. The general aim for this thesis is to provide a better understanding of how hantaviruses might cause pathogenesis. This was done by studying if hantavirus-infection protects cells from apoptosis and if hantaviruses can change normal cytotoxic lymphocyte functions.

3 RESULTS AND DISCUSSION

Inhibition of chemically-induced apoptosis

Previous studies have shown that hantaviruses do not cause apoptosis *in vitro* (Hardestam et al., 2005), although, they might cause apoptosis in bystander cells (Markotic et al., 2003). If seen by a virus point of view, inhibition or at least delay of apoptosis can be crucial, since the virus needs a host cell for its propagation. Therefore, investigation was made to see if hantaviruses can inhibit apoptosis. To study this, hantavirus-infected cells and uninfected cells were treated with the kinase inhibitor STS. Apoptosis was observed in STS-treated uninfected cells, in contrast, hantavirus-infection protected cells from STS-induced apoptosis (Figure 5). Caspase 3-activity was studied, and in comparison to uninfected cells, hantavirus-infected cells had lower levels of caspase 3 activity post STS-treatment (**paper I**).

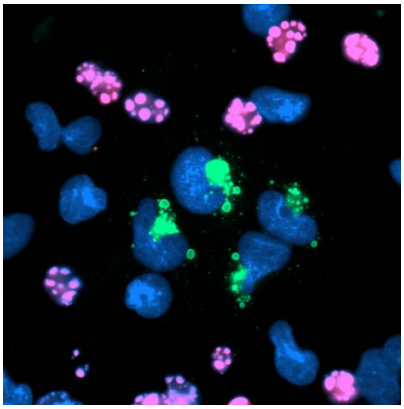

Figure 5. Epithelial cells infected with hantavirus (green) treated with STS and TUNEL (red) was measured. Nuclei were counterstained with DAPI (blue).

Further, the direct effect on caspase 3 by hantaviruses was explored. As the hantavirus N protein is multifunctional (Ye et al., 2001; Li et al., 2002; Mir and Panganiban 2008; Taylor et al., 2009a; Ontiveros et al., 2010), we proposed that the N protein might also have functions that somehow affected caspase 3. *In silico* studies revealed putative caspase 3 cleavage sites in hantavirus N protein, and interaction between the N protein and caspase 3 was established by showing that caspase 3 could cleave the N protein. Moreover, ANDV N protein in infected cells was cleaved during STS-treatment and this cleavage was inhibited by addition of the pan caspase inhibitor Z-VAD prior to STS-treatment. Importantly, we showed that recombinant N protein inhibits caspase 3 activity. Caspase 3 is a cysteine protease that cleave proteins after aspartic acid, and the canonical cleavage site is DEVD (aspartic acid-glutamic acid-valine-aspartic acid). $DLID_{285}$ (aspartic acid-leucine-isoleucine-aspartic acid) was identified as the caspase 3 site in ANDV N protein. The inhibition of STS-mediated apoptosis could also be seen in wild type ANDV N protein transfected cells. However, if a

mutation was introduced in the caspase 3 site, from $DLID_{285}$ to $DLIA_{285}$ (aspartic acid-leucine-isoleucine-aspartic acid), the apoptosis-resistance was reverted (**paper I**). Other studies also show that hantavirus N protein is important for modulating apoptosis, certain deletions in HTNV N protein render transfected cells prone to apoptosis (Ontiveros et al., 2010).

In previous studies, attempts to induce apoptosis in hantavirus-infected cells to investigate the effect of this on the virus-infected cell were not reported. Direct effect on apoptosis induction during virus-infection was studied, where it was believed that hantaviruses caused apoptosis (Kang et al., 1999; Li et al 2004; Li et al., 2005). It has also been suggested that hantavirus might cause apoptosis in bystander cells (Markotic et al., 2003). Thus, by inducing apoptosis with STS, we could study if hantavirus infected cells are susceptible or resistance to apoptosis. Hantavirus-infection of both primary cells and cell lines protects them from chemically induced apoptosis. Taken together, it could be proposed that hantavirus N protein might act as a decoy that inhibits caspase 3-dependent apoptosis (**paper I**). In this aspect, it has been shown that Junin virus, another hemorrhagic fever causing virus, uses its nucleoprotein as a decoy to inhibit the induction of apoptosis during infection (Wolff et al., 2013).

Inhibition of cytotoxic granule-mediated apoptosis

In an effort to place this inhibition of apoptosis in a biological context, studies were performed to determine if cytotoxic lymphocytes may induce apoptosis in hantavirus-infected cells. NK cells, isolated from healthy donors, were used as a model for cytotoxic lymphocytes and infected primary human endothelial cells were used as the target cells. NK cells were activated with IL-2, and HLA class I on the uninfected and hantavirus-infected target cells was blocked. Activated NK cells were co-incubated with HLA class I-blocked target cells and degranulation of the cytotoxic granula was measured by studying CD107 surface expression on the NK cells. Similar degranulation towards both hantavirus-infected and uninfected cells was seen in when HLA class I was blocked on the target cells. These NK cells were able to kill uninfected cells by inducing apoptosis in them, but hantavirus-infected cells on the other hand were not killed by the NK cells, showing that hantaviruses protect infected cells from induction of apoptosis by NK cells. In line with this findings, it was also shown that NK cells failed to induce caspase 3 activity in hantavirus-infected endothelial cells (**paper I**).

Inhibition of caspase 3 activity might explain how hantaviruses inhibit STS-mediated apoptosis but it does not entirely account for inhibition of cytotoxic granule-mediated cell death. Granzyme B, a key player during cytotoxic granule dependent caspase 3-mediated apoptosis, is also an inducer of cell death independently of caspase 3 activation (Heibein et al., 1999), indicating that blocking granzyme B activity could be important for hantaviruses. For example, adenovirus L4-100K assembly protein and CrmA potently and directly inhibit

granzyme B (Tewari et al., 1995; Quan et al., 1995; Andrade et al., 2001). As hantavirus N protein inhibits caspase 3 activity, could it also harbor a way to inhibit granzyme B activity? *In silico* experiments revealed putative granzyme B sites in the N protein (our unpublished data). Interaction between hantavirus N protein and granzyme B was established, as granzyme B could cleave the hantavirus N protein at several sites. ANDV N protein strongly inhibited granzyme B activity and this inhibitory effect lasted for a long time (**paper I**). Granzyme B can be translocated to the nucleus, possibly with the aid of importin, where it may cause cell death (Trapani et al., 1996; Jans et al., 1996, Blink et al., 2005). Hantavirus N protein interact with importin-α and prevents its transport capacity to the nucleus (Taylor et al., 2009a). Taken together, N protein can directly inhibit granzyme B (**paper I**) and maybe have the ability to indirectly limit its transport to the nucleus.

By showing that hantaviruses effectively blocks the function of granzyme B and caspase 3, it can be concluded that the cytotoxic lymphocytes, e.g. NK cells, and $CD8^+$ T cells, that use cytotoxic granule-mediated pathway to induce apoptosis in infected cells, will not be able to eradicate hantavirus-infected cells (**paper I**). A few mechanisms for granzyme B inhibition by viruses have been proposed. For instance, serine proteinase inhibitor (serprin)-mediated granzyme B inhibition, where the serpin irreversibly binds its target. The best known viral serpin is the cowpox virus encoded CrmA (Chowdhury and Lieberman 2008). CrmA acts as serpin for caspases too (Ray et al., 1992). Another mechanism was shown by Andrade and colleagues: adenovirus L4-100K protein efficiently blocks granzyme B activity by acting as a decoy, as it takes very long time for granzyme to cleave this protein (Andrade et al., 2001). Both CrmA and adenovirus L4-100K protein act directly on granzyme B, but other mechanisms have been suggested as well. For example, parainfluenza virus type 3 can inhibit granzyme B in infected T-cells by blocking granzyme B mRNA transcription (Sieg et al., 1995). Hantaviruses, only encode four to five proteins, in contrast to poxvirus and adenovirus which express 20-200 viral proteins. In this regard, it is astonishing that a small virus like hantavirus could also harbor the similar potential as the viruses that expresses many more proteins. Whether hantavirus N protein acts as a serpin or a decoy for granzyme B is currently unknown. It would be interesting to identify all the granzyme B cleavage sites in the hantavirus N protein and explore which site(s) that might be important for granzyme B inhibition. Interestingly, adenovirus L4-100K protein, which is cleaved into several fragments only need one of the cleavage sites to inhibit granzyme B. If one may speculate, it seems likely that hantavirus N protein, abundantly expressed in an infected cell and being cleaved by granzyme B, may perhaps act as a decoy for granzyme B, similarly as I also proposed for N protein mediated caspase 3 inhibition.

In contrast to what is presented in this thesis, hantavirus nucleocapsid expressing target cells have been shown to be killed by T-cells. In these cases the hantavirus protein expression was dependent on viral vectors, rather than hantavirus-infection (Van Epps et al., 1999; Van Epps et al., 2002; Safronetz et al., 2009). So, this raises the question, why are cells expressing hantavirus N protein not protected from T-cell mediated killing? One possibility is that virus replication is needed to block cytotoxic lymphocyte mediated cell death. Cytotoxic

lymphocytes use granzyme H to circumvent adenovirus L4-100K protein mediated granzyme B resistance, via cleaving the virus protein, thus incapacitating the virus protein (Andrade et al., 2007). If this or something similar could be the case also for hantavirus N expressing cells remains to be elucidated. Further explanation could be that cytotoxic lymphocytes use other ways than the cytotoxic granule pathway to kill target cells. However in this case the N protein expressing cells might still be protected, since N protein inhibits caspase 3 activity. Nevertheless, if caspase 3 independent cell death is induced, this might render N expressing cells susceptible to lysis by the CTLs.

Inhibition of a death ligand/receptor pathway

Showing inhibition of cytotoxic granule dependent apoptosis explains that one major pathway used by cytotoxic lymphocytes to induce cell death is blocked by hantaviruses. Another major pathway used by these lymphocytes to kill cells is the death ligand/receptor pathway. One of the main death ligands used to induce apoptosis is TRAIL. To study if TRAIL-induced apoptosis is also inhibited by hantavirus-infection, endothelial cells were infected with HTNV and four days post infection they were treated with TRAIL. TRAIL caused cell death in uninfected cells but as expected hantavirus-infected cells were not killed (**paper II**).

To find out how this inhibition of TRAIL-induced apoptosis occurred different key molecules of the death receptor mediated pathway were studied. It was demonstrated in **paper I** that hantavirus N protein can inhibit caspase 3 activity, indicating that this inhibition might be important for inhibition of TRAIL-induced apoptosis. However, while TRAIL triggered activation of caspase 8 and caspase 3 in uninfected cells it did not in the HTNV-infected cells, therefore it was concluded that inhibition of caspase 3 is not needed for hantavirus-mediated inhibition of TRAIL-induced apoptosis (**paper II**). TRAIL can induce cell death by MOMP pathway via cytochrome C release to the cytoplasm, and without the aid of caspases (Suliman et al 2001). So release of cytochrome C was studied. TRAIL caused cytochrome C release in uninfected, but not in the infected cells, showing that inhibition cytochrome C release is also not necessary for hantavirus-mediated inhibition of TRAIL-induced apoptosis (**paper II**).

Viruses such as hepatitis B virus and adenovirus, deregulate the TRAIL death receptors DR4 and DR5 (Benedict et al., 2001; Du et al., 2009). Hence, we suggested that hantavirus-infection might cause changes in DR4 and DR5 levels in infected cells. DR4 was not detected in any of the endothelial cells, infected or uninfected. Similar levels of full-length DR5 was detected in both HTNV-infected and uninfected cells. Moreover, an additional smaller DR5 band was detected in the infected endothelial cells, which remarkably was not seen in the uninfected cells. Specific cellular localization is important for death receptors in order for them to function normally. In this case, the receptors should be on the cell surface to be able to interact with TRAIL. DR5 expression on the surface of hantavirus-infected cells decreased

over time, whereas levels of DR5 on the uninfected cells remained unchanged. Hence, TRAIL cannot induce cell death in hantavirus-infected cells (**paper II**).

Aberrant localization of death receptor 5

It has been suggested that increased levels of DR5 during ER stress in the cytosol may cause apoptosis without the binding of its cognate ligand TRAIL (Lu et al., 2014). Since the total amount of full-length DR5 is not changed during hantavirus infection, and might be increased as an additional band is formed, should DR5 in that case cause induction of apoptosis in the infected cells? As hantavirus infection does not cause apoptosis *per se*, it was speculated that the virus might cause aberrant localization of DR5. Cell fractionation assay showed that the levels of DR5 in the cytosol was decreased during infection (**paper II**). TRAIL resistance in certain cancer cells have been credited to aberrant localization of DR4 and/or DR5 from the cell surface to the cell nucleus (Leithner et al., 2009; Chen et al., 2012). Thus, the next step was to investigate if this also was the case for hantavirus-infected cells. Indeed, DR5 was detected in the nucleus of hantavirus-infected cells to a higher extent than in uninfected cells (**paper II**). It is remarkable that a virus that causes acute transient infection in humans, harbors such a function which previously has only been demonstrated in cancer cells (Leithner et al., 2009; Chen et al., 2012).

Death receptors might gain other functions due to mislocalisation. For instance, DR5 in the nucleus might cause cell proliferation (Haselmann et al., 2014). Cell proliferation is considered as one of the hallmarks of cancer, together with resistance to cell death and others (Hanahan and Weinberg, 2011). This piques curiosity, since following HFRS the risk for lymphoma increases (Klingström et al., 2014). As hantavirus-infected cells are protected from cell death (**paper I** and **II**), one might ask, is this long term consequence of HFRS directly due to virus-infection or are other factors involved? The notion that a transient infection may be the origin of cancer is very thought provocative. Elucidating this issue in the future could indeed be very interesting. Other viruses known to cause cancer are Epstein Barr virus, human herpes virus 8, human T-cell leukemia virus type 1 and hepatitis C virus, all of these causes either chronic or latent infection (Carrillo-Infante et al., 2007), and this seems to argue against the notion that hantaviruses could possibly be oncogenic. Although, in natural hosts, hantavirus-infection is chronic (Jonson et al., 2010). Could this mean that hantaviruses might be oncogenic? More studies are needed to explain this matter.

NK cells activated by hantaviruses

NK cell levels are elevated during HFRS (Björkström et al., 2011). To explore the NK cell activation in patients during HFRS, 16 Swedish PUUV-infected patients were studied. Peripheral blood monocytes (PBMCs) samples were collected during acute and convalescence phase of HFRS (Figure 6). $CD56^{dim}$ and $CD56^{bright}$ NK cells were evaluated by

analyzing the early activation marker CD69. During acute phase of HFRS $CD56^{dim}$ NK cells exhibited high expression of CD69, which declined over time during the convalescence phase. Expression of CD69 on $CD56^{bright}$ cells was somewhat higher during the acute phase but not to the same extent as on $CD56^{dim}$ cells. Other NK cell activating receptors such as NKG2D, 2B4, and the natural cytotoxicity receptors NKp30 and NKp46 were elevated on the $CD56^{dim}$ cells but not on $CD56^{bright}$ cells while the activating receptors DNAM-1, CD161, and CD16, were not changed. Intracellular levels of granzyme B and perforin were increased in both $CD56^{dim}$ and $CD56^{bright}$ cells (**paper III**).

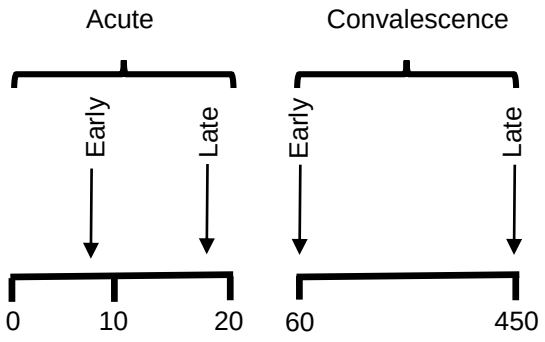

Figure 6. 16 PUUV-infected patients were sampled during acute (days 0-20) and early convalescence (days 60-450) and PBMCs were isolated.

The activation of $CD56^{dim}$ cells was closely studied *in vitro* to explain possible mechanism of how this activation may occur. Resting primary NK cells were isolated from healthy donors and exposed to HTNV to induce activation. These NK cells were not infected by HTNV and nor were they activated after direct exposure to virus particles, thus suggesting that HTNV cannot directly cause activation of $CD56^{dim}$ cells. Next step was to examine if HNTV infected endothelial and epithelial cells could somehow activate NK cells. Isolated primary NK cells were co-incubated with HTNV-infected primary endothelial and epithelial cells. As activation markers, the expression levels of CD69 and CD38 expression on both $CD56^{dim}$ and $CD56^{bright}$ cells were measured. Clear activation of $CD56^{dim}$ cells were seen, as indicated by increase in CD69 and CD38 levels. $CD56^{bright}$ cells also showed activation but to a much lower level than that seen in $CD56^{dim}$ cells. To show if this activation was cell to cell contact dependent or due to some factors in the supernatant, the NK cells were separated from the target cells using a transwell system. NK cells separated by transwells showed no obvious signs of activation, which suggests that the activation is cell to cell contact dependent (**paper III**).

Activation through interleukin-15

Virus-infection can cause changes in IL-15 expression (Fawaz et al., 1999; Zhou et al., 2007; Zdrenghea et al., 2012; Jabri and Abadie 2015), and IL-15 is essential for NK cell activation (Jabri and Abadie 2015). mRNA levels of both IL-15 and IL-15Rα were increased in HTNV-infected endothelial and epithelial cells compared to uninfected cells (**paper III**). The surface levels of IL-15 and IL-15Rα were measured by flow cytometry and as with mRNA levels, levels of IL-15 and IL-15Rα were increased on the virus-infected endothelial and epithelial cells (**paper III**). Additionally, to test the significance of IL-15, anti-IL-15 neutralizing antibodies were used to block IL-15 on HTNV-infected cells surface and then NK cells were co-incubated with the infected cells. Blocking of IL-15 on the infected cells inhibited NK cells activation, as displayed by decreased CD69 expression. Therefore, it was concluded that trans-presentation of IL-15 on the hantavirus-infected cell surface is an important factor for activation of NK cells, at least *in vitro* (**paper III**).

It has been shown that several viruses, both DNA and RNA (positive and negative sense RNA), including Human herpesvirus-6, Human herpesvirus-7, herpes simplex virus, Epstein-Barr virus, respiratory syncitial virus, vesicular stomatitis virus, influenza virus, reovirus, and rotavirus, induce IL-15 (Flamand et al., 1996; Atedzoe et al., 1997; Fawaz et al., 1999; Zhou et al., 2007; Zdrenghea et al., 2012). In this regard, it is not very surprising that hantavirus also cause induction of IL-15. This shows that IL-15 is a common key cytokine expressed during many virus-infections. Supporting the findings from **paper III** are reports that show that both IL-15 is elevated in HFRS patients (Björkström et al., 2011) and also in SNV-infected non-human primates (Safronetz et al., 2014).

An unexpected function of hantavirus activated NK cells

HFRS patients exhibit increased levels of granzyme B, perforin, lactate dehydrogenase, cleaved cytokeratin 18, and cell free DNA (Klingström et al., 2006; Outinen et al., 2012), indicating tissue damage during disease. As shown so far in this thesis and by others, hantaviruses protects cells from programmed cell death (**paper I** and **II**, Ontiveros et al., 2010). How might these tissue damage be explained then? Studies show that cytotoxic lymphocytes are highly activated during human hantavirus-infection (Kilpatrick et al., 2004; Björkström et al., 2011; Lindberg et al., 2011). *In vitro*, NK cells are activated by hantavirus-infected cells via IL-15 and IL-15Rα (**paper III**). Could these NK cells be behind the cause of the observed damage?

As shown above, NK cells are activated by hantavirus-infected cells (**paper III**). To explore if these hantavirus-activated NK cells had gained any functional ability, effector responses against NK cell-sensitive K562 cells were tested. After co-incubation with HTNV-infected cells NK cells showed increased degranulation, IFN-γ-, and TNF-responses towards K562 cells, resulting in increased specific lysis of the K562 cells. The response towards

endothelial cells was also tested. Here, the NK cells were activated with either IL-15 or IFN-α and co-incubated with HTNV-infected or uninfected endothelial cells. The activated NK cells displayed increased degranulation, IFN-γ-, and TNF-responses towards uninfected endothelial cells. Similar but considerably lower responses by activated NK cells towards HTNV-infected cells were seen. This difference was attributed to increased levels of HLA class I on the HTNV-infected cell surface, as blocking of HLA class I in both uninfected and infected cells increased the NK cell responses towards the hantavirus infected cells (**paper III**).

Finally, could $CD56^{dim}$ NK cells activated by HTNV-infected endothelial cells gain additional funtions? To investigate this, NK cells were co-incubated with uninfected cells or HTNV-infected cells. Twenty-four hours post incubation NK cells were transferred to new cells, either infected or uninfected and co-incubated for five hours (Figure 7). Then the NK cells were removed and the target endothelial cells were studied. Despite normal HLA class I levels, uninfected endothelial cells were killed by NK cells that were activated by HTNV-infected cells. The infected cells were protected due to increased levels of HLA class I, and if targeted also through inhibition of granzyme B and TRAIL-mediated cell death. Taken together, this suggests that NK cells are activated by IL-15 on HTNV-infected cells, and this activation might cause NK cells to break the normal HLA class I-mediated self-tolerance and kill uninfected cells (**paper III**). The specific mechanism of how NK cells activated by hantavirus-infected cells can kill uninfected cells has not been explored in this thesis. It would be interesting to see by which means the activated NK cells kill the uninfected endothelial cells.

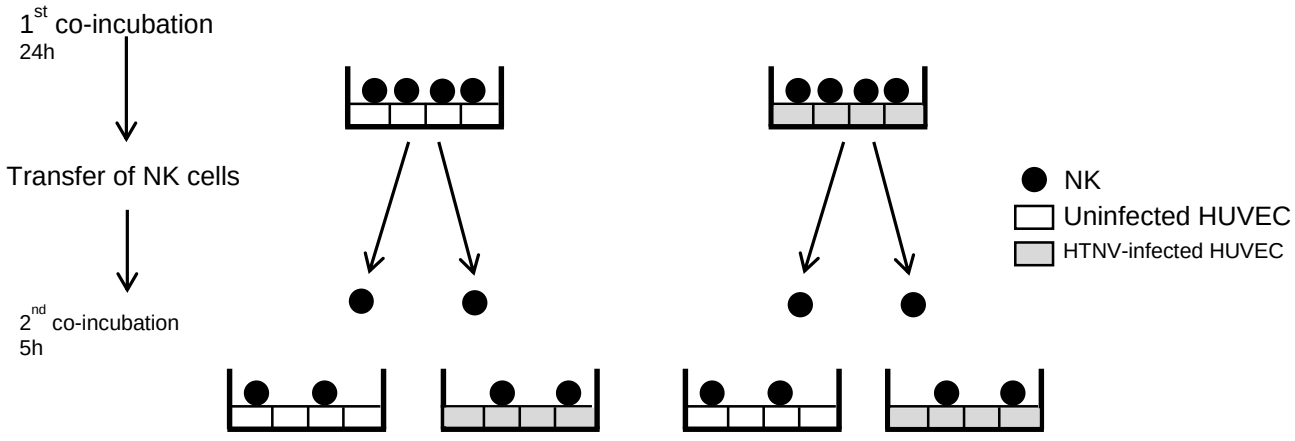

Figure 7. Schematic picture of the experimental setup. NK cells were co-incubated with uninfected or HTNV-infected HUVECs for 24 hours and then transferred to new sets of cells (uninfected or HTNV-infected) and again co-incubated for five hours.

It is not uncommon that virus infection increases NK cell toxicity (Flamand et al., 1996; Atedzoe et al., 1997; Fawaz et al., 1999), and this was also shown for HTNV-infection (**paper III**). Nevertheless, to the best of my knowledge, it has never been shown that activation of NK cells directly or indirectly through virus-infection causes NK cells to kill uninfected cells. Moreover, it was striking that this killing occurred on target uninfected cells despite their normal levels of HLA class I.

Thus, a hypothesis for hantavirus-mediated pathogenesis might be proposed:

NK cells activated by hantavirus-infected cells might kill bystander uninfected cells which might partly explain the vascular leakage seen during HFRS and HCPS.

Altogether, the three papers presented in this thesis show different ways that hantaviruses use to inhibit the killing effect of cytotoxic lymphocytes. Further, hantavirus-infected cells activates NK cells and cause NK cells to target and destroy uninfected cells.

CONCLUSIONS

The studies presented in this thesis shows that hantavirus-infected cells are protected from cytotoxic lymphocyte mediated programmed cell death (**paper I** and **II**), and that NK cells are activated by hantavirus-induced IL-15/IL-15Rα expression (**paper III**).

In detail, this thesis demonstrate that hantavirus-infected cells are protected from cytotoxic granule mediated pathway. This was attributed to virus N protein mediated inhibition of granzyme B and caspse 3. Hantavirus N protein inhibit caspase 3 mediated apoptosis, and the site $DLID_{285}$ in the N protein was shown to be important for this inhibition (**paper I**).

Hantavirus-infected cells are protected from TRAIL-mediated apoptosis. Virus-infection causes down-regulation of DR5 on the infected cell surface. DR5 is translocated to the nucleus during hantavirus-infection (**paper II**).

During acute HFRS $CD56^{dim}$ NK cells are activated. *In vitro*, these NK cells are activated by hantavirus-infected cells. This activation is cell to cell contact dependent and is explained by IL-15/IL-15Rα expression on the infected cell surface. These activated NK cells induce apoptosis in uninfected cells with normal HLA class I levels (**paper III**).

Taken together, hantavirus-infection confers resistance to cytotoxic lymphocyte mediated apoptosis by inhibition of granzyme B, caspse 3 and down-regulation of cell surface DR5. The virus affects NK cells in such way, that the NK cells get activated and then starts to kill uninfected cells (Figure 8).

These works have been attempted to shed more light to the understanding of hantavirus pathogenesis. Although, to understand the entire characteristic of HFRS and HCPS caused pathogenesis more basic research combined with translational research will be required.

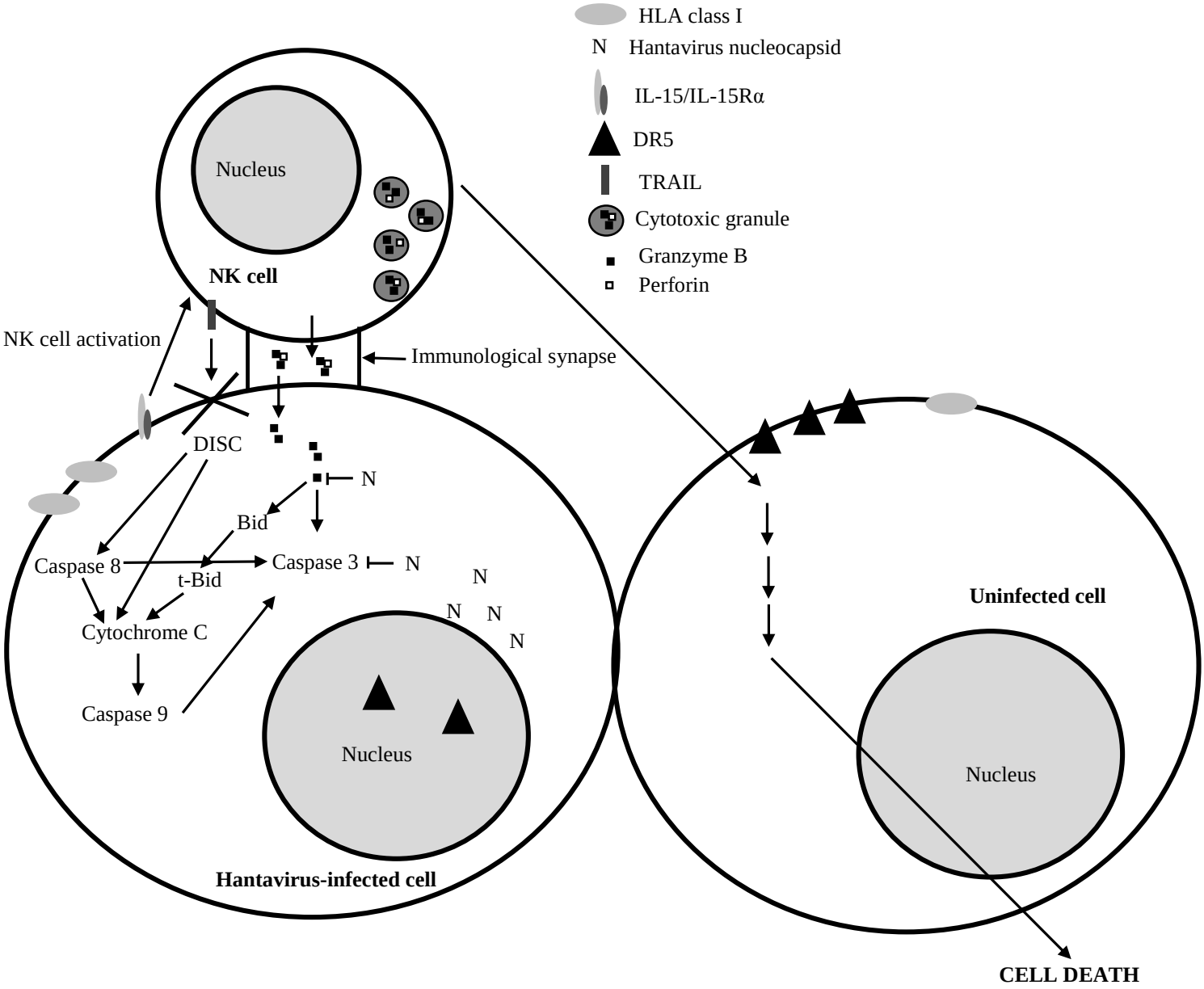

Figure 8. Illustration of hantaviruses inhibit cytotoxic lymphocyte mediated killing and how they activate NK cell, which cause cell death of uninfected cell.

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5 REFERENCES

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. **2001**. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature. 18;413(6857):732-8.

Alff PJ, Gavrilovskaya IN, Gorbunova E, Endriss K, Chong Y, Geimonen E, Sen N, Reich NC, Mackow ER. **2006**. The pathogenic NY-1 hantavirus G1 cytoplasmic tail inhibits RIG-I- and TBK-1-directed interferon responses. J Virol. 80(19):9676-86.

Alff PJ, Sen N, Gorbunova E, Gavrilovskaya IN, Mackow ER. **2008**. The NY-1 hantavirus Gn cytoplasmic tail coprecipitates TRAF3 and inhibits cellular interferon responses by disrupting TBK1-TRAF3 complex formation. J Virol. 82(18):9115-22.

Alimonti JB, Shi L, Baijal PK, Greenberg AH. **2001**. Granzyme B induces BID-mediated cytochrome c release and mitochondrial permeability transition. J Biol Chem. 9;276(10):6974-82.

Andrade F, Bull HG, Thornberry NA, Ketner GW, Casciola-Rosen LA, Rosen A. **2001**. Adenovirus L4-100K assembly protein is a granzyme B substrate that potently inhibits granzyme B-mediated cell death. Immunity. 14(6):751-61.

Andrade F, Casciola-Rosen LA, Rosen A. **2004**. Granzyme B-induced cell death. Acta Haematol. 111(1-2):28-41.

Andrade F, Fellows E, Jenne DE, Rosen A, Young CS. **2007**. Granzyme H destroys the function of critical adenoviral proteins required for viral DNA replication and granzyme B inhibition. EMBO J. 18;26(8):2148-57.

Antonen J, Leppänen I, Tenhunen J, Arvola P, Mäkelä S, Vaheri A, Mustonen J. **2013**. A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. Scand J Infect Dis. 45(6):494-6.

Asada H, Tamura M, Kondo K, Dohi Y, Yamanishi K. **1988**. Cell-mediated immunity to virus causing haemorrhagic fever with renal syndrome: generation of cytotoxic T lymphocytes. J Gen Virol. 69 (Pt 9):2179-88.

Atedzoe BN, Ahmad A, Menezes J. **1997**. Enhancement of natural killer cell cytotoxicity by the human herpesvirus-7 via IL-15 induction. J Immunol. 15;159(10):4966-72.

Atkinson EA, Barry M, Darmon AJ, Shostak I, Turner PC, Moyer RW, Bleackley RC. **1998**. Cytotoxic T lymphocyte-assisted suicide. Caspase 3 activation is primarily the result of the direct action of granzyme B. J Biol Chem. 14;273(33):21261-6.

Avsic-Zupanc T, Xiao SY, Stojanovic R, Gligic A, van der Groen G, LeDuc JW. **1992**. Characterization of Dobrava virus: a Hantavirus from Slovenia, Yugoslavia. J Med Virol. 38(2):132-7.

Baigildina AA, Khaiboullina SF, Martynova EV, Anokhin VA, Lombardi VC, Rizvanov AA. **2015**. Inflammatory cytokines kinetics define the severity and phase of nephropathia epidemica. Biomark Med. 9(2):99-107.

Banchereau J, Steinman RM. **1999**. Dendritic cells and the control of immunity. Nature. 19;392(6673):245-52.

Banda NK, Bernier J, Kurahara DK, Kurrle R, Haigwood N, Sekaly RP, Finkel TH. **1992**. Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. J Exp Med. 1;176(4):1099-106.

Barchet W, Cella M, Odermatt B, Asselin-Paturel C, Colonna M, Kalinke U. **2002**. Virus-induced interferon alpha production by a dendritic cell subset in the absence of feedback signaling in vivo. J Exp Med. 18;195(4):507-16.

Benedict CA, Norris PS, Prigozy TI, Bodmer JL, Mahr JA, Garnett CT, Martinon F, Tschopp J, Gooding LR, Ware CF. **2001**. Three adenovirus E3 proteins cooperate to evade apoptosis by tumor necrosis factor-related apoptosis-inducing ligand receptor-1 and -2. J. Biol. Chem. 276:3270–3278.

Björkström NK, Fauriat C, Bryceson YT, Sandberg JK, Ljunggren HG, Malmberg KJ. **2010**. Analysis of the KIR repertoire in human NK cells by flow cytometry. Methods Mol Biol. 612:353-64.

Björkström NK, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M, Michaëlsson J, Malmberg KJ, Klingström J, Ahlm C, Ljunggren HG. **2011**. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J Exp Med. 17;208(1):13-21.

Blink EJ, Jiansheng Z, Hu W, Calanni ST, Trapani JA, Bird PI, Jans DA. **2005**. Interaction of the nuclear localizing cytolytic granule serine protease granzyme B with importin alpha or beta: modulation by the serpin inhibitor PI-9. J Cell Biochem. 1;95(3):598-610.

Bodmer JL, Holler N, Reynard S, Vinciguerra P, Schneider P, Juo P, Blenis J, Tschopp J. **2000**. TRAIL receptor-2 signals apoptosis through FADD and caspase-8. Nature Cell Biology 2(4):241-3.

Bondu V, Schrader R, Gawinowicz MA, McGuire P, Lawrence DA, Hjelle B, Buranda T. **2015**. Elevated cytokines, thrombin and PAI-1 in severe HCPS patients due to Sin Nombre virus. Viruses. 10;7(2):559-89.

Borges AA, Campos GM, Moreli ML, Moro Souza RL, Saggioro FP, Figueiredo GG, Livonesi MC, Moraes Figueiredo LT. **2008**. Role of mixed Th1 and Th2 serum cytokines on pathogenesis and prognosis of hantavirus pulmonary syndrome. Microbes Infect. 10(10-11):1150-7.

Brocato RL, Hammerbeck CD, Bell TM, Wells JB, Queen LA, Hooper JW. **2014**. A lethal disease model for hantavirus pulmonary syndrome in immunosuppressed Syrian hamsters infected with Sin Nombre virus. J Virol. 88(2):811-9.

Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni T, Penttinen K, Oker-Blom N, Lähdevirta J. **1980**. Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. J Infect Dis. 141(2):131-4.

Bryceson YT, March ME, Ljunggren HG, Long EO. **2006**. Activation, coactivation, and costimulation of resting human natural killer cells. Immunol Rev. 214:73-91.

Bryceson YT, Long EO. **2008**. Line of attack: NK cell specificity and integration of signals. Curr Opin Immunol. 20(3):344-52.

Caputo A, James MN, Powers JC, Hudig D, Bleackley RC. **1994**. Conversion of the substrate specificity of mouse proteinase granzyme B. Nat Struct Biol. 1(6):364-7.

Carey DE, Reuben R, Panicker KN, Shope RE, Myers RM. **1971**. Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. Indian J Med Res. 59(11):1758-60.

Carrillo-Infante C, Abbadessa G, Bagella L, Giordano A. **2007**. Viral infections as a cause of cancer (review). Int J Oncol. 30(6):1521-8.

Castillo C, Naranjo J, Sepúlveda A, Ossa G, Levy H. **2001**. Hantavirus pulmonary syndrome due to Andes virus in Temuco, Chile: clinical experience with 16 adults. Chest. 120(2):548-54.

Chaplin DD. **2010**. Overview of the immune response. J Allergy Clin Immunol. 125(2 Suppl 2):S3-23.

Chelbi-Alix MK, Wietzerbin J. **2007**. Interferon, a growing cytokine family: 50 years of interferon research. Biochimie. 89(6-7):713-8.

Chen J-J, Shen H-C. J, Rivera Rosado L. A, Zhang Y, Di X, Zhang B. **2012**. Mislocalization of death receptors correlates with cellular resistance to their cognate ligands in human breast cancer cells. Oncotarget. 3(8):833-42.

Chinnaiyan AM, Hanna WL, Orth K, Duan H, Poirier GG, Froelich CJ, Dixit VM. **1996**. Cytotoxic T-cell-derived granzyme B activates the apoptotic protease ICE-LAP3. Curr Biol. 1;6(7):897-9.

Choi Y, Kwon YC, Kim SI, Park JM, Lee KH, Ahn BY. **2008**. A hantavirus causing hemorrhagic fever with renal syndrome requires gC1qR/p32 for efficient cell binding and infection. Virology. 25;381(2):178-83.

Chow J, Franz KM, Kagan JC. **2015**. PRRs are watching you: Localization of innate sensing and signaling regulators. Virology. 479-480:104-9.

Chowdhury D, Lieberman J. **2008**. Death by a thousand cuts: granzyme pathways of programmed cell death. Annu Rev Immunol. 26:389-420.

Clarke P, Meintzer SM, Gibson S, Widmann C, Garrington TP, Johnson GL, Tyler KL. **2000**. Reovirus-induced apoptosis is mediated by TRAIL. J Virol. 74(17):8135-9.

Clemens MJ. **2003**. Interferons and apoptosis. J Interferon Cytokine Res. 23(6):277-92.

Cohen GM. **1997**. Caspases: the executioners of apoptosis. Biochem J. 15;326 (Pt 1):1-16.

Collison A, Foster PS, Mattes J. **2009**. Emerging role of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) as a key regulator of inflammatory responses. Clin Exp Pharmacol Physiol. 36(11):1049-53.

Connolly-Andersen AM, Ahlm K, Ahlm C, Klingström J. **2013**. Puumala virus infections associated with cardiovascular causes of death. Emerg Infect Dis. 19(1):126-8.

Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA. **2001**. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood. 97(10):3146-51.

Cory S, Adams JM. **2002**. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer. 2(9):647-56.

D'Amours D, Sallmann FR, Dixit VM, Poirier GG. **2001**. Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. J Cell Sci. 114(Pt 20):3771-8.

Darmon AJ, Nicholson DW, Bleackley RC. **1995**. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature. 5;377(6548):446-8.

Darmon AJ, Ley TJ, Nicholson DW, Bleackley RC. **1996**. Cleavage of CPP32 by granzyme B represents a critical role for granzyme B in the induction of target cell DNA fragmentation. J Biol Chem. 6;271(36):21709-12.

Delbridge AR, Valente LJ, Strasser A. **2012**. The role of the apoptotic machinery in tumor suppression. Cold Spring Harb Perspect Biol. 1;4(11). pii: a008789.

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. **2004**. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 5;303(5663):1529-31.

Dobbelstein M, Shenk T. **1996**. Protection against apoptosis by the vaccinia virus SPI-2 (B13R) gene product. J Virol. 70(9):6479-85.

Du J, Liang X, Liu Y, Qu Z, Gao L, Han L, Liu S, Cui M, Shi Y, Zhang Z, Yu L, Cao L, Ma C, Zhang L, Chen Y, Sun W. **2009**. Hepatitis B virus core protein inhibits TRAIL-induced apoptosis of hepatocytes by blocking DR5 expression. Cell Death Differ. 16:219–229.

Duan H, Orth K, Chinnaiyan AM, Poirier GG, Froelich CJ, He WW, Dixit VM. **1996**. ICE-LAP6, a novel member of the ICE/Ced-3 gene family, is activated by the cytotoxic T cell protease granzyme B. J Biol Chem. 12;271(28):16720-4.

Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, Ksiazek TG, Rollin PE, Nichol S, Umland ET, Moolenaar RL, Reef SE, Nolte KB, Gallaher MM, Butler JC, Breiman RF, and the Hantavirus Study Group. **1994**. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. The Hantavirus Study Group. N Engl J Med. 7;330(14):949-55.

Ermler ME, Traylor Z, Patel K, Schattgen SA, Vanaja SK, Fitzgerald KA, Hise AG. **2014**. Rift Valley fever virus infection induces activation of the NLRP3 inflammasome. Virology. 20;449:174-80.

Falschlehner C, Emmerich CH, Gerlach B, Walczak H. **2007**. TRAIL signalling: decisions between life and death. Int J Biochem Cell Biol. 39(7-8):1462-75.

Falschlehner C, Schaefer U, Walczak H. **2009**. Following TRAIL's path in the immune system. Immunology. 127(2):145-54.

Fauriat C, Long EO, Ljunggren HG, Bryceson YT. **2010**. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood. 18;115(11):2167-76.

Fawaz LM, Sharif-Askari E, Menezes J. **1999**. Up-regulation of NK cytotoxic activity via IL-15 induction by different viruses: a comparative study. J Immunol. 15;163(8):4473-80.

Flamand L, Stefanescu I, Menezes J. **1996**. Human herpesvirus-6 enhances natural killer cell cytotoxicity via IL-15. J Clin Invest. 15;97(6):1373-81.

Flick K, Hooper JW, Schmaljohn CS, Pettersson RF, Feldmann H, Flick R. **2003**. Rescue of Hantaan virus minigenomes. Virology. 15;306(2):219-24.

Fuchs Y, Steller H. **2011**. Programmed cell death in animal development and disease. Cell. 11;147(4):742-58.

Galluzzi L, Brenner C, Morselli E, Touat Z, Kroemer G. **2008**. Viral control of mitochondrial apoptosis. PLoS Pathog. 30;4(5):e1000018.

Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, Dawson TM, Dawson VL, El-Deiry WS, Fulda S, Gottlieb E, Green DR, Hengartner MO, Kepp O, Knight RA, Kumar S, Lipton SA, Lu X, Madeo F, Malorni W, Mehlen P, Nuñez G, Peter ME, Piacentini M, Rubinsztein DC, Shi Y, Simon HU, Vandenabeele P, White E, Yuan J, Zhivotovsky B, Melino G, Kroemer G. **2012**. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ. 19(1):107-20.

Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, Alnemri ES, Altucci L, Andrews D, Annicchiarico-Petruzzelli M, Baehrecke EH, Bazan NG, Bertrand MJ, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Bredesen DE, Brenner C, Campanella M, Candi E, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, Di Daniele N, Dixit VM, Dynlacht BD, El-Deiry WS, Fimia GM, Flavell RA, Fulda S, Garrido C, Gougeon ML, Green DR, Gronemeyer H, Hajnoczky G, Hardwick JM, Hengartner MO, Ichijo H, Joseph B, Jost PJ, Kaufmann T, Kepp O, Klionsky DJ, Knight RA, Kumar S, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, López-Otín C, Lugli E, Madeo F, Malorni W, Marine JC, Martin SJ, Martinou JC, Medema JP, Meier P, Melino S, Mizushima N, Moll U, Muñoz-Pinedo C, Nuñez G, Oberst A, Panaretakis T, Penninger JM, Peter ME, Piacentini M, Pinton P, Prehn JH, Puthalakath H, Rabinovich GA, Ravichandran KS, Rizzuto R, Rodrigues CM, Rubinsztein DC, Rudel T, Shi Y, Simon HU, Stockwell BR, Szabadkai G, Tait SW, Tang HL, Tavernarakis N, Tsujimoto Y, Vanden Berghe T, Vandenabeele P, Villunger A, Wagner EF, Walczak H, White E, Wood WG, Yuan J, Zakeri Z, Zhivotovsky B, Melino G, Kroemer G. **2015**. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ. 22(1):58-73.

Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER. **1998**. beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A. 9;95(12):7074-9.

Gavrilovskaya IN, Brown EJ, Ginsberg MH, Mackow ER. **1999**. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J Virol. 73(5):3951-9.

Gavrilovskaya IN, Gorbunova EE, Mackow NA, Mackow ER. **2008**. Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine 1-phosphate inhibit hantavirus-directed permeability. J Virol. 82(12):5797-806.

Goping IS, Barry M, Liston P, Sawchuk T, Constantinescu G, Michalak KM, Shostak I, Roberts DL, Hunter AM, Korneluk R, Bleackley RC. **2003**. Granzyme B-induced apoptosis requires both direct caspase activation and relief of caspase inhibition. Immunity. 18(3):355-65.

Groen J, Gerding M, Koeman JP, Roholl PJ, van Amerongen G, Jordans HG, Niesters HG, Osterhaus AD. **1995**. A macaque model for hantavirus infection. J Infect Dis. 172(1):38-44.

Guo WP, Lin XD, Wang W, Tian JH, Cong ML, Zhang HL, Wang MR, Zhou RH, Wang JB, Li MH, Xu J, Holmes EC, Zhang YZ. **2013**. Phylogeny and origins of hantaviruses harbored by bats, insectivores, and rodents. PLoS Pathog. 9(2):e1003159.

Habjan M, Andersson I, Klingström J, Schümann M, Martin A, Zimmermann P, Wagner V, Pichlmair A, Schneider U, Mühlberger E, Mirazimi A, Weber F. **2008**. Processing of genome 5' termini as a strategy of negative-strand RNA viruses to avoid RIG-I-dependent interferon induction. PLoS One. 30;3(4):e2032.

Hallin GW, Simpson SQ, Crowell RE, James DS, Koster FT, Mertz GJ, Levy H. **1996**. Crit Care Med. 24(2):252-8.

Hammerbeck CD, Hooper JW. **2011**. T cells are not required for pathogenesis in the Syrian hamster model of hantavirus pulmonary syndrome. J Virol. 85(19):9929-44.

Hanahan D, Weinberg RA. **2011**. Hallmarks of cancer: the next generation. Cell. 4;144(5):646-74.

Handke W, Oelschlegel R, Franke R, Krüger DH, Rang A. **2009**. Hantaan virus triggers TLR3-dependent innate immune responses. J Immunol. 1;182(5):2849-58.

Haselmann V, Kurz A, Bertsch U, Hübner S, Olempska-Müller M, Fritsch J, Häsler R, Pickl A, Fritsche H, Annewanter F, Engler C, Fleig B, Bernt A, Röder C, Schmidt H, Gelhaus C, Hauser C, Egberts J-H,

Heneweer C, Rohde AM, Böger C, Knippschild U, Röcken C, Adam D, Walczak H, Schütze S, janssen O, Wulczyn FG, Wajant H, Kalthoff H, Trauzold. **2014**. Nuclear death receptor TRAIL-R2 inhibits maturation of let-7 and promotes proliferation of pancreatic and other tumor cells. Gastroenterology. 146: 278-290.

Hardestam J, Klingström J, Mattsson K, Lundkvist A. **2005**. HFRS causing hantaviruses do not induce apoptosis in confluent Vero E6 and A-549 cells. J Med Virol. 76(2):234-40.

Hart LS, Ornelles D, Koumenis C. **2007.** The adenoviral E4orf6 protein induces atypical apoptosis in response to DNA damage. J Biol Chem 282: 6061–6067.

Hautala N, Kauma H, Rajaniemi SM, Sironen T, Vapalahti O, Pääkkö E, Karttunen A, Ilonen J, Rytky S, Vainio O, Vaheri A, Hautala T. **2012**. Signs of general inflammation and kidney function are associated with the ocular features of acute Puumala hantavirus infection. Scand J Infect Dis. 44(12):956-62.

Heibein JA, Barry M, Motyka B, Bleackley RC. **1999**. Granzyme B-induced loss of mitochondrial inner membrane potential (Delta Psi m) and cytochrome c release are caspase independent. J Immunol. 1;163(9):4683-93.

Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S. **2004**. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 5;303(5663):1526-9.

Hepojoki J, Strandin T, Lankinen H, Vaheri A. **2012**. Hantavirus structure--molecular interactions behind the scene. J Gen Virol. 93(Pt 8):1631-44.

Hesselgesser J, Taub D, Baskar P, Greenberg M, Hoxie J, Kolson DL, Horuk R. **1998**. Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. Curr Biol. 7;8(10):595-8.

Hooper JW, Larsen T, Custer DM, Schmaljohn CS. **2001**. A lethal disease model for hantavirus pulmonary syndrome. Virology. 10;289(1):6-14.

Jabri B, Abadie V. **2015**. IL-15 functions as a danger signal to regulate tissue-resident T cells and tissue destruction. Nat Rev Immunol. 15(12):771-83.

Jacobs SR, Damania B. **2012**. NLRs, inflammasomes, and viral infection. J Leukoc Biol. 92(3):469-77.

Jans DA, Jans P, Briggs LJ, Sutton V, Trapani JA. **1996**. Nuclear transport of granzyme B (fragmentin-2). Dependence of perforin in vivo and cytosolic factors in vitro. J Biol Chem. 29;271(48):30781-9.

Jeremias I, Herr I, Boehler T, Debatin KM. **1998**. TRAIL/Apo-2-ligand-induced apoptosis in human T cells. Eur J Immunol. 28(1):143-52.

Jiang H, Wang PZ, Zhang Y, Xu Z, Sun L, Wang LM, Huang CX, Lian JQ, Jia ZS, Li ZD, Bai XF. **2008**. Hantaan virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon, interleukin-6 and tumor necrosis factor-alpha. Virology. 10;380(1):52-9.

Jin M, Park J, Lee S, Park B, Shin J, Song KJ, Ahn TI, Hwang SY, Ahn BY, Ahn K. **2002**. Hantaan virus enters cells by clathrin-dependent receptor-mediated endocytosis. Virology. 1;294(1):60-9.

Johnson KM. **2001**. Hantaviruses: history and overview. Curr Top Microbiol Immunol. 256:1-14.

Jonsson CB, Schmaljohn CS. **2001**. Replication of hantaviruses. Curr Top Microbiol Immunol. 256:15-32.

Jonsson CB, Hooper J, Mertz G. **2008**. Treatment of hantavirus pulmonary syndrome. Antiviral Res. 78(1):162-9.

Jonsson CB, Figueiredo LT, Vapalahti O. **2010**. A global perspective on hantavirus ecology, epidemiology, and disease. Clin Microbiol Rev. 23(2):412-41.

Jost S, Altfeld M. **2013**. Control of human viral infections by natural killer cells. Annu Rev Immunol. 31:163-94.

Jääskeläinen KM, Kaukinen P, Minskaya ES, Plyusnina A, Vapalahti O, Elliott RM, Weber F, Vaheri A, Plyusnin A. **2007**. Tula and Puumala hantavirus NSs ORFs are functional and the products inhibit activation of the interferon-beta promoter. J Med Virol. 79(10):1527-36.

Jääskeläinen KM, Plyusnina A, Lundkvist A, Vaheri A, Plyusnin A. **2008**. Tula hantavirus isolate with the full-length ORF for nonstructural protein NSs survives for more consequent passages in interferon-competent cells than the isolate having truncated NSs ORF. Virol J. 11;5:3. doi: 10.1186/1743-422X-5-3.

Kabsch K, Alonso A. **2002**. The human papillomavirus type 16 E5 protein impairs TRAIL- and FasL-mediated apoptosis in HaCaT cells by different mechanisms. J Virol. 76(23):12162-72.

Kanerva M, Mustonen J, Vaheri A. **1998**. Pathogenesis of puumala and other hantavirus infections. Rev Med Virol. 8(2):67-86.

Kang JI, Park SH, Lee PW, Ahn BY. **1999**. Apoptosis is induced by hantaviruses in cultured cells. Virology. 10;264(1):99-105.

Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, Faraoni R, Floyd M, Hunt JP, Lockhart DJ, Milanov ZV, Morrison MJ, Pallares G, Patel HK, Pritchard S, Wodicka LM, Zarrinkar PP. **2008**. A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol. 26(1):127-32.

Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S. **2006**. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature. 4;441(7089):101-5.

Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T, Akira S. **2008**. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J Exp Med. 7;205(7):1601-10.

Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S. **2005**. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol. 6(10):981-8.

Kawanishi M, Tada-Oikawa S, Kawanishi S. **2002**. Epstein-Barr virus BHRF1 functions downstream of Bid cleavage and upstream of mitochondrial dysfunction to inhibit TRAIL-induced apoptosis in BJAB cells. Biochem Biophys Res Commun. 27;297(3):682-7.

Kayagaki N, Yamaguchi N, Nakayama M, Kawasaki A, Akiba H, Okumura K, Yagita H. **1999**. Involvement of TNF-related apoptosis-inducing ligand in human CD4+ T cell-mediated cytotoxicity. J Immunol. 1;162(5):2639-47.

Kelly KJ, Plotkin Z, Dagher PC. **2001**. Guanosine supplementation reduces apoptosis and protects renal function in the setting of ischemic injury. J Clin Invest. 108(9):1291-8.

Kerr JF, Wyllie AH, Currie AR. **1972**. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 26(4):239-57.

Khaiboullina SF, Morzunov SP, Boichuk SV, Palotás A, St Jeor S, Lombardi VC, Rizvanov AA. **2013**. Death-domain associated protein-6 (DAXX) mediated apoptosis in hantavirus infection is counter-balanced by activation of interferon-stimulated nuclear transcription factors. Virology. 1;443(2):338-48.

Kilpatrick ED, Terajima M, Koster FT, Catalina MD, Cruz J, Ennis FA. **2004**. Role of specific CD8+ T cells in the severity of a fulminant zoonotic viral hemorrhagic fever, hantavirus pulmonary syndrome. J Immunol. 1;172(5):3297-304.

Klaus JP, Eisenhauer P, Russo J, Mason AB, Do D, King B, Taatjes D, Cornillez-Ty C, Boyson JE, Thali M, Zheng C, Liao L, Yates JR 3rd, Zhang B, Ballif BA, Botten JW. **2013**. The intracellular cargo receptor ERGIC-53 is required for the production of infectious arenavirus, coronavirus, and filovirus particles. Cell Host Microbe. 13;14(5):522-34.

Klingström J, Plyusnin A, Vaheri A, Lundkvist A. **2002**. Wild-type Puumala hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in cynomolgus macaques. J Virol. 76(1):444-9.

Klingström J, Falk KI, Lundkvist A. **2005**. Delayed viremia and antibody responses in Puumala hantavirus challenged passively immunized cynomolgus macaques. Arch Virol. 150(1):79-92.

Klingström J, Hardestam J, Stoltz M, Zuber B, Lundkvist A, Linder S, Ahlm C. **2006**. Loss of cell membrane integrity in puumala hantavirus-infected patients correlates with levels of epithelial cell apoptosis and perforin. J Virol. 80(16):8279-82.

Klingström J, Stoltz M, Hardestam J, Ahlm C, Lundkvist A. **2008**. Passive immunization protects cynomolgus macaques against Puumala hantavirus challenge. Antivir Ther. 13(1):125-33.

Klingström J, Ahlm C. **2011**. Hantavirus protein interactions regulate cellular functions and signaling responses. Expert Rev Anti Infect Ther. 9(1):33-47.

Klingström J, Granath F, Ekbom A, Björkström NK, Ljunggren HG. **2014**. Increased risk for lymphoma following hemorrhagic fever with renal syndrome. Clin Infect Dis. 15;59(8):1130-2.

Kotelkin A, Prikhod'ko EA, Cohen JI, Collins PL, Bukreyev A. **2003**. Respiratory syncytial virus infection sensitizes cells to apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand. J Virol. 77(17):9156-72.

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP. **2003**. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol. 4(1):69-77.

Krautkrämer E, Zeier M. **2008**. Hantavirus causing hemorrhagic fever with renal syndrome enters from the apical surface and requires decay-accelerating factor (DAF/CD55). J Virol. 82(9):4257-64.

Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, Blagosklonny MV, El-Deiry WS, Golstein P, Green DR, Hengartner M, Knight RA, Kumar S, Lipton SA, Malorni W, Nuñez G, Peter ME, Tschopp J, Yuan J, Piacentini M, Zhivotovsky B, Melino G; Nomenclature Committee on Cell Death 2009. **2009**. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ. 16(1):3-11.

Kukkonen SK, Vaheri A, Plyusnin A. **2004**. Tula hantavirus L protein is a 250 kDa perinuclear membrane-associated protein. J Gen Virol. 85(Pt 5):1181-9.

Kukkonen SK, Vaheri A, Plyusnin A. **2005**. L protein, the RNA-dependent RNA polymerase of hantaviruses. Arch Virol. 150(3):533-56.

Kyriakidis I, Papa A. **2013**. Serum TNF-α, sTNFR1, IL-6, IL-8 and IL-10 levels in hemorrhagic fever with renal syndrome. Virus Res. 175(1):91-4.

Kärre K, Ljunggren HG, Piontek G, Kiessling R. **1986**. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature. 20-26;319(6055):675-8.

Lalwani P, Raftery MJ, Kobak L, Rang A, Giese T, Matthaei M, van den Elsen PJ, Wolff T, Krüger DH, Schönrich G. **2013**. Hantaviral mechanisms driving HLA class I antigen presentation require both RIG-I and TRIF. Eur J Immunol. 43(10):2566-76.

Lanier LL. **2003**. Natural killer cell receptor signaling. Curr Opin Immunol. 15(3):308-14.

Lanier LL. **2008**. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. 9(5):495-502.

Latus J, Kitterer D, Segerer S, Artunc F, Alscher MD, Braun N. **2015**. Determination of procalcitonin levels in patients with nephropathia epidemica - a useful tool or an unnecessary diagnostic procedure? Kidney Blood Press Res. 40(1):22-30.

Lavoie JN, Nguyen M, Marcellus RC, Branton PE, Shore GC. **1998**. E4orf4, a novel adenovirus death factor that induces p53-independent apoptosis by a pathway that is not inhibited by zVAD-fmk. J Cell Biol. 9;140(3):637-45.

Lavrik IN, Golks A, Krammer PH. **2005**. Caspases: pharmacological manipulation of cell death. J Clin Invest. 115(10):2665-72.

Lee HW, Lee PW, Johnson KM. **1978**. Isolation of the etiologic agent of Korean hemorrhagic fever. J Infect Dis. 137:298–308.

Lee HW, Baek LJ, Johnson KM. **1982**. Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. J Infect Dis. 146(5):638-44.

Lee MH, Lalwani P, Raftery MJ, Matthaei M, Lütteke N, Kirsanovs S, Binder M, Ulrich RG, Giese T, Wolff T, Krüger DH, Schönrich G. **2011**. RNA helicase retinoic acid-inducible gene I as a sensor of Hantaan virus replication. J Gen Virol. 92(Pt 9):2191-200.

Leithner K, Stacher E, Wurm R, Ploner F, Quehenberger, Wohlkoenig C, Bálint Z, Polachova J, Olschewski A, Samonigg, Popper H. H, Olschewski H. **2009**. Nuclear and cytoplasmic death receptor 5 as prognostic factors in patients with non-small cell lung cancer treated with chemotherapy. Lung Cancer. 65: 98-104.

Lettre G, Hengartner MO. **2006**. Developmental apoptosis in C. elegans: a complex CEDnario. Nat Rev Mol Cell Biol. 7(2):97-108.

Levine JR, Prescott J, Brown KS, Best SM, Ebihara H, Feldmann H. **2010**. Antagonism of type I interferon responses by new world hantaviruses. J Virol. 84(22):11790-801.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. **1997**. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 14;91(4):479-89.

Li XD, Mäkelä TP, Guo D, Soliymani R, Koistinen V, Vapalahti O, Vaheri A, Lankinen H. **2002**. Hantavirus nucleocapsid protein interacts with the Fas-mediated apoptosis enhancer Daxx. J Gen Virol. 83(Pt 4):759-66.

Li XD, Kukkonen S, Vapalahti O, Plyusnin A, Lankinen H, Vaheri A. **2004**. Tula hantavirus infection of Vero E6 cells induces apoptosis involving caspase 8 activation. J Gen Virol. 85(Pt 11):3261-8.

Li XD, Lankinen H, Putkuri N, Vapalahti O, Vaheri A. **2005**. Tula hantavirus triggers pro-apoptotic signals of ER stress in Vero E6 cells. Virology. 1;333(1):180-9.

Li Y, Wang W, Wang JP, Pan L, Zhang Y, Yu HT, Jiang W, Wang PZ, Bai XF. **2012**. Elevated vascular endothelial growth factor levels induce hyperpermeability of endothelial cells in hantavirus infection. J Int Med Res. 40(5):1812-21.

Lieberman J. **2003**. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. Nat Rev Immunol. 3(5):361-70.

Lin W, Kim SS, Yeung E, Kamegaya Y, Blackard JT, Kim KA, Holtzman MJ, Chung RT. **2006**. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. J Virol. 80(18):9226-35.

Linderholm M, Bjermer L, Juto P, Roos G, Sandström T, Settergren B, Tärnvik A. **1993**. Local host response in the lower respiratory tract in nephropathia epidemica. Scand J Infect Dis. 25(5):639-46.

Lindgren T, Ahlm C, Mohamed N, Evander M, Ljunggren HG, Björkström NK. **2011**. Longitudinal analysis of the human T cell response during acute hantavirus infection. J Virol. 85(19):10252-60.

Liu X, Kim CN, Yang J, Jemmerson R, Wang X. **1996**. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell. 12;86(1):147-57.

Ljunggren HG, Kärre K. **1990**. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today. 11(7):237-44.

López N, Padula P, Rossi C, Lázaro ME, Franze-Fernández MT. **1996**. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. Virology. 1;220(1):223-6.

Lozach PY, Mancini R, Bitto D, Meier R, Oestereich L, Overby AK, Pettersson RF, Helenius A. **2010**. Entry of bunyaviruses into mammalian cells. Cell Host Microbe. 25;7(6):488-99.

Lu M, Lawrence DA, Marsters S, Acosta-Alvear D, Kimmig P, Mendez AS, Paton AW, Paton JC, Walter P, Ashkenazi A. **2014**. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis. Science. 4;345(6192):98-101.

Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A. **2003**. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. J Exp Med. 4;198(3):513-20.

Löber C1, Anheier B, Lindow S, Klenk HD, Feldmann H. **2001**. The Hantaan virus glycoprotein precursor is cleaved at the conserved pentapeptide WAASA. Virology. 25;289(2):224-9.

Ma Y, Liu B, Yuan B, Wang J, Yu H, Zhang Y, Xu Z, Zhang Y, Yi J, Zhang C, Zhou X, Yang A, Zhuang R, Jin B. **2012**. Sustained high level of serum VEGF at convalescent stage contributes to the renal recovery after HTNV infection in patients with hemorrhagic fever with renal syndrome. Clin Dev Immunol. 2012:812386.

Manigold T, Vial P. **2014**. Human hantavirus infections: epidemiology, clinical features, pathogenesis and immunology. Swiss Med Wkly. 20;144:w13937.

Manns J, Daubrawa M, Driessen S, Paasch F, Hoffmann N, Löffler A, Lauber K, Dieterle A, Alers S, Iftner T, Schulze-Osthoff K, Stork B, Wesselborg S. **2011**. Triggering of a novel intrinsic apoptosis pathway by

the kinase inhibitor staurosporine: activation of caspase-9 in the absence of Apaf-1. FASEB J. 25(9):3250-61.

Marcellus RC, Teodoro JG, Wu T, Brough DE, Ketner G, Shore GC, Branton PE. **1996**. Adenovirus type 5 early region 4 is responsible for E1A-induced p53-independent apoptosis. J Virol. 70(9):6207-15.

Marcellus RC, Lavoie JN, Boivin D, Shore GC, Ketner G, Branton PE. **1998**. The early region 4 orf4 protein of human adenovirus type 5 induces p53-independent cell death by apoptosis. J Virol. 72(9):7144-53.

Markotic A, Hensley L, Geisbert T, Spik K, Schmaljohn C. **2003**. Hantaviruses induce cytopathic effects and apoptosis in continuous human embryonic kidney cells. J Gen Virol. 84(Pt 8):2197-202.

Markotić A, Hensley L, Daddario K, Spik K, Anderson K, Schmaljohn C. **2007**. Pathogenic hantaviruses elicit different immunoreactions in THP-1 cells and primary monocytes and induce differentiation of human monocytes to dendritic-like cells. Coll Antropol. 31(4):1159-67.

Marsac D, García S, Fournet A, Aguirre A, Pino K, Ferres M, Kalergis AM, Lopez-Lastra M, Veas F. **2011**. Infection of human monocyte-derived dendritic cells by ANDES Hantavirus enhances pro-inflammatory state, the secretion of active MMP-9 and indirectly enhances endothelial permeability. Virol J. 13;8:223.

Martin SJ, Amarante-Mendes GP, Shi L, Chuang TH, Casiano CA, O'Brien GA, Fitzgerald P, Tan EM, Bokoch GM, Greenberg AH, Green DR. **1996**. The cytotoxic cell protease granzyme B initiates apoptosis in a cell-free system by proteolytic processing and activation of the ICE/CED-3 family protease, CPP32, via a novel two-step mechanism. EMBO J. 15;15(10):2407-16.

Martinou I, Missotten M, Fernandez PA, Sadoul R, Martinou JC. **1998**. Bax and Bak proteins require caspase activity to trigger apoptosis in sympathetic neurons. Neuroreport. 5;9(1):15-9.

Marq JB, Kolakofsky D, Garcin D. **2010**. Unpaired 5' ppp-nucleotides, as found in arenavirus double-stranded RNA panhandles, are not recognized by RIG-I. J Biol Chem. 11;285(24):18208-16.

Marq JB, Hausmann S, Veillard N, Kolakofsky D, Garcin D. **2011**. Short double-stranded RNAs with an overhanging 5' ppp-nucleotide, as found in arenavirus genomes, act as RIG-I decoys. J Biol Chem. 25;286(8):6108-16.

Matthys VS, Cimica V, Dalrymple NA, Glennon NB, Bianco C, Mackow ER. **2014**. Hantavirus GnT elements mediate TRAF3 binding and inhibit RIG-I/TBK1-directed beta interferon transcription by blocking IRF3 phosphorylation. J Virol. 88(4):2246-59.

Macneil A, Nichol ST, Spiropoulou CF. **2011**. Hantavirus pulmonary syndrome. Virus Res. 162(1-2):138-47.

McAuley JL, Tate MD, MacKenzie-Kludas CJ, Pinar A, Zeng W, Stutz A, Latz E, Brown LE, Mansell A. **2013**. Activation of the NLRP3 inflammasome by IAV virulence protein PB1-F2 contributes to severe pathophysiology and disease. PLoS Pathog. 9(5):e1003392.

McAllister RC, Jonsson CB. **2014**. Hantaviruses: past, present and future. Future Virology. 9(1), 87–99.

McElroy AK, Bray M, Reed DS, Schmaljohn CS. **2002**. Andes virus infection of cynomolgus macaques. J Infect Dis. 15;186(12):1706-12.

McInerney GM, Karlsson Hedestam GB. **2009**. Direct cleavage, proteasomal degradation and sequestration: three mechanisms of viral subversion of type I interferon responses. J Innate Immun. 1(6):599-606.

Medema JP, Toes RE, Scaffidi C, Zheng TS, Flavell RA, Melief CJ, Peter ME, Offringa R, Krammer PH. **1997**. Cleavage of FLICE (caspase-8) by granzyme B during cytotoxic T lymphocyte-induced apoptosis. Eur J Immunol. 27(12):3492-8.

Metkar SS, Wang B, Ebbs ML, Kim JH, Lee YJ, Raja SM, Froelich CJ. **2003**. Granzyme B activates procaspase-3 which signals a mitochondrial amplification loop for maximal apoptosis. J Cell Biol. 17;160(6):875-85.

Meyer BJ, Schmaljohn CS. **2000**. Persistent hantavirus infections: characteristics and mechanisms. Trends Microbiol. 8(2):61-7.

Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, Tschopp J. **2005**. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 20;437(7062):1167-72.

Mir MA, Panganiban AT. **2008**. A protein that replaces the entire cellular eIF4F complex. EMBO J. 3;27(23):3129-39.

Miyamoto M, Fujita T, Kimura Y, Maruyama M, Harada H, Sudo Y, Miyata T, Taniguchi T. **1988**. Regulated expression of a gene encoding a nuclear factor, IRF-1, that specifically binds to IFN-beta gene regulatory elements. Cell. 9;54(6):903-13.

Moore M, Horikoshi N, Shenk T. **1996**. Oncogenic potential of the adenovirus E4orf6 protein. Proc Natl Acad Sci U S A. 15;93(21):11295-301.

Mori M, Rothman AL, Kurane I, Montoya JM, Nolte KB, Norman JE, Waite DC, Koster FT, Ennis FA. **1999**. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. J Infect Dis. 179(2):295-302.

Morzunov SP, Khaiboullina SF, St Jeor S, Rizvanov AA, Lombardi VC. **2015**. Multiplex Analysis of Serum Cytokines in Humans with Hantavirus Pulmonary Syndrome. Front Immunol. 31;6:432.

Mou DL, Wang YP, Huang CX, Li GY, Pan L, Yang WS, Bai XF. **2006**. Cellular entry of Hantaan virus A9 strain: specific interactions with beta3 integrins and a novel 70kDa protein. Biochem Biophys Res Commun. 13;339(2):611-7.

Murphy TL, Grajales-Reyes GE, Wu X, Tussiwand R, Briseño CG, Iwata A, Kretzer NM, Durai V, Murphy KM. **2015**. Transcriptional Control of Dendritic Cell Development. Annu Rev Immunol. 34:4.1–4.27.

Moutouh L, Estaquier J, Richman DD, Corbeil J. **1998**. Molecular and cellular analysis of human immunodeficiency virus-induced apoptosis in lymphoblastoid T-cell-line-expressing wild-type and mutated CD4 receptors. J Virol. 72(10):8061-72.

Mäkelä S, Jaatinen P, Miettinen M, Salmi J, Ala-Houhala I, Huhtala H, Hurme M, Pörsti I, Vaheri A, Mustonen J. **2010**. Hormonal deficiencies during and after Puumala hantavirus infection. Eur J Clin Microbiol Infect Dis. 29(6):705-13.

Nan Y, Nan G, Zhang YJ. **2014**. Interferon induction by RNA viruses and antagonism by viral pathogens. Viruses. 12;6(12):4999-5027.

Nasirudeen AM, Wong HH, Thien P, Xu S, Lam KP, Liu DX. **2011**. RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection. PLoS Negl Trop Dis. 4;5(1):e926.

Nevels M, Täuber B, Kremmer E, Spruss T, Wolf H, Dobner T. **1999**. Transforming potential of the adenovirus type 5 E4orf3 protein. J Virol. 73(2):1591-600.

Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ. **1993**. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science. 5;262(5135):914-7.

Nichol ST. Bunyaviruses. **2001.** In: Knipe DM, Howley PM, eds. Field's Virol Vol 2, 4th ed Philadelphia, Pa Lippincott Williams Wilkins. 2001. p. 1603–33.

Ning YJ, Wang M, Deng M, Shen S, Liu W, Cao WC, Deng F, Wang YY, Hu Z, Wang H. **2014**. Viral suppression of innate immunity via spatial isolation of TBK1/IKKε from mitochondrial antiviral platform. J Mol Cell Biol. 6(4):324-37.

Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, Merlin TL, McFeeley PJ, Umland ET, Zumwalt RE. **1995**. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. Hum Pathol. 26(1):110-20.

Ontiveros SJ, Li Q, Jonsson CB. **2010**. Modulation of apoptosis and immune signaling pathways by the Hantaan virus nucleocapsid protein. Virology. 5;401(2):165-78.

Op De Beeck A, Caillet-Fauquet P. **1997**. The NS1 protein of the autonomous parvovirus minute virus of mice blocks cellular DNA replication: a consequence of lesions to the chromatin? J Virol. 71(7):5323-9.

Orth K, Chinnaiyan AM, Garg M, Froelich CJ, Dixit VM. **1996**. The CED-3/ICE-like protease Mch2 is activated during apoptosis and cleaves the death substrate lamin A. J Biol Chem. 12;271(28):16443-6.

Outinen TK, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Hurme M, Paakkala AS, Pörsti IH, Syrjänen JT, Mustonen JT. **2010**. The severity of Puumala hantavirus induced nephropathia epidemica can be better evaluated using plasma interleukin-6 than C-reactive protein determinations. BMC Infect Dis. 25;10:132.

Outinen TK, Kuparinen T, Jylhävä J, Leppänen S, Mustonen J, Mäkelä S, Pörsti I, Syrjänen J, Vaheri A, Hurme M. **2012**. Plasma cell-free DNA levels are elevated in acute Puumala hantavirus infection. PLoS One. 7(2):e31455.

Ow YP, Green DR, Hao Z, Mak TW. **2008**. Cytochrome c: functions beyond respiration. Nat Rev Mol Cell Biol. 9(7):532-42.

Paulus C, Krauss S, Nevels M. **2006**. A human cytomegalovirus antagonist of type I IFN-dependent signal transducer and activator of transcription signaling. Proc Natl Acad Sci U S A. 7;103(10):3840-5.

Pepini T, Gorbunova EE, Gavrilovskaya IN, Mackow JE, Mackow ER. **2010**. Andes virus regulation of cellular microRNAs contributes to hantavirus-induced endothelial cell permeability. J Virol. 84(22):11929-36.

Petak I, Vernes R, Szucs KS, Anozie M, Izeradjene K, Douglas L, Tillman DM, Phillips DC, Houghton JA. **2003**. A caspase-8-independent component in TRAIL/Apo-2L-induced cell death in human rhabdomyosarcoma cells. Cell Death Differ. 10(6):729-39.

Plyusnin A, Vapalahti O, Vaheri A. **1996**. Hantaviruses: genome structure, expression and evolution. J Gen Virol. 77 (Pt 11):2677-87.

Pothlichet J, Meunier I, Davis BK, Ting JP, Skamene E, von Messling V, Vidal SM. **2013**. Type I IFN triggers RIG-I/TLR3/NLRP3-dependent inflammasome activation in influenza A virus infected cells. PLoS Pathog. 9(4):e1003256.

Prescott J, Hall P, Acuna-Retamar M, Ye C, Wathelet MG, Ebihara H, Feldmann H, Hjelle B. **2010**. New World hantaviruses activate IFNlambda production in type I IFN-deficient vero E6 cells. PLoS One. 17;5(6):e11159.

Prescott J, Safronetz D, Haddock E, Robertson S, Scott D, Feldmann H. **2013**. The adaptive immune response does not influence hantavirus disease or persistence in the Syrian hamster. Immunology. 140(2):168-78.

Quan LT, Caputo A, Bleackley RC, Pickup DJ, Salvesen GS. **1995**. Granzyme B is inhibited by the cowpox virus serpin cytokine response modifier A. J Biol Chem. 5;270(18):10377-9.

Raftery MJ, Kraus AA, Ulrich R, Krüger DH, Schönrich G. **2002**. Hantavirus infection of dendritic cells. J Virol. 76(21):10724-33.

Ramanathan HN, Chung DH, Plane SJ, Sztul E, Chu YK, Guttieri MC, McDowell M, Ali G, Jonsson CB. **2007**. Dynein-dependent transport of the hantaan virus nucleocapsid protein to the endoplasmic reticulum-Golgi intermediate compartment. J Virol. 81(16):8634-47.

Ramanathan HN, Jonsson CB. **2008**. New and Old World hantaviruses differentially utilize host cytoskeletal components during their life cycles. Virology. 25;374(1):138-50.

Randall RE, Goodbourn S. **2008**. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol. 89(Pt 1):1-47.

Rao L, Debbas M, Sabbatini P, Hockenbery D, Korsmeyer S, White E. **1992**. The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. Proc Natl Acad Sci U S A. 15;89(16):7742-6.

Rasche FM, Uhel B, Krüger DH, Karges W, Czock D, Hampl W, Keller F, Meisel H, von Müller L. **2004**. Thrombocytopenia and acute renal failure in Puumala hantavirus infections. Emerg Infect Dis. 10(8):1420-5.

Ravkov EV, Nichol ST, Peters CJ, Compans RW. **1998**. Role of actin microfilaments in Black Creek Canal virus morphogenesis. J Virol. 72(4):2865-70.

Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, Salvesen GS, Pickup DJ. **1992**. Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. Cell. 15;69(4):597-604.

Reynes JM, Carli D, Boukezia N, Debruyne M, Herti S. **2015**. Tula hantavirus infection in a hospitalised patient, France, June 2015. Euro Surveill. 17;20(50).

Riedl SJ, Shi Y. **2004**. Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol. 5(11):897-907.

Riquelme R, Riquelme M, Torres A, Rioseco ML, Vergara JA, Scholz L, Carriel A. **2003**. Hantavirus pulmonary syndrome, southern Chile. Emerg Infect Dis. 9(11):1438-43.

Rodriguez JJ, Wang LF, Horvath CM. **2003**. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. J Virol. 77(21):11842-5.

Roulston A, Marcellus RC, Branton PE. **1999**. Viruses and apoptosis. Annu Rev Microbiol. 53:577-628.

Sadeghi M, Eckerle I, Daniel V, Burkhardt U, Opelz G, Schnitzler P. **2011**. Cytokine expression during early and late phase of acute Puumala hantavirus infection. BMC Immunol. 16;12:65.

Safronetz D, Hegde NR, Ebihara H, Denton M, Kobinger GP, St Jeor S, Feldmann H, Johnson DC. **2009**. Adenovirus vectors expressing hantavirus proteins protect hamsters against lethal challenge with andes virus. J Virol. 83(14):7285-95.

Safronetz D, Zivcec M, Lacasse R, Feldmann F, Rosenke R, Long D, Haddock E, Brining D, Gardner D, Feldmann H, Ebihara H. **2011**. Pathogenesis and host response in Syrian hamsters following intranasal infection with Andes virus. PLoS Pathog. 7(12):e1002426.

Safronetz D, Ebihara H, Feldmann H, Hooper JW. **2012**. The Syrian hamster model of hantavirus pulmonary syndrome. Antiviral Res. 95(3):282-92.

Safronetz D, Prescott J, Feldmann F, Haddock E, Rosenke R, Okumura A, Brining D, Dahlstrom E, Porcella SF, Ebihara H, Scott DP, Hjelle B, Feldmann H. **2014**. Pathophysiology of hantavirus pulmonary syndrome in rhesus macaques. Proc Natl Acad Sci U S A. 13;111(19):7114-9.

Saito S, Murata T, Kanda T, Isomura H, Narita Y, Sugimoto A, Kawashima D, Tsurumi T. **2013**. Epstein-Barr virus deubiquitinase downregulates TRAF6-mediated NF-κB signaling during productive replication. J Virol. 87(7):4060-70.

Sakahira H, Enari M, Nagata S. **1998**. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature. 1;391(6662):96-9

Saksida A, Wraber B, Avšič-Županc T. **2011**. Serum levels of inflammatory and regulatory cytokines in patients with hemorrhagic fever with renal syndrome. BMC Infect Dis. 23;11:142.

Sasaki M, Miyazaki K, Koga Y, Kimura G, Nomoto K, Yoshida H. **2002**. Calcineurin-dependent mitochondrial disturbances in calcium-induced apoptosis of human immunodeficiency virus gp160-expressing CD4+ cells. J Virol. 76(1):416-20.

Sato K, Hida S, Takayanagi H, Yokochi T, Kayagaki N, Takeda K, Yagita H, Okumura K, Tanaka N, Taniguchi T, Ogasawara K. **2001**. Antiviral response by natural killer cells through TRAIL gene induction by IFN-alpha/beta. Eur J Immunol. 31(11):3138-46.

Scaffidi C1, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME. **1998**. Two CD95 (APO-1/Fas) signaling pathways. EMBO J. 16;17(6):1675-87.

Schmaljohn CS, Hasty SE, Harrison SA, Dalrymple JM. **1983a**. Characterization of Hantaan virions, the prototype virus of hemorrhagic fever with renal syndrome. J Infect Dis. 148(6):1005-12.

Schmaljohn CS, Dalrymple JM. **1983b**. Analysis of Hantaan virus RNA: evidence for a new genus of bunyaviridae. Virology. 131(2):482-91.

Schlee M. **2013**. Master sensors of pathogenic RNA - RIG-I like receptors. Immunobiology. 218(11):1322-35.

Schneider P, Bodmer JL, Thome M, Hofmann K, Holler N, Tschopp J. **1997a**. Characterization of two receptors for TRAIL. FEBS Lett. 27;416(3):329-34.

Schneider P, Thome M, Burns K, Bodmer JL, Hofmann K, Kataoka T, Holler N, Tschopp J. **1997b**. TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-kappaB. Immunity. 7(6):831-6.

Schroder K, Tschopp J. **2010**. The Inflammasomes. Cell. 19;140(6):821-32.

Schönrich G, Rang A, Lütteke N, Raftery MJ, Charbonnel N, Ulrich RG. **2008**. Hantavirus-induced immunity in rodent reservoirs and humans. Immunol Rev. 225:163-89.

Sedger LM, Shows DM, Blanton RA, Peschon JJ, Goodwin RG, Cosman D, Wiley SR. **1999**. IFN-gamma mediates a novel antiviral activity through dynamic modulation of TRAIL and TRAIL receptor expression. J Immunol. 15;163(2):920-6.

Segovia J, Sabbah A, Mgbemena V, Tsai SY, Chang TH, Berton MT, Morris IR, Allen IC, Ting JP, Bose S. **2012**. TLR2/MyD88/NF-κB pathway, reactive oxygen species, potassium efflux activates NLRP3/ASC inflammasome during respiratory syncytial virus infection. PLoS One. 7(1):e29695.

Seth RB, Sun L, Ea CK, Chen ZJ. **2005**. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell. 122(5):669-82.

Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J, Ostrander C, Dong D, Shin J, Presnell S, Fox B, Haldeman B, Cooper E, Taft D, Gilbert T, Grant FJ, Tackett M, Krivan W, McKnight G, Clegg C, Foster D, Klucher KM. **2003**. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol. 4(1):63-8.

Shi B, De Girolami U, He J, Wang S, Lorenzo A, Busciglio J, Gabuzda D. **1996a**. Apoptosis induced by HIV-1 infection of the central nervous system. J Clin Invest. 1;98(9):1979-90.

Shi L, Chen G, MacDonald G, Bergeron L, Li H, Miura M, Rotello RJ, Miller DK, Li P, Seshadri T, Yuan J, Greenberg AH. **1996b**. Activation of an interleukin 1 converting enzyme-dependent apoptosis pathway by granzyme B. Proc Natl Acad Sci U S A. 1;93(20):11002-7.

Shin OS, Kumar M, Yanagihara R, Song JW. **2013**. Hantaviruses induce cell type- and viral species-specific host microRNA expression signatures. Virology. 446(1-2):217-24.

Shresta S, Pham CT, Thomas DA, Graubert TA, Ley TJ. **1998**. How do cytotoxic lymphocytes kill their targets? Curr Opin Immunol. 10(5):581-7.

Shrivastava-Ranjan P, Rollin PE, Spiropoulou CF. **2010**. Andes virus disrupts the endothelial cell barrier by induction of vascular endothelial growth factor and downregulation of VE-cadherin. J Virol. 84(21):11227-34.

Shtrichman R, Kleinberger T. **1998**. Adenovirus type 5 E4 open reading frame 4 protein induces apoptosis in transformed cells. J Virol. 72(4):2975-82.

Sieg S, Xia L, Huang Y, Kaplan D. **1995**. Specific inhibition of granzyme B by parainfluenza virus type 3. J Virol. 69(6):3538-41.

Sironen T, Klingström J, Vaheri A, Andersson LC, Lundkvist A, Plyusnin A. **2008**. Pathology of Puumala hantavirus infection in macaques. PLoS One. 21;3(8):e3035.

Sloan E, Henriquez R, Kinchington PR, Slobedman B, Abendroth A. **2012**. Varicella-zoster virus inhibition of the NF-κB pathway during infection of human dendritic cells: role for open reading frame 61 as a modulator of NF-κB activity. J Virol. 86(2):1193-202.

Smadel JE. **1953**. Epidemic hemorrhagic fever. Am J Public Health Nations Health. 43(10):1327-30.

Spiropoulou CF, Albariño CG, Ksiazek TG, Rollin PE. **2007**. Andes and Prospect Hill hantaviruses differ in early induction of interferon although both can downregulate interferon signaling. J Virol. 81(6):2769-76.

Stegmann KA, Björkström NK, Veber H, Ciesek S, Riese P, Wiegand J, Hadem J, Suneetha PV, Jaroszewicz J, Wang C, Schlaphoff V, Fytili P, Cornberg M, Manns MP, Geffers R, Pietschmann T, Guzmán CA, Ljunggren HG, Wedemeyer H. **2010**. Interferon-alpha-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. Gastroenterology. 138(5):1885-97.

Steinman RM, Cohn ZA. **1973**. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med. 1;137(5):1142-62.

Stoltz M, Ahlm C, Lundkvist A, Klingström J. **2007**. Lambda interferon (IFN-lambda) in serum is decreased in hantavirus-infected patients, and in vitro-established infection is insensitive to treatment with all IFNs and inhibits IFN-gamma-induced nitric oxide production. J Virol. 81(16):8685-91.

Stoltz M, Klingström J. **2010**. Alpha/beta interferon (IFN-alpha/beta)-independent induction of IFN-lambda1 (interleukin-29) in response to Hantaan virus infection. J Virol. 84(18):9140-8.

Strandin T, Hepojoki J, Laine O, Mäkelä S, Klingström J, Lundkvist Å, Julkunen I, Mustonen J, Vaheri A. **2015**. Interferons Induce STAT1-dependent Expression of Tissue Plasminogen Activator, a Pathogenicity Factor in Puumala Hantavirus Disease. J Infect Dis. 23. pii: jiv764.

Sträter J, Walczak H, Pukrop T, Von Müller L, Hasel C, Kornmann M, Mertens T, Möller P. **2002**. TRAIL and its receptors in the colonic epithelium: a putative role in the defense of viral infections. Gastroenterology. 122(3):659-66.

Suliman A, Lam A, Datta R, Srivastava RK. **2001**. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene 19;20(17):2122-33.

Sundström KB, Nguyen Hoang AT, Gupta S, Ahlm C, Svensson M, Klingström J. **2016**. Andes Hantavirus-infection Of A 3D Human Lung Tissue Model Reveals A Late Peak In Progeny Virus Production Followed By Increased Levels Of Pro-inflammatory Cytokines And VEGF-A. PLoS One. Conditionally accepted 10.1371/journal.pone.0149354.

Sutton VR, Vaux DL, Trapani JA. **1997**. Bcl-2 prevents apoptosis induced by perforin and granzyme B, but not that mediated by whole cytotoxic lymphocytes. J Immunol. 15;158(12):5783-90.

Sutton VR, Davis JE, Cancilla M, Johnstone RW, Ruefli AA, Sedelies K, Browne KA, Trapani JA. **2000**. Initiation of apoptosis by granzyme B requires direct cleavage of bid, but not direct granzyme B-mediated caspase activation. J Exp Med. 20;192(10):1403-14.

Talamonti L, Padula PJ, Canteli MS, Posner F, Marczeski FP, Weller C. **2011**. Hantavirus pulmonary syndrome: encephalitis caused by virus Andes. J Neurovirol. 17(2):189-92.

Talanian RV, Yang X, Turbov J, Seth P, Ghayur T, Casiano CA, Orth K, Froelich CJ. **1997**. Granule-mediated killing: pathways for granzyme B-initiated apoptosis. J Exp Med. 20;186(8):1323-31.

Taylor RC, Cullen SP, Martin SJ. **2008**. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol. 9(3):231-41.

Taylor SL, Frias-Staheli N, García-Sastre A, Schmaljohn CS. **2009a**. Hantaan virus nucleocapsid protein binds to importin alpha proteins and inhibits tumor necrosis factor alpha-induced activation of nuclear factor kappa B. J Virol. 83(3):1271-9.

Taylor SL, Krempel RL, Schmaljohn CS. **2009b**. Inhibition of TNF-alpha-induced activation of NF-kappaB by hantavirus nucleocapsid proteins. Ann N Y Acad Sci. 1171 Suppl 1:E86-93.

Taylor SL, Wahl-Jensen V, Copeland AM, Jahrling PB, Schmaljohn CS. **2013**. Endothelial cell permeability during hantavirus infection involves factor XII-dependent increased activation of the kallikrein-kinin system. PLoS Pathog. 9(7):e1003470.

Teodoro JG, Branton PE. **1997**. Regulation of p53-dependent apoptosis, transcriptional repression, and cell transformation by phosphorylation of the 55-kilodalton E1B protein of human adenovirus type 5. J Virol. 71(5):3620-7.

Terajima M, Hayasaka D, Maeda K, Ennis FA. **2007**. Immunopathogenesis of hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome: Do CD8+ T cells trigger capillary leakage in viral hemorrhagic fevers? Immunol Lett. 15;113(2):117-20.

Tewari M, Telford WG, Miller RA, Dixit VM. **1995**. CrmA, a poxvirus-encoded serpin, inhibits cytotoxic T-lymphocyte-mediated apoptosis. J Biol Chem. 29;270(39):22705-8.

Trapani JA, Browne KA, Smyth MJ, Jans DA. **1996**. Localization of granzyme B in the nucleus. A putative role in the mechanism of cytotoxic lymphocyte-mediated apoptosis. J Biol Chem. 23;271(8):4127-33.

Trapani JA, Sutton VR. **2003**. Granzyme B: pro-apoptotic, antiviral and antitumor functions. Curr Opin Immunol. 15(5):533-43.

Tschopp J, Schroder K. **2010**. NLRP3 inflammasome activation: The convergence of multiple signaling pathways on ROS production? Nat Rev Immunol. 10(3):210-5.

Tsergouli K, Papa A. **2013**. Vascular endothelial growth factor levels in dobrava/belgrade virus infections. Viruses. 10;5(12):3109-18.

Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S, Mustonen J. **2013**. Uncovering the mysteries of hantavirus infections. Nat Rev Microbiol. 11(8):539-50.

Vaheri A, Strandin T, Jääskeläinen AJ, Vapalahti O, Jarva H, Lokki ML, Antonen J, Leppänen I, Mäkelä S, Meri S, Mustonen J. **2014**. Pathophysiology of a severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. Antiviral Res. 111:23-5.

Van Epps HL, Schmaljohn CS, Ennis FA. **1999**. Human memory cytotoxic T-lymphocyte (CTL) responses to Hantaan virus infection: identification of virus-specific and cross-reactive CD8(+) CTL epitopes on nucleocapsid protein. J Virol. 73(7):5301-8.

Van Epps HL, Terajima M, Mustonen J, Arstila TP, Corey EA, Vaheri A, Ennis FA. **2002**. Long-lived memory T lymphocyte responses after hantavirus infection. J Exp Med. 2;196(5):579-88.

Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A. **2003**. Hantavirus infections in Europe. Lancet Infect Dis. 3(10):653-61.

Vera-Otarola J, Solis L, Soto-Rifo R, Ricci EP, Pino K, Tischler ND, Ohlmann T, Darlix JL, López-Lastra M. **2012**. The Andes hantavirus NSs protein is expressed from the viral small mRNA by a leaky scanning mechanism. J Virol. 86(4):2176-87.

Vestweber D. **2007**. VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. Arterioscler Thromb Vasc Biol. 28(2):223-32.

Vial PA, Valdivieso F, Mertz G, Castillo C, Belmar E, Delgado I, Tapia M, Ferrés M. **2006**. Incubation period of hantavirus cardiopulmonary syndrome. Emerg Infect Dis. 12(8):1271-3.

Vial PA, Valdivieso F, Ferres M, Riquelme R, Rioseco ML, Calvo M, Castillo C, Díaz R, Scholz L, Cuiza A, Belmar E, Hernandez C, Martinez J, Lee SJ, Mertz GJ; Hantavirus Study Group in Chile. **2013**. High-dose intravenous methylprednisolone for hantavirus cardiopulmonary syndrome in Chile: a double-blind, randomized controlled clinical trial. Clin Infect Dis. 57(7):943-51.

Virtanen JO, Jääskeläinen KM, Djupsjöbacka J, Vaheri A, Plyusnin A. **2010**. Tula hantavirus NSs protein accumulates in the perinuclear area in infected and transfected cells. Arch Virol. 155(1):117-21.

Wang C, Youle RJ. **2009**. The role of mitochondria in apoptosis*. Annu Rev Genet. 43:95-118.

Wang SZ, Smith PK, Lovejoy M, Bowden JJ, Alpers JH, Forsyth KD. **1998**. The apoptosis of neutrophils is accelerated in respiratory syncytial virus (RSV)-induced bronchiolitis. Clin Exp Immunol. 114(1):49-54.

Wang M, Zhu Y, Wang J, Lv T, Jin B. **2011**. Identification of three novel CTL epitopes within nucleocapsid protein of Hantaan virus. Viral Immunol. 24(6):449-54.

Wang K, Ni L, Wang S, Zheng C. **2014**. Herpes simplex virus 1 protein kinase US3 hyperphosphorylates p65/RelA and dampens NF-κB activation. J Virol. 88(14):7941-51.

Wells RM, Sosa Estani S, Yadon ZE, Enria D, Padula P, Pini N, Mills JN, Peters CJ, Segura EL. **1997**. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia. Emerg Infect Dis. 3(2):171-4.

Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, Debatin KM, Krammer PH. **1995**. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature. 8;375(6531):497-500.

Wolff S, Becker S, Groseth A. **2013**. Cleavage of the Junin virus nucleoprotein serves a decoy function to inhibit the induction of apoptosis during infection. J Virol. 87(1):224-33.

Wolff S, Groseth A, Meyer B, Jackson D, Strecker T, Kaufmann A, Becker S. **2016**. The New World Arenavirus Tacaribe induces caspase-dependent apoptosis in infected cells. J Gen Virol. 14. doi: 10.1099/jgv.0.000403.

Wu M, Xu Y, Lin S, Zhang X, Xiang L, Yuan Z. **2007**. Hepatitis B virus polymerase inhibits the interferon-inducible MyD88 promoter by blocking nuclear translocation of Stat1. J Gen Virol. 88(Pt 12):3260-9.

Xie M, Dong Y, Zhou Y, Ren H, Ji Y, Lv S. **2013**. Levels of HTNV-specific CD8+ T lymphocytes in PBMC from the patients with hemorrhagic fever with renal syndrome. Intern Emerg Med. 8(6):503-8.

Xing J, Ni L, Wang S, Wang K, Lin R, Zheng C. **2013**. Herpes simplex virus 1-encoded tegument protein VP16 abrogates the production of beta interferon (IFN) by inhibiting NF-κB activation and blocking IFN regulatory factor 3 to recruit its coactivator CBP. J Virol. 87(17):9788-801.

Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB. **2005**. VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol Cell. 16;19(6):727-40.

Yanagihara R, Amyx HL, Lee PW, Asher DM, Gibbs CJ Jr, Gajdusek DC. **1988**. Experimental hantavirus infection in nonhuman primates. Arch Virol. 101(1-2):125-30.

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. **1997**. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science. 21;275(5303):1129-32.

Yang X, Stennicke HR, Wang B, Green DR, Jänicke RU, Srinivasan A, Seth P, Salvesen GS, Froelich CJ. **1998**. Granzyme B mimics apical caspases. Description of a unified pathway for trans-activation of executioner caspase-3 and -7. J Biol Chem. 18;273(51):34278-83.

Yano Y, Hayashi Y, Nakaji M, Nagano H, Seo Y, Ninomiya T, Yoon S, Wada A, Hirai M, Kim SR, Yokozaki H, Kasuga M. **2003**. Different apoptotic regulation of TRAIL-caspase pathway in HBV- and HCV-related hepatocellular carcinoma. Int J Mol Med. 11(4):499-504.

Ye L, Liu Y, Yang S, Liao W, Wang C. **2001**. Increased expression of Hsp70 and co-localization with nuclear protein in cells infected with the Hantaan virus. Chin Med J (Engl). 114(5):535-9.

Ye W, Lei Y, Yu M, Xu Y, Cao M, Yu L, Zhang L, Li P, Bai W, Xu Z, Zhang F. **2015**. NLRP3 inflammasome is responsible for Hantavirus inducing interleukin-1β in THP-1 cells. Int J Mol Med. 35(6):1633-40.

Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. **2004**. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol. 5(7):730-7.

Yu L, Ye L, Zhao R, Liu YF, Yang SJ. **2009**. HSP70 induced by Hantavirus infection interacts with viral nucleocapsid protein and its overexpression suppresses virus infection in Vero E6 cells. Am J Transl Res. 15;1(4):367-80.

Yu HT, Jiang H, Zhang Y, Nan XP, Li Y, Wang W, Jiang W, Yang DQ, Su WJ, Wang JP, Wang PZ, Bai XF. **2012**. Hantaan virus triggers TLR4-dependent innate immune responses. Viral Immunol. 25(5):387-93.

Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS, Rollin PE, Ksiazek TG, Nichol ST, Mahy B, Peters CJ. **1995**. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol. 146(3):552-79.

Zamai L, Ahmad M, Bennett IM, Azzoni L, Alnemri ES, Perussia B. **1998**. Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. J Exp Med. 21;188(12):2375-80.

Zamzami N, Kroemer G. **2003**. Apoptosis: mitochondrial membrane permeabilization--the (w)hole story? Curr Biol. 21;13(2):R71-3.

Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. **1997**. The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell. 17;91(2):243-52.

Zauli G, Secchiero P. **2006**. The role of the TRAIL/TRAIL receptor system in hematopoiesis and endothelial cell biology. Cytokine Growth Factor Rev. 17:245–257.

Zdrenghea MT, Telcian AG, Laza-Stanca V, Bellettato CM, Edwards MR, Nikonova A, Khaitov MR, Azimi N, Groh V, Mallia P, Johnston SL, Stanciu LA. **2012**. RSV infection modulates IL-15 production and MICA levels in respiratory epithelial cells. Eur Respir J. 39(3):712-20.

Zelená H, Mrázek J, Kuhn T. **2013**. Tula hantavirus infection in immunocompromised host, Czech Republic. Emerg Infect Dis. 19(11):1873-5.

Zhang Y, Liu B, Ma Y, Yi J, Zhang C, Zhang Y, Xu Z, Wang J, Yang K, Yang A, Zhuang R, Jin B. **2014**. Hantaan virus infection induces CXCL10 expression through TLR3, RIG-I, and MDA-5 pathways correlated with the disease severity. Mediators Inflamm. 2014:697837.

Zhao J, He S, Minassian A, Li J, Feng P. **2015**. Recent advances on viral manipulation of NF-κB signaling pathway. Curr Opin Virol. 15:103-11.

Zhou R, Wei H, Sun R, Tian Z. **2007**. Recognition of double-stranded RNA by TLR3 induces severe small intestinal injury in mice. J Immunol. 1;178(7):4548-56.

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. **1997**. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell. 1997 Aug 8;90(3):405-13.

"You either die a hero or you live long enough to see yourself become the villain"

- Harvey Dent from The dark knight