Influenza virus affects apoptosis: Identifying targets for therapy

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Summary.

Influenza virus infection is an infectious disease, especially dangerous for immunocompromised patients and the elderly. This disease is the cause of many fatalities each year during the annual flu outbreaks and can potentially result in the deaths of millions if a dangerous pandemic strain appears in the population. The body's immune system fights the influenza infection via several mechanisms, one being the induction of apoptosis, the regulated form of cell death. However, the influenza infection also seems capable of influencing the apoptosis pathways, probably to improve the virus propagation. Identification of the effect influenza infection has on the apoptosis pathways could lead to new ways to fight or prevent the infection. Influenza infection affects an anti-apoptotic pathway, the viral protein NS1 causes an activation of the P13K/Akt pathway. This occurs mostly in the earlier stages of the infection. Influenza infection also has effects on the pro-apoptotic pathways by inducing p53, TRAIL, FasL, caspase-3 and cytochrome c release from the mitochondria, which are all factors that lead to an increase in apoptosis in the cell, during the later stages of infection. Most of these effects appear to be a reaction of the host's body to the influenza infection, only the release of cytochrome c seems to be a direct response to a viral protein, PB1-F2. The upregulations caused by the host do seem to be profitable for the virus, since downregulation of TRAIL, FasL and caspase-3 all cause the viral propagation to decrease. Thus, influenza appears to activate anti-apoptotic pathways to prevent early clearance of infected cells and induce apoptosis through pro-apoptotic pathways later in the infection to provide necessary factors for efficient virus propagation. Targeting the factors influenza affects could lead to new ways to fight influenza infection, although much research is necessary before this is a realistic approach.

Contents.

Introduction.

Human viral influenza is one of the most important infectious diseases present in the human population. It is a highly contagious disease that can spread from person to person via aerosol droplets which infect the epithelial cells of the respiratory tract (6). Transmission can also occur from contact with infected animals for some influenza virus strains (1). In young adults without health problems an influenza infection commonly results in one to two weeks of debilitating illness with symptoms much akin to the common cold (19), like moderate to high fevers, sore throat, coughing, muscle pains, weakness and general discomfort. But influenza is a much more dangerous disease, which can certainly lead to life-threatening complications, such as pneumonia and deaths. Especially in patients with respiratory or cardio-vascular disease, young children and the elderly influenza virus infection tends to take a much more serious turn (19). Moreover, historically epidemics and pandemics of influenza viruses are responsible for the deaths of millions of people (1), for example, the Spanish Flu of 1918-1919 resulted in a worldwide mortality of 20-40 million people, many of whom were young and healthy individuals (19).

Influenza is caused by three types of RNA viruses, influenza types A, B and C, which can be distinguished from each other by the serological responses to their internal proteins. All these virus types belong to the orthomyxoviridae family. Influenza type A is the most important of the three types, responsible for the annual flu outbreaks. Each influenza A virion is constructed with eight negative single-stranded RNA-segments (Fig. 1), encoding eleven proteins (1). Influenza A can be further subtyped based on the major antigenic specificities of their surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA) (19). The HA proteins are able to bind the virus with the host cell and cause fusion with the cell and the NA proteins are responsible for assisting in the release of newly formed viral particles from the infected cells. These proteins are used to specify the subtype H and N formulae for strains of influenza A virus, resulting in the familiar H_xN_x virus indications (6). Due to its antigenic variability, influenza type A infection is also by far the most serious, since this variability can allow the virus to escape the human bodies' neutralizing antibodies, and most research on influenza viruses is therefore done with influenza A strains (19).

Once infection of the human host has occurred, a series of immune responses is triggered in order to attempt to fight of the virus infection. Humoral antibodies of complementary specificity to the HA and NA antigens of the virus are generated by the host. The antibodies aimed at the HA neutralize the infectivity of the virus and those aimed at the NA interfere with the release of newly formed virus particles, thus restricting the virus. The cell-mediated immune response is mostly based on CD8+ cytotoxic T-lymphocytes responses, these will appear in about three to four days after infection has occurred. The T-cells will detect virus-infected cells and cause lysis via perforin-mediated pathways. Class II CD4+ T helper lymphocytes will facilitate both the humoral and cellular responses and can also have cytolytic activity (1, 6). Furthermore, the body uses apoptosis induction in the infected cells in order to clear the body of these cells. However, the influenza virus itself is also capable of inducing cell death in its infected cells. This happens mostly by way of apoptosis, though some infections can also cause necrosis in certain cells. Multiple factors in apoptosis pathways have been identified that are affected by influenza virus infection, and lead to activation of intrinsic or extrinsic apoptotic pathways (11).

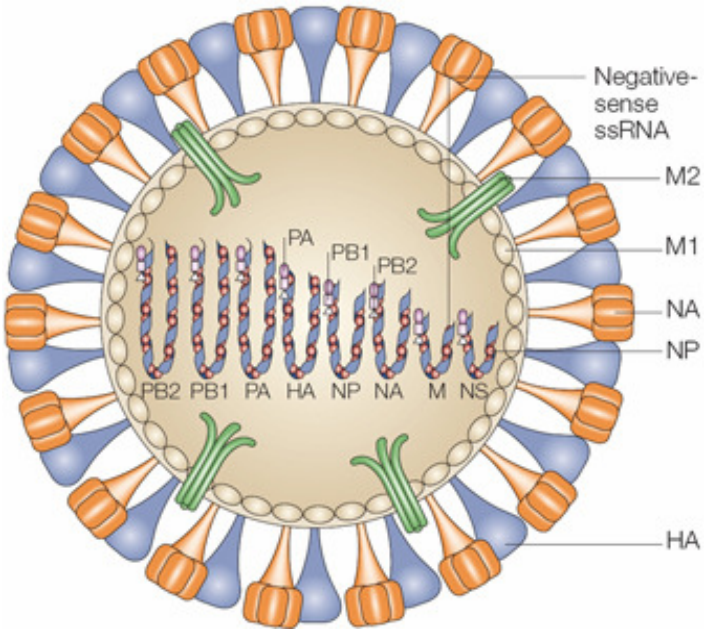

Figure 1. Structure of an influenza A virion (7). The influenza virion consists of a viral envelope, containing two surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). The envelope also contains a M2 ion channel. Beneath the envelope lies the matrix (M1 protein) which covers the viral genetic material, 8 single stranded RNA segments. Each of the looped RNA gene segments is encapsidated by nucleoprotein (NP) and associated with three polymerase proteins (PA, PB1 and PB2), which form a polymerase complex for transcription (6).

Whether the apoptosis is induced by the virus itself, or induced by the host and subsequently abused by the virus is often unclear. The timing of the effects may give a clue towards this, since the host is more likely to induce apoptosis early in the infection, to remove the infected cells from the body and may cause less apoptosis later to prevent too much tissue damage. The virus on the other hand will not induce apoptosis early, since this would impair the infection process, and may cause more apoptosis later, furthering the pathogenesis. Influenza infection can also lead to the activation of anti-apoptotic pathways as well as activation of the pro-apoptotic ones, this effect is most likely caused directly by the virus since the host organism has no profit of such a mechanism (21).

The effects influenza virus infection has on the apoptosis pathways are likely to be profitable for the infection, somehow resulting in higher replication and survival of the virus. Knowledge of the roles of these mechanisms in influenza infection could lead to understanding how and why these apoptosis pathways are affected by the influenza virus. A next step could then be to use this information in order to counter the effect in influenza infected cells, leading possibly to new ways to treat the virus infection. In this thesis the mechanisms that underlie the effect influenza virus infection has both on pro-and anti-apoptotic pathways and their roles in viral replication will be discussed in order to attempt to elucidate new targets to use in the fight against influenza virus infection.

1. The apoptosis pathways.

Cell death can occur in several ways, the most common of these are apoptosis and necrosis. Necrosis is the more passive form of cell death and most often associated with inflammation. Apoptosis on the other hand is an energy-dependent process leading to more organized cell death without inflammation (9). Cells that are undergoing apoptosis can be recognized by morphological changes like nuclear fragmentation, membrane blebbing and formation of apoptotic bodies. These apoptotic bodies are small sealed membrane vesicles, the process of formation of these vesicles in cell death prevents leakage of the cellular content, since some of the cellular components can be toxic and cause inflammation. This happens with necrosis, which is characterized by fast swelling of the cytoplasm and rupture of the plasma membrane, causing the cellular content to come free and cause inflammation (10).

Apoptosis can occur via two distinct pathways, though these pathways can potentially also influence each other in some ways, the intrinsic pathway and the extrinsic pathway (Fig. 2). Both these pathways lead to activation of the aspartate-specific cysteine proteases (caspases), which are the main executioners of the apoptotic response in the cell (5).

The intrinsic apoptosis pathway is mostly regulated by the Bcl-2 protein family, these proteins control the release of cytochrome c from the mitochondria. Within the Bcl-2 family there are both proteins that act pro-apoptotic, like Bcl-2-associated X protein (BAX), p53 upregulated modulator of apoptosis (PUMA) and Noxa, and proteins that act anti-apoptotic, like Bcl-2. A subset of this family are proteins that are p53 targets. This tumor suppressor protein is most commonly known for its ability to induce apoptosis in cancer cells. Activation of p53, usually in response to internal cues like cellular stress or DNA damage, will induce the pro-apoptotic Bcl-2 proteins BAX, PUMA and Noxa, which will then form hetero- or homodimers and cause release of cytochrome c from the mitochondria (5). In the cytosol, cytochrome c will bind to an adapter molecule called apoptotic protease-activating factor 1 (Apaf-1), which is also induced by p53, and this bond will induce a conformational change of the Apaf-1 molecule. Apaf-1, cytochrome c and procaspase-9 will then form a complex known as the apoptosome. This complex will cleave procaspase-9 and thus form the active caspase-9, which in turn will activate downstream effector caspases, caspase-3, caspase-6 and caspase-7, and lead to the execution phase of apoptosis (12).

The extrinsic apoptosis pathway works via death ligands, the most important of these being FasL and TNF-related apoptosis inducing ligand (TRAIL). TRAIL and FasL are both endogenous proteins often expressed on immune cells, or present in soluble form. These death ligands bind to their receptors on the cell surface, Fas for FasL and death receptor 4 (DR4) and death receptor 5 (DR5) for TRAIL (12). TRAIL binds as a homotrimer to DR4 and DR5, which results in trimerization of the receptors and assembly of a death-inducing signaling complex (DISC). At the DISC, Fas-associated death domain (FADD) acts as a bridge between the death receptor complex and initiator caspase-8. Caspase-8 will be activated and subsequently activate the effector caspases, caspase-3, caspase-6 and caspase-7, and this will initiate the apoptosis (3). Fas/FasL induces apoptosis in a similar fashion. The tumor suppressor protein p53, which affects the intrinsic pathway, is also capable of influencing the extrinsic pathway. It can do so by induction of several genes, namely those encoding for Fas and DR5 proteins and besides increasing transcription of Fas p53 also enhances the trafficking of this protein to the cell membrane. This results in higher levels of Fas and DR5 on the cell surface, causing the cells to be more susceptible to the death ligands (5).

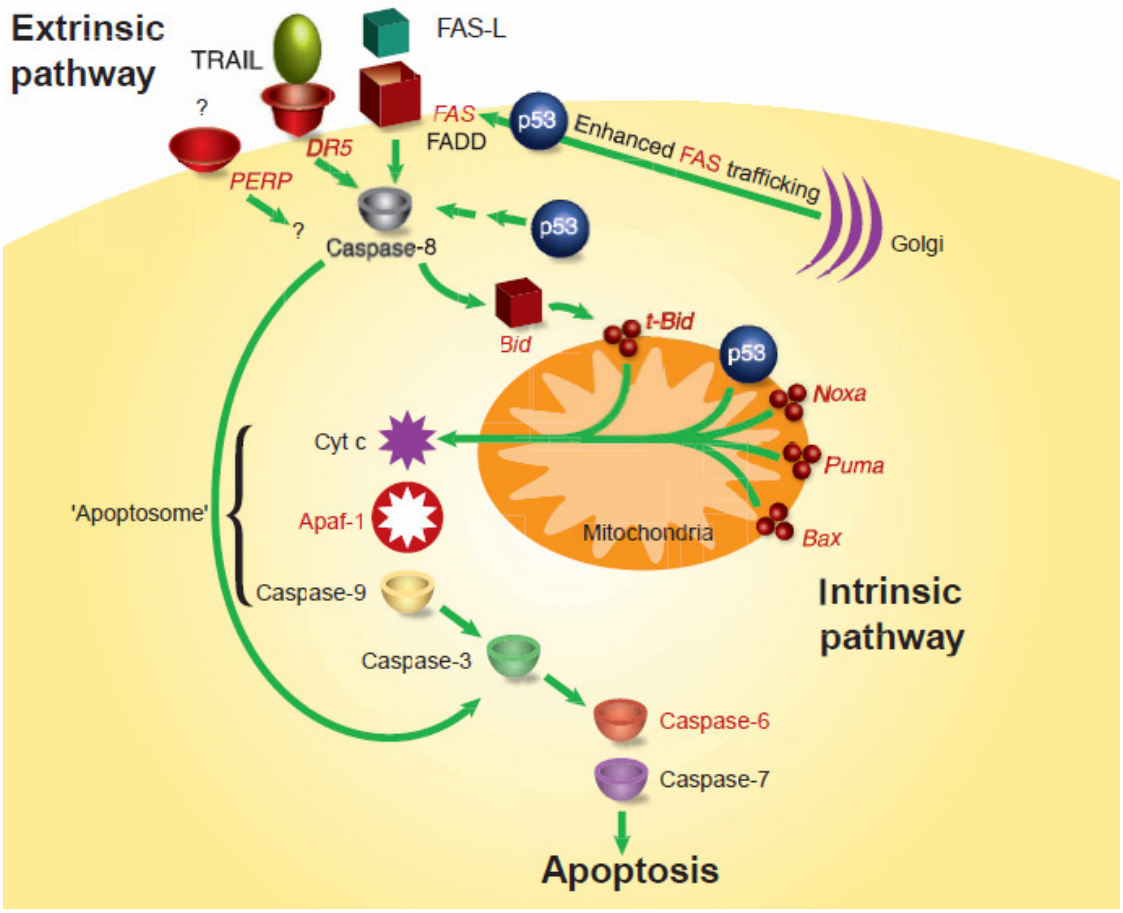

Figure 2. The apoptosis pathways (5). The intrinsic and extrinsic apoptosis pathways, as described in the text.

2. How influenza infection affects anti-apoptotic pathways.

In early stages of the infection, when the host organism attempts to remove the virus infected cells by way of apoptosis, the influenza virus seems to be able to counter this mechanism, by activating anti-apoptotic pathways. While it is known that many viruses have anti-apoptotic proteins, for influenza infection only one pathway has been extensively described, the P13K-Akt pathway. The P13K-Akt pathway is a strong intracellular anti-apoptotic pathway, which mediates its effect amongst others by inhibition of pro-apoptotic factors, like procaspase-9 and activation of anti-apoptotic factors like MDM2, which binds p53 (21).

Several effects were found on this pathway, especially on P13K and its downstream regulator Akt, after infection of cells with influenza A virus. P13K activation was found to be increased in cells infected with influenza, the activity was increased in very early stages of the infection and increased again shortly thereafter and then maintained through middle to late stages of the infection (4). The phosphorylated, and active, form of Akt was also detected in the infected cells in a higher degree than in the control situation. This upregulation of phosphorylated Akt was found in the early to middle stages of the influenza infection (21). Since phosphorylation of Akt depends strictly on the activity of P13K, these findings confirm the activation of P13K by

influenza infection. The same effect was found after infection with several different virus strains. This implies that infection with influenza A virus activates the P13K/Akt pathway in infected cells. The upregulation of the P13K/Akt pathway is induced by the influenza type A non-structural protein 1 (NS1), this is a multifunctional influenza protein expressed mostly in early stages of infection. A virus in which this protein was deleted (deltaNS1) was found to induce more apoptosis than a wild-type virus (20). Cells infected with the deltaNS1 virus barely showed increase of P13K after the initial very early activation, nor showed an upregulation of Akt phosphorylation. Furthermore, infected cells showed an earlier induction of apoptosis than the cells infected with a wild-type virus (4, 21).

The interactions between the NS1 protein and P13K have been investigated in detail. P13K consists of two subunits, a regulatory (p85) and an enzymatic subunit (p110). Activation of P13K can occur by binding of a Src Homology (SH) domain of p85 to phosphorylated tyrosine-kinase receptors or G-protein coupled receptors. NS1 also seems to be able to interact with the p85 subunit of P13K and thus activate it, since specific p85 antibodies counter the effect of NS1 on P13K. The NS1 protein of influenza A has two functional domains, a RNA binding domain and an effector domain, and three binding motifs, two motifs for SH3 binding and one motif for SH2 binding (Fig. 3). The P13K subunit p85 has two isoforms, α and β, and NS1 can bind directly to the SH2 and SH3 domains of p85β.

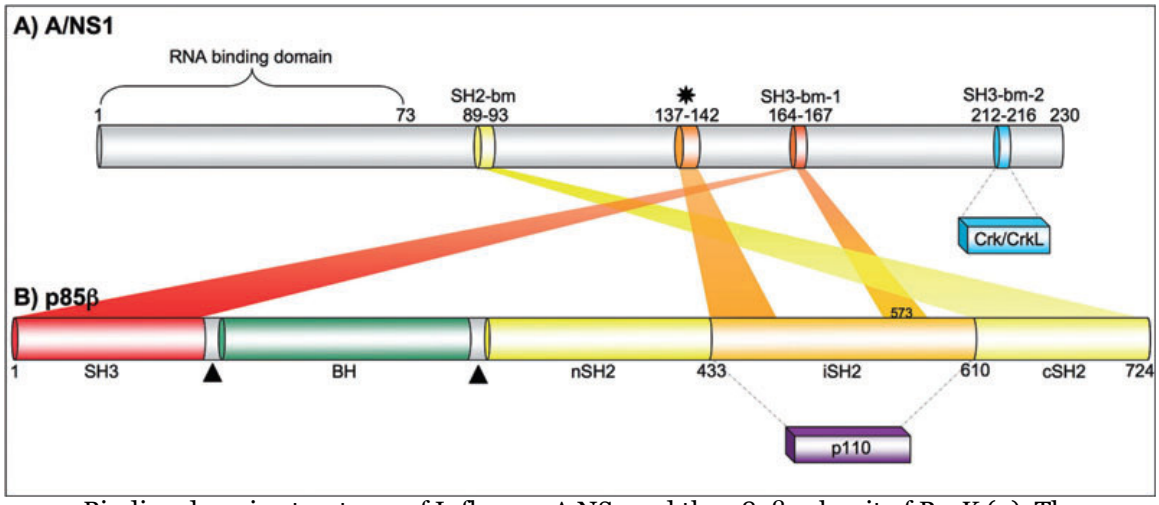

Figure 3. Binding domain structures of Influenza A NS1 and the p85β subunit of P13K (4). The presumed motifs for A/NS1 interaction with p85β or Crk/CrkL are depicted.

However, the complex-formation of p85α with NS1 only occurred in infected cells, and not in cells where both the P13K and p85α proteins were expressed from plasmids. This suggests the possibility that co-factors are required for binding with this isoform, making p85β the only direct binding target for NS1. The adaptor proteins Crk and CrkL have been identified as binding partners for NS1. NS1 binds Crk/CrkL via a SH3 binding motif, and disruption of this bond results in decreased activity of P13K (4). Unfortunately this is only useful for some influenza A strains, since this second SH3 binding motif is often not preserved in human influenza. The very early activation of P13K cannot be due to this mechanism since very little NS1 is present at that point. A possible explanation for this very early activation is the presence of double-stranded RNA, which is also known to be able to induce P13K activation (4, 14).

3. How influenza infection affects pro-apoptotic pathways.

Many studies have been done to investigate the effect influenza infection has on the apoptosis pathways in infected cells. Especially the pro-apoptotic pathways have been thoroughly investigated. Influenza infection results in activation of these pathways in multiple ways, inducing apoptosis in the infected cells. Although it is not clear for every pathway whether the influenza virus is the cause of this induction, or the apoptosis is caused as a reaction of the host cells, it is clear that apoptosis has roles in both the defense against influenza and the pathogenesis of influenza. Apoptosis is used by the immune system to clear infected cells but is also at least partially responsible for the loss of alveolar epithelial cells in the lungs, which can cause pneumonia.

The p53 tumor suppressor protein is one of the targets that influenza infection influences to induce apoptosis in infected cells. This protein was found to be elevated in the lungs of infected animals and after this discovery tests were done on different human cell types, like primary human lung bronchial epithelial cells (15). After infection with influenza there were higher levels of p53 present, compared with the control situation. The transcription activity was also confirmed to be upregulated in response to influenza infection, since specific p53 regulated genes were increasingly expressed. To confirm that p53 was the cause of the increased apoptosis that was observed, a situation was created with inhibited p53 activity. The apoptosis levels here were significantly lower, confirming that influenza induced upregulation of p53 was the cause of the apoptosis levels observed in infected cells. Furthermore, inhibiting p53 also resulted in an increase of viral titers, suggesting it is involved in the host's defense mechanism against the virus infection (15). The upregulation of p53 occurred independently of cell type or viral strain, indicating that p53 is a protein commonly activated during influenza induced cell death. The upregulation of p53 did not only occur at the late stage of infection, when apoptosis is induced, but in a biphasic pattern. An initial elevation occurred at the beginning phase of the infection, and a second elevation at middle to late stage infection. While the second elevation was shown to occur in response to viral replication, the first elevation took place even in cells infected with a virus that was incapable of replication (13), suggesting that the initial elevation of p53 is caused by virus adsorption alone. Since no specific viral protein appears to be involved in this upregulation of p53, it is likely to be solely a response of the host's defense mechanisms.

The p53 protein is known to influence both the intrinsic and extrinsic apoptosis pathways, causing increased levels of apoptosis through both these pathways. Influenza infection appears to also be capable of inducing apoptosis through these pathways individually, independently of the p53 protein.

The fact that influenza infection is capable of affecting the intrinsic apoptosis pathway has been confirmed through experimental research. After infection with influenza downregulation of the anti-apoptotic protein Bcl-2, upregulation of the pro-apoptotic protein BAX, release of cytochrome c from the mitochondria and upregulation of caspase-9 was found (2). All these are factors known to play a role in the intrinsic mitochondria-mediated apoptosis pathway. These results were obtained using swine flu strains, which are genetically and antigenically similar to human influenza viruses. But the same results were not found for all the cell types and virus strains that were tested. Implying that, while influenza is capable of influencing apoptosis through the intrinsic pathways, this induced cell death may differ in regulation between cell types and viral strains.

It is unclear what mechanisms cause these effects on factors of the intrinsic apoptosis pathway, and whether they are caused by the virus or the host. Only one way has so far been identified through which the influenza virus can actively induce apoptosis through the intrinsic pathway, namely PB1-F2, which is an influenza virus gene product. This gene product can activate the apoptotic pathway at the mitochondria regulator step by inducing release of cytochrome c (11). Treatment of mitochondria with PB1-F2 resulted in a concentration dependent release of cytochrome c, suggesting a direct effect of PB1-F2 on the mitochondrial membrane permeabilization. The PB1-F2 protein was found to interact with two mitochondrial proteins, adenine nucleotide translocator 3 (ANT3) and voltage-dependent anion channel 1 (VDAC1), both part of the permeability transition pore complex (18). ANT3 is a mitochondrial protein localized to the inner membrane and plays a role in apoptosis induction by creating nonspecific pores in this membrane. Using ANT3 blockers resulted in an attenuation of PB1-F2 induced membrane depolarization and reduction of apoptosis. VDAC1 is also a protein involved in the membrane pore complex, but localizes to the mitochondrial outer membrane where it forms pores. Three possible mechanisms of the permeability induction of PB1-F2 have been proposed: direct permeabilization by multimeric complexes of PB1-F2, separate effects at the inner and outer membrane in conjunction with ANT3 and VDAC1 and formation of a bridge between the two mitochondrial proteins caused by PB1-F2 which enhances the formation of pores in both membranes (18).

The apoptosis induction through the extrinsic pathway has mostly been described in relation with the death receptor ligands. TRAIL, one of the death receptor ligands, is usually only effective on tumor cells. Normal cells are not very susceptible to TRAIL, which makes it possible for TRAIL to be present in areas of the body in a stable concentration. The human lung for example has an abundance of TRAIL present, and when infection with an influenza virus occurs the levels of TRAIL mRNA rise even further. Also, TRAIL is induced on the cell surface of Natural Killer (NK) cells and T-lymphocytes (8). TRAIL expression on NK-cells and T-cells is regulated by T-cell receptor signaling and cytokines. Most likely the increase of TRAIL is mediated via induction of interferon (IFN), as reaction to the viral infection by the host. The death receptors, especially DR5, were also upregulated as a response to influenza infection and sensitivity of virus infected cells to TRAIL increased as well (8). These data all imply that TRAIL is upregulated in response to virus infection in order to clear the infected cells from the body. However, TRAIL induction also appears to further the pathogenesis of influenza. Avian influenza virus capable of infecting humans, H5N1, is able to infect human monocyte–derived macrophages (MDMs), and induce an up-regulation of TRAIL and TNF-α, which is another death receptor ligand. The TRAIL that is produced by these MDMs may contribute to the severe lung damage and lymphopenia, dangerously low amounts of lymphocytes in the blood, observed clinically in H5N1 patients (22).

One way through which influenza infection may cause the upregulation of TRAIL is NF-κB. This transcription factor is activated by multiple families of viruses, including influenza viruses. In general it acts as an antiviral factor, by inducing inflammation and immune responses. NF-κB is also known to be a regulator of apoptosis, usually by up-regulating anti-apoptotic factors. However, NF-κB appears to also control gene products as TRAIL and FasL, both products which are involved in the extrinsic apoptosis pathway as death receptor ligands. Strangely, virus propagation was not enhanced upon NF-κB inhibition. In fact, the opposite effect occurred and the propagation was impaired, suggesting that NF-κB is a necessary factor for the influenza virus. Inhibition of NF-κB also resulted in a block of virus-induced TRAIL synthesis, and a significant decrease of FasL expression. Inhibitors of TRAIL and FasL resulted in a dose dependent decrease of virus production when used individually (17). This implies that, while NF-κB has antiviral effects, its proviral effects appear to be more outspoken. Influenza infection

induces NF-κB, which induces TRAIL and FasL and apoptotic processes, and all three factors act in a proviral manner.

Thus it seems that while the host initiates apoptosis in infected cells to rid the body of infection, the influenza infection itself also induces apoptosis in the cells and for some effects it is yet unclear whether they are a result of host or viral mechanisms. Downregulation of TRAIL and FasL had a negative effect on virus propagation. Another factor that seems to be necessary for efficient virus propagation is caspase-3. This is an effector caspase activated at the very end of both the intrinsic and the extrinsic apoptosis pathways. Inhibition of caspase-3 led to a significant decrease in virus titers, and further evidence was found in infecting a cell line without caspase-3 expression with influenza A virus, which resulted in very poor replication of the virus. Manually supplying the cells with procaspase-3 however caused the replication to increase significantly (16). This all suggests that caspase-3 is necessary for the propagation of influenza virus. The reason influenza virus needs caspase-3 may lie in the genome export. The influenza virus needs to export its genome in the form of ribonucleoprotein (RNP) complexes from the nucleus to the cytoplasm of the infected cell. The cytoplasmic accumulation of these complexes is inhibited after blocking caspase-3. Caspase-3 can affect the nuclear pores, probably allowing for diffusion of the RNPs to the cytoplasm, caspase-3 then supports virus replication by enhancing RNP migration to the cytoplasm (16).

4. The role of the effects on apoptosis in virus viability

What use are all these effects for the influenza virus itself? While it is at best debatable whether a virus is a living being, it does need to replicate and survive, otherwise many viruses would have disappeared long ago. Therefore, it needs to stay present in a host organism long enough to replicate and infect another, or multiple other, potential hosts. The effects an influenza virus infection has on the apoptosis pathways in the cell will therefore probably somehow have positive effects on the replication or survival of the virus. These interests of the virus naturally conflict with those of the host organism, which will try to prevent the replication and survival of the influenza virus. Therefore, it would be logical to see opposite effects on the apoptosis pathways of the virus and the host.

In early to middle stages of the infection opposite effect are indeed present. The effect the influenza virus seems to have is anti-apoptotic, since an anti-apoptotic pathway is activated by the viral protein NS1. Apoptosis is of course used by the host as a defense mechanism, in order to kill cells infected with influenza virus. The host will therefore activate pro-apoptotic mechanisms early in the infection. Countering these mechanisms will then provide the virus with the opportunity to replicate. Early apoptosis induction in the infected cells would quickly eradicate the virus in the host, leaving no possibility for virus replication. Stopping the apoptosis induction is then a very profitable mechanism for the influenza virus.

In later stages of the infection however the virus and the host both give similar effects. The virus starts to give the complete opposite effect than in early stages of the infection. In many different ways it affects pro-apoptotic pathways resulting in apoptosis induction of the infected cells. At first this does not appear to make much sense, since induction of apoptosis in influenza infected cells is exactly what the host organism also attempts to do, and the virus seems to be assisting in its own eradication. A closer look however gives a logical explanation for this effect. Viral induction of apoptosis seems to be essential for replication of the virus itself. Several factors known to be involved in inducing apoptosis, like TRAIL and caspase-3, are also factors

necessary for virus propagation. Blocking these factors will reduce viral titers. So, influenza virus infection causes apoptosis in late stages of infection for its own good, to aid its own propagation. The induction of apoptosis by the host in late stages of infection may be helping the virus propagation more then fighting it, since it provides the virus with factors necessary for its propagation.

Conclusion.

In this thesis the effects influenza virus infection has on the pro- and anti-apoptotic pathways were described, in the context of usefulness for the virus. Influenza virus infection, in early stages, gives an anti-apoptotic effect. This is most likely to eliminate the apoptosis response the host organism will give as reaction on the infection. This allows the virus to remain and replicate in the host. In later stages of the infection the virus affects pro-apoptotic pathways in order to cause apoptosis in the infected cells. This appears to be a necessary step for the virus, to provide factors needed for viral propagation, and thus also leads to better virus survival. A precise understanding of these mechanisms could be very useful for creating new ways to fight influenza infections. All mechanisms and effects a virus has, that are useful for the virus itself, are also potentially ways to combat the infection.

The anti-apoptotic effect influenza has much potential for finding new ways to treat influenza infection. If this effect could be blocked, in early stages of the infection, it could potentially lower the chances that the infection will take hold. While it is difficult in healthy people to identify very early infections, in immunocompromised subjects, who are watched more closely, this could be a way to help fight the infection. If the virus is unable to counter the early apoptotic effects in response to infection, the body might more easily rid itself of the infection. However, the P13K-Akt pathway is but one pathway identified to have an anti-apoptotic effect activated by a virus protein. Viruses, like influenza, could potentially have more then one anti-apoptotic effect, through entirely different pathways that lead to similar effects. Blocking only one pathway may then not give the results desired. However, since the effect influenza has on the P13K/Akt pathway has been described in great detail and is clearly a result of a specific viral protein, this is a very interesting target.

The effect influenza has on the pro-apoptotic pathways might not be the best to counteract, since apoptosis induction is also an important part of the host defense mechanism. However, there are many factors involved in the apoptosis pathways and it might be possible to block the effect of the virus without impairing the immune system itself too much. Caspase-3 seems a likely target, since it has been shown that blocking this will reduce virus titers and it is a factor essential for efficient propagation. Also, caspase-3 is a part of both the intrinsic and extrinsic apoptosis pathways, meaning that it will reduce apoptosis induced through both pathways. However, blocking caspase-3 will also lead to slower removal of the virus infected cells by apoptosis, letting the virus spread inside the body. This might eventually lead to negative effects for the patients. For blocking p53, TRAIL and FasL the same argument holds true, since these are all also involved in the clearance of virus infected cells. NF-κB is also a difficult target since inhibiting this transcription factor will also inhibit its anti-viral effects and its upregulation of TRAIL and FasL. The PB1-F2 protein is a viral protein, thus finding a way to counter the effects of this protein will not affect the apoptosis induced by the host itself. Inhibiting the protein may lead to less apoptosis induction through the intrinsic pathway, however the virus will in later stages of the infection still be able to abuse apoptosis factors like caspase-3 and TRAIL that the hosts own defense mechanisms will provide.

It is still a long way to developing medication based on the knowledge and potential targets described in this thesis. All experiments were done in cell cultures or animals, which gives a good idea of function in general but is hardly a guarantee of getting the same effect in human beings. There are many unknown factors that might affect these pathways and mechanisms, and extensive cell line and animal research is still necessary before attempts in human patients could even be tried. Attempts could be made to inhibit the targets of influenza infection and their downstream effectors, NF-κB, TRAIL, FasL, p53, caspase-3, P13K and Akt in animals infected

with influenza, to see if there is a positive effect on the progress of the disease. Alternatively, research could be done to find a way to block the viral proteins, PB1-F2 and NS1, to prevent these from affecting the apoptosis pathways. However, basic research to improve the knowledge of the precise mechanisms involved in the ways influenza affects apoptosis in the host cells is still necessary. When the mechanisms are well understood it might be easier to find a way to block the profit the virus has of the apoptosis induction and still leave the hosts defense mechanisms intact to provide clearance of infected cells. If all ways that influenza influences anti-apoptotic pathways are understood, these could be blocked early in the infection to allow for rapid clearance of the infection in the body. This could be very profitable for the world, since influenza is still a dangerous disease, costing many lives each year, and vaccination is difficult due to the variability of the virus.

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