Influenza A virus-induced apoptosis and virus propagation

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ABSTRACT

Influenza A viruses (IAVs) are respiratory pathogens that cause severe morbidity and mortality worldwide. They affect cellular processes such as proliferation, protein synthesis, autophagy, and apoptosis. Although apoptosis is considered an innate cellular response to invading infectious pathogens, IAVs have evolved to encode viral proteins that modulate host cellular apoptosis in ways that support efficient viral replication and propagation. An understanding of the modulation of host responses is essential to the development of novel therapeutics for the treatment of IAV infections. In this review, we discuss the IAV lifecycle, biology, and strategies employed by the virus to modulate apoptosis to enhance viral survival and establishment of an infection.

1 INFLUENZA A VIRUS-INDUCED APOPTOSIS AND PROPAGATION

1.1. THE INFLUENZA A VIRUS (IAV)

Influenza viruses are pathogenic micro-organisms that cause severe morbidity and mortality worldwide. Annually, seasonal influenza virus infections are associated with between 250,000–300,000 deaths worldwide, and account for approximately 3,000,000 cases of severe illness (1). The hallmark of IAV infection is inflammation; infection is characterized by sudden onset of fever, headache, cough, sore throat, and muscle aches. The fever and acute symptoms associated with infection may last between 7–10 days, and are accompanied by persistent fatigue. Most seasonal IAV infections are symptomatic, however, severe disease may result in lung inflammation and associated tissue injury due to dysregulation of the immune response (2). Those at increased risk of mortality from IAV infection include pregnant women, individuals with immunosuppressive conditions such as human immunodeficiency virus infection/acquired immunodeficiency syndrome, infants, the elderly, and patients with chronic conditions (e.g. chronic obstructive pulmonary disease, renal, and hematological diseases) (3, 4). Increased risk from IAV infection in these subgroups may, in part, be explained by weakened immune systems that ineffectively clear viral pathogens.

Influenza viruses comprise three of the five genera of the Orthomyxoviridae family, which includes Influenza A, Influenza B, and Influenza C, each with a different pathogenicity and host range (5-7). Of interest in this review, IAV can cause frequent seasonal epidemics with severe outcomes. IAV particles contain a 13 Kbp negative-sense RNA genome, made up of eight single-stranded segments, encased in a lipid bilayer. This 8-segmented genome encodes approximately 14 proteins, some of which have been identified only in recent years (8). The segmented genome of IAV allows for easy recombination and antigenic shift, resulting in novel antigens that are immunologically-naïve to the human population (9). During antigenic shift, two different strains of the same virus, or two or more strains of different viruses, combine to form a new subtype with a mixture of surface antigens; in contrast, antigenic drift results from naturally-occurring mutations that arise during viral replication and the absence of proof-reading function of the viral RNA polymerase . Thus, the ability of

IAV to rapidly evolve can result in highly pathogenic viral strains, and novel treatment regimens are needed for the management of IAV infections and for the mitigation of pandemic situations (10).

1.1.1. The IAV structure and contents

The segmented genome of IAV encodes hemagglutinin (HA), neuraminidase (NA), matrix proteins 1 and 2 and M2-related protein (M1, M2, and M42, respectively), nuclear export protein (NEP, also known as NS2), nucleoprotein (NP), non-structural protein 1 (NS1), three polymerase acid proteins (PA-X, PA-N115 and PA-N182), two polymerase basic protein 1 proteins (PB1-F2 and PB1-N40) and polymerase basic protein 2 (PB2) (6, 11, 12). The viral polymerase is comprised of PA, PB1, and PB2 proteins in complex with NP, and is required for the assembly of viral ribonucleoprotein (vRNP) and for viral RNA synthesis (13, 14).

The structure of IAV is shown in Figure 1. HA and NA are glycoproteins found on the surface of the viral particle (virion), and which appear as spikes when viewed under the electron microscope (15, 16). M2 is an ion channel protein which is also present on the virion surface, but in relatively lower quantities. The M1 protein forms a matrix beneath the viral envelope and holds together the vRNPs; in addition, M1 and NEP/NS2 are reported to mediate nuclear export of vRNPs (17, 18). The non-virulence factor, NS1, plays a role in inhibiting host immune responses during viral infection (19). When packaged together and budded from an infected cell, these viral proteins form an infectious particle.

IAV replicates mainly in the epithelial cells of the nose, throat, and lungs; however, it is also able to replicate in immune cells such as alveolar macrophages and dendritic cells (6). The IAV replication cycle (Figure 2) can be divided into distinct, multi-step processes, which include the attachment and entry of the viral particle into the host cell, viral RNA nuclear import, viral RNA transcription and nuclear export, viral protein synthesis, and the assembly and budding of the viral particle (20).

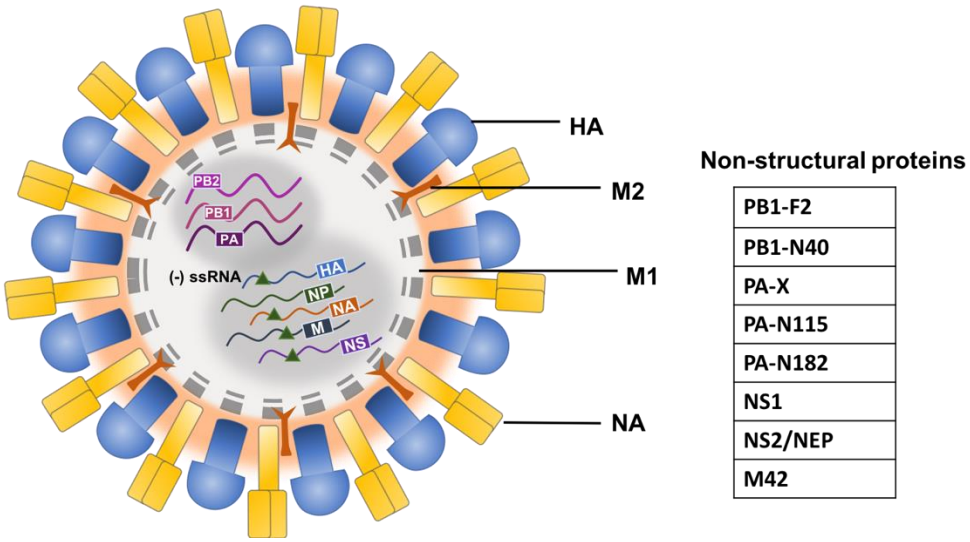

Figure 1 | The influenza A virion structure and genetic contents. The influenza A virus is comprised of a lipid bilayer embedded with two glycoproteins, neuraminidase (NA) and hemagglutinin (HA). The transmembrane M2 ion channel protein is also interspersed throughout the viral envelope, while the M1 protein forms an internal matrix to support vRNPs. The vRNPs consist of the segmented viral genome and nucleoprotein (NP) in association with the polymerase acid proteins (PA) and polymerase basic proteins 1 and 2 (PB1 and PB2).

1.1.2. Viral attachment and entry into the host cell

Host specificity is conferred by the HA proteins tethered to the lipid bilayer of IAV, which recognize and bind to specific N- or O-substituted derivatives of neuraminic acid, known as sialic acid, on the cell surface. IAV strains that are pathogenic to humans recognize α (2,6) linkages, whereas those infecting avian and equine species prefer an α (2,3) linkage (6, 21). Swine IAV strains binds to sialic acid moieties with either linkage, making swine a useful and convenient reservoir for both human and avian influenza, and allowing for periodic zoonotic infections. Upon sialic acid binding on the host cell surface, IAV is internalized by receptor-mediated endocytosis, where the acidic conditions (pH 5–6) within the endosome promote two significant events that result in the uncoating of vRNPs into the host cell cytoplasm (22). Firstly, a conformational change in HA exposes a fusion protein which enables the viral envelope to fuse with the endosomal membrane; secondly, hydrogen ions (H^+) are pumped from the endosome into the virus particle via the M2 ion channel embedded within the viral envelope (9).

1.1.3. vRNP nuclear import

IAV replication is dependent upon host cell factors, as such, vRNPs require nuclear translocation for the replication and translation of new viral proteins. Once inside the host cell cytoplasm, nuclear localization signals (NLS) present on the viral NP, part of the vRNP, recognize and bind to the host nuclear import machinery, which mediates entry into the nucleus (23, 24) and subsequent viral replication.

1.1.4. vRNP transcription and nuclear export

The negative-sense RNA genome of IAV requires that it is converted to positive sense to serve as a template for successful transcription. Human cells do not possess an RNA-dependent RNA polymerase (RdRp) capable of catalyzing the replication of RNA from RNA templates; thus, IAV has evolved its own polymerase to perform this function, and the IAV RdRp is able to initiate RNA synthesis on the negative-sense viral RNA genome (20). The PB2 subunit of the RNA complex first recognizes and cleaves host cell pre-mRNA that are capped. The cleaved mRNA cap then serves as a primer for the transcription of the viral negative-sense RNA, catalyzed by PB1 (25, 26). The newly synthesized positive-sense RNA strand may then either serve as a template for the synthesis of new virions, or is exported by cellular exportins to cytoplasmic ribosomes for translation into viral proteins (9). Nuclear export of vRNPs via nuclear pores appears to be mediated by NP binding to chromosomal maintenance 1 (CRM1), also known as exportin 1 (27, 28). Additional evidence also implicates NEP/NS2 and M1 proteins in the cytoplasmic export of vRNPs following transcription (17, 18).

1.1.5. Viral protein synthesis

In the cytoplasm, exported viral mRNA are translated into viral proteins in a cap-dependent manner, a process facilitated by cellular guanine-rich sequence factor 1 (GRSF1), which mediates recruitment of viral mRNA containing 5’ untranslated region (UTR) GRSF1 binding sites to the ribosomes (29, 30).

IAV genomic segments 1, 4, 5, and 6) encode a single protein each, whereas due to alterntative splicing, segments 2, 3, 7, and 8 encode two or more proteins . Segment

2 encodes PB1-F2 and PB1-N40, while the polymerase acidic proteins PA-X, PA-N115 and PA-N182, are encoded on segment 3. Of interest, segment 7 of IAV has been reported to produce four mRNA transcripts. The unspliced transcript encodes M1, while M2 is encoded on the spliced mRNA2 transcript. More recently, the mRNA4 transcript from segment 7 has been shown to produce a functional variant of M2, termed M42 (31). , Segment 8 encodes the two non-structural proteins, NS1 and NEP/NS2, within different reading frames.

1.1.6. Assembly of the virion, budding and release

All eight segmented genomes of IAV (Figure 1) must be present for a virion to become a fully infectious particle (9). Two models have been proposed to explain the mechanisms by which the viral segments are packaged into virions: (i) the random incorporation model and (ii) the specific incorporation model (20, 32). The former suggests that packaging of viral segments occurs randomly, and that only those virions with all eight segments present may become infectious particles (33). On the other hand, the latter model purports the presence of distinct signals within each of the viral RNA segments that dictate packaging of all eight components into a single virion (32).

The IAV must now be transported to the cell surface to be released or bud from the host cell. For budding to occur, the trans-golgi network (TGN), together with the coat protein I (COPI) complex and GTPase Rab8, traffic newly synthesized HA, NA and M2 proteins to the apical plasma membrane (34, 35). At the plasma membrane, the membrane-embedded NA, HA and M2 proteins assemble with viral genomic segments to form a virion, and M1 and M2 proteins mediate final closure and budding (36-38). In addition, NA releases IAV from sialic acid residues on the host cell surface, enabling subsequent infection of neighboring cells. Cellular host factors also appear to contribute important roles to the budding and release of infectious viral particles; for example, casein kinase 2 (CK2) significantly decreased the budding of IAV from infected MDCK cells (39).

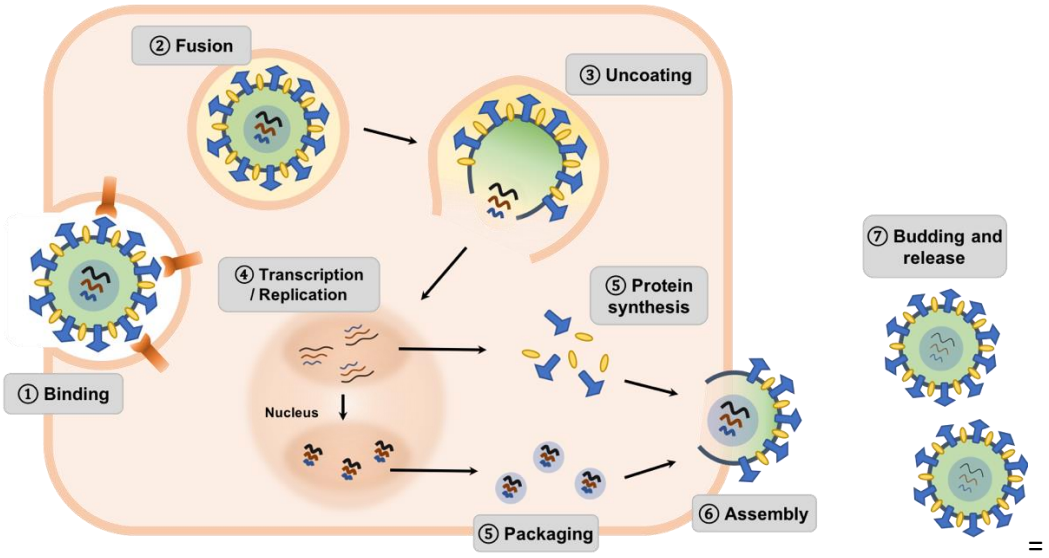

Figure 2 | The influenza A viral lifecycle. The lifecycle begins by binding of the virus to sialic acid residues on the surface of receptive cells. This is followed by endocytosis of the virus into the endosomal compartment, where low pH mediates release of the segmented viral genome into the cytoplasm. The viral genome is consequently translocated to the nucleus where it is transcribed and assembled into vRNPs. The transcripts are then exported to the cytoplasm by viral M1 and NEP/NS2 for protein synthesis. Viral particles assemble at the cell membrane and the newly-formed viral progeny bud into the extracellular space to infect neighboring cells.

1.2. VIRAL SURVIVAL STRATEGIES

From the initial attachment of the virus to the host cell, to replication and budding, viruses have co-evolved with host immune responses and have developed mechanisms to avoid detection and to manipulate host factors to facilitate their own replication and infectivity (14, 40). IAV regulates the expression of host cell proteins including G-protein coupled receptors, Annexin A1, and cytokines (41-43). In particular, IAV employs a plethora of strategies to avoid detection by both the innate and adaptive immune systems to establish an infection. Notable strategies include modulation of apoptosis (44-47), inhibition of type I interferon (IFN) production and antiviral signaling (14, 19) (48, 49), regulation of autophagy (50, 51) and effects on B cell antibody production (52). In this article, we review in detail the effects of IAV-induced apoptosis on viral propagation and replication.

1.3. APOPTOSIS

Apoptosis is a highly regulated form of programmed cell death that occurs as a homeostatic control of cell growth, or as a cellular immune response to invading pathogens (53). It is characterized by cell shrinkage, membrane blebbing, DNA fragmentation, chromosomal condensation, and caspase activation (54). To date, two primary mechanisms have been proposed to explain activation of the apoptotic pathway: (i) the intrinsic or mitochondrial pathway, and (ii) the extrinsic or death receptor pathway (Figure 3) (53, 55).

1.3.1. The intrinsic apoptosis pathway

Drivers of the intrinsic apoptosis pathway include factors such as nitric oxide, cytochrome c, and second mitochondria-derived activator of caspases (SMAC). Nitric oxide modulates apoptosis by altering mitochondrial membrane potential or permeability, (56-58) which facilitates mitochondrial cytochrome c release. Upon release, cytochrome c initiates apoptosis by binding to apoptosis protease activating factor-1 (APAF-1) and ATP; this forms a complex with pro-caspase 9, which is then cleaved into its active caspase 9 form. In turn, caspase 9 cleaves its effector, pro-caspase 3 (59). SMAC, on the other hand, is a mitochondrial protein which also localizes in the cytosol when cells commence apoptosis (60). In the cytosol, SMAC binds to inhibitor of apoptosis proteins (AIPs) to remove their inhibitory activity and initiate caspase 9 activation (61).

1.3.2. The extrinsic apoptosis pathway

In mammals, the extrinsic pathways that initiate apoptosis involve ligand-receptor interactions that occur at the cell surface. Two models have been proposed to explain direct apoptotic activation, both of which involve death receptors belonging to the tumor necrosis factor (TNF) receptor gene superfamily (62, 63). These death receptors possess an intracellular death domain that plays an essential role in transducing death signals from the cell surface to intracellular pathways to trigger apoptosis. Widely-studied models include the first apoptosis signal (FAS) receptor-FAS ligand (FASR/FASL) model and the TNF-α-TNF receptor 1 (TNFα/TNFR1) model (53).

FAS ligand binds to its transmembrane receptor, FASR, also known as APO-1 or CD95, (64, 65) to recruit FAS-associated death domain protein (FADD); this is followed by an association with pro-caspase 8 to form the death-inducing signaling complex (DISC), and ultimately leads to the activation of caspase 8 (53). On the other hand, binding of TNFα to TNFR1 results in an interaction with TNFR1-associated death domain protein (TRADD), which recruits FADD and receptor-interacting protein (RIP) (63, 66). Using its death effector domain, FADD associates with pro-caspase 8 to form the DISC, resulting in caspase 8 activation and apoptosis.

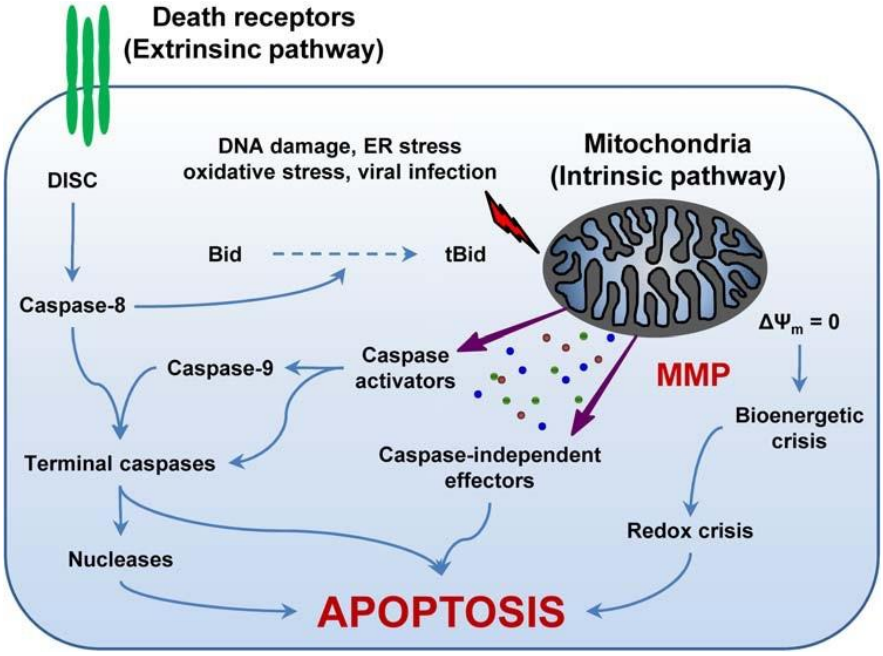

Figure 3 | Pathways involved in the activation of apoptosis. Apoptosis occurs via two main pathways (intrinsic and extrinsic). Pro-apoptotic factors such as DNA damage, ER stress, and infections activate intrinsic apoptotic pathways by altering mitochondrial membrane permeability. This results in the release of factors that induce apoptosis. The extrinsic pathway involves the activation of cell surface death receptors including FASR or TNFR1, which initiate a cascade of events that culminate in cell death. tBID (the truncated form of BID) interconnects the two apoptotic pathways; activation of caspase 8 by DISC in the extrinsic pathway results in BID truncation and can alter mitochondria permeability. (Adapted from Viral control of mitochondrial apoptosis. - Galluzzi et. al., 2008)
BID, BH3-interacting domain death agonist; DISC, death-inducing signaling complex; ER, endoplasmic reticulum; MMP, matrix metalloprotease; tBID, truncated BID.

1.4. IAV AND APOPTOSIS

During IAV infection, programmed cell death by infected host cells is thought to be a defensive mechanism to inhibit viral replication. However, over the years, viruses have evolved strategies that modulate host apoptotic responses to facilitate their own survival and propagation (67).

One of the first studies to elegantly demonstrate the anti-apoptotic and pro-apoptotic functions of IAV was by Zhirnov and Klenk in 2007 (68). IAV was shown to inhibit apoptosis during the initial phase of infection by upregulating the anti-apoptotic phosphoinositide-3-kinase–protein kinase B (PI3K-AKT) pathway, and in the later phase of infection, by suppressing this pathway and upregulating the pro-apoptotic p53 pathway. In this manner, the virus can prevent cellular apoptosis and permit sufficient time for the replication and production of viral proteins and for virion formation. Once the virus initiates apoptosis, virions are released from the cell, allowing for neighboring cells to be infected. In recent years, several studies have described different roles of various viral proteins in modulating host apoptosis.

1.5. VIRAL PROTEINS AND THEIR APOPTOTIC EFFECTS

In recent years, several studies have described different roles of the various viral in modulating apoptosis. The next section (and table 1) discussions and summarizes the IAV proteins and their functions in apoptosis

1.5.1. IAV nucleoprotein (NP)

IAV NP is a viral protein that functions to promote apoptosis through the attenuation of ring finger 43 (RNF43) protein expression. RNF43 is a member of the ring finger family of ubiquitin ligases, which are highly expressed in human colorectal and hepatocellular carcinomas, and exerts its anti-apoptotic function by destabilizing p53 (69). NP targets the interaction between RNF43 with p53, leading to improved stability of p53 and the subsequent induction of apoptosis, correlating with increased viral replication (70). In addition, IAV NP can induce apoptosis by interacting directly with host apoptotic inhibitor 5 (API5) (71), a protein demonstrated to prevent E2F transcription factor 1 (E2F1)-dependent apoptosis under growth-deficient conditions

(72). Hence, interaction of API5 with IAV NP increases the availability of E2F1, which initiates cellular apoptosis.

A further critical piece of evidence supporting the role of IAV NP in inducing host cellular apoptosis is its interaction with clusterin. Clusterin is a heterodimeric protein thought to play a role in apoptosis and in the clearance of cellular debris (73). Clusterin prevents apoptosis by binding to pro-apoptotic bcl-2-associated X protein (BAX), thereby inhibiting BAX translocation into the mitochondria. BAX is localized in the cytosol in most healthy cells; upon the trigger of apoptosis, BAX is activated and undergoes a conformational change, which allows for mitochondrial docking and later translocation (74, 75). Interaction of NP with clusterin reduces its association with BAX, thus allowing for mitochondrial translocation and subsequent apoptosis (47). The role of NP in apoptosis thus appears to favor viral replication and suggests that it may present a suitable target for anti-IAV therapies.

1.5.2. IAV non-structural protein 1 (NS1)

IAV NS1 appears to possess a dual function in regulating apoptosis during infection. While some reports have implicated NS1 in promoting cellular apoptosis, contrary evidence also suggests that NS1 may suppress apoptotic pathways to allow for full virus maturation during the earlier phases of infection. In the early 2000s, studies by two groups demonstrated an inhibitory role of IAV NS1 in apoptosis of cultured cells; mutant IAV lacking the NS1 gene induced apoptosis more rapidly than wildtype virus (45, 76). This NS1-dependent repression of apoptosis may be explained, in part, by an ability to inhibit the production of antiviral type I IFN, which facilitates apoptosis (77). Type I IFNs are critical antiviral molecules produced by cells that are infected by viruses (78). Although essential to inhibiting viral infection and replication, excessive production of type I IFNs has been shown to promote lung injury, causing tissue destruction and resulting in apoptosis (79, 80). The ability of IAV NS1 to interfere with the type I IFN induction pathway may therefore explain its anti-apoptotic effects.

In contrast, recent reports indicate a role for IAV NS1 in the induction of host cell apoptosis, highlighting the complexity of IAV-mediated modulation of host antiviral mechanisms (81, 82). In a study using MDCK and HeLa cells, expression of NS1 was found sufficient to induce apoptosis (82).Interestingly, however, mutations of the *NS1* gene alone (NS13'SS DM (with no NS1 produced), NS1 M2 mutant RK19/20 AA and NS1 NS1Δ117-161 deletion mutants were not able inhibit apoptosis. These findings imply that other viral proteins may also play a role in IAV-induced apoptosis, and that this process also requires cooperation with other host or viral factors. This phenomenon can be observed through reverse genetics inserting NS1, NA, M1, M2 and PB2 genes from different strains of IAV – H3N2 (A/PR8/34-A/England/939/69 clone 7a) H1N1 (A/Fiji/15899/83) and H3N2 (A/Victoria/3/75) which induce decreasing levels of apoptosis, respectively. Inserting the A/Fiji NS1 genes into A/PR8 or A/Victoria reduced the apoptosis compared to the parent H3N2 strain. Inserting several genes together from A/Fiji (M, NA and HA) reduced apoptosis which was not seen when the genes were inserted individually, highlighting the importance of the combination of viral genes in the regulation of apoptosis

In summary, IAV NS1 plays an intricate yet a vital role in IAV maturation and replication. Targeting NS1 at different stages of the viral lifecycle presents a therapeutic opportunity for the treatment of IAV infection.

1.5.3. IAV neuraminidase (NA)

The role of IAV NA in host cell apoptosis is conflicting and was first elucidated in the 1990s, when it was shown that an antibody against NA but not HA inhibited the activation of transforming growth factor TGF-β, a known inducer of apoptosis, by influenza virus (83). A clone of IAV with high NA activity induced apoptosis to a greater extent when compared with an NA-attenuated strain, yet yielding similar infection rates and levels, indicating that the NA-induced apoptosis may not be important in viral infection . Furthermore, apoptosis can be partially blocked using three different anti-NA compounds only when administered during viral entry but not after. (84).. NA is now a recognized therapeutic target for most IAVs (85); drugs including Relenza™ and Tamiflu™ inhibit the activity of NA, preventing viral budding and thus decreasing infectivity and viral replication in neighboring cells (86).

1.5.4. IAV polymerase basic protein (PB)1-F2

PB1-F2, translated via the +1 open reading frame of IAV PB1, constitutes an 87 amino acid protein that has also been studied for its role in the induction of apoptosis and promoting viral replication. PB1-F2 was first identified in a search for other open reading frames encoding IAV proteins recognizable by $CD8^+$ T cells (87), and its expression was found to induce apoptosis in human monocytes. Furthermore, IAV strains harboring mutated *PB1-F2* induced less apoptosis in infected monocytes compared to infections with viruses encoding wildtype protein (87). PB1-F2 has also been shown to play a pro-apoptotic role by sensitizing cells to apoptotic signals such as TNFα; expression of PB1-F2 in HEK 293T cells increased their sensitivity to apoptotic ligands including TNF-related apoptosis-inducing ligand (TRAIL), TNFα, and cisplatin (88). Treatment of purified mitochondria from mouse livers with PB1-F2 resulted in increased cytochrome c release and disruption of mitochondrial potential (88). Taken together, these data suggest that PB1-F2 may increase cellular apoptosis by dysregulating mitochondrial potential, allowing the release of cytochrome c and caspase activators into the cytoplasm, both of which play key roles in apoptosis (67).

1.5.5. IAV matrix protein 1 (M1)

During IAV infection, stress responses (e.g. heat shock protein 70 [HSP70]) are activated which can inhibit viral replication (89). The viral M1 protein is crucial to the modulation of cellular HSP70 activity, and M1 silencing demonstrated reduced caspase 3- and caspase 9-dependent viral-induced apoptosis (90). HSP70 exerts its anti-apoptotic effects by binding to APAF-1, resulting in the formation of an apoptosome (a large protein structure formed during apoptosis) and the subsequent disruption of apoptosis initiation. Binding of M1 to HSP70 reduces its interaction with APAF-1, thus making it accessible for caspase activation and the subsequent activation of apoptosis (90).

In conclusion, IAV possesses sophisticated mechanisms that can either inhibit or initiate cellular apoptosis. This process appears to be tightly coordinated in a viral replication stage- and time-dependent manner, which ensures that virus maturation occurs in such a way so as to facilitate viral replication. However, it is also important

to consider that some studies report no association between viral spread and apoptosis, which may be time and cell dependent [90].

1.6. IAV-induced interferon and apoptosis

In the late 1950s, IFNs – low molecular weight compounds produced in virally-infected cells - were discovered by their ability to limit IAV replication [78]. However, over the years, accumulating evidence has implicated IFNs in contributing to disease pathogenesis and inflammatory responses detrimental to the host [79, 95, 96]. Such contradictory evidence makes further studies into the role of IFNs during IAV infection desirable.

IAV are potent inducers of IFNs. IAV is recognized by host cytosolic and membrane-bound pattern recognition receptors (PRRs), including retinoic acid-inducible gene I (RIG-I) and toll-like receptor 7 (TLR7), respectively, the latter of which detects the single-stranded viral RNA genome (91, 92). Upon recognition of the viral genome, activation of these receptors initiates a signaling cascade resulting in the production of pro-inflammatory cytokines such as TNF, interleukin 6, and type I IFNs (93, 94). Of interest, double-stranded RNA (dsRNA), a replicative intermediate during IAV infection (95), and/or IAV result in the rapid phosphorylation of tank binding kinase I (TBK1), which, in turn, phosphorylates IFN regulatory factor 3 (IRF3) and/or 7 (IRF7). Phosphorylation of IRF3 results in nuclear translocation, and the subsequent induction of type I IFNs (41, 96).

IFNs have been shown to mediate pro-apoptotic effects, and one of the earliest reports demonstrated increased rates of apoptosis in the presence of both dsRNA and IFN (99). Further studies have since elucidated the mechanism by which IFNs mediate apoptosis and have shown a requirement for signaling via the IFN-$\alpha\beta$-receptor (IFNAR) (100, 101). Apoptosis resulting from IAV infection may occur through an indirect mechanism, whereby viral induction of IFNs activates IFNAR signaling to amplify the apoptotic functions of viral proteins. By increasing the rate of apoptosis via either

direct or indirect mechanisms, IAV is able to enhance infectivity of neighboring cells, thus facilitating viral replication and propagation.

Type I IFNs are potent in reducing IAV replication, yet, as previously mentioned, IFNs are also implicated in severe host morbidity (79). The severe effects associated with type I IFNs following IAV infection have been attributed to increased lung damage, a consequence of elevated apoptosis. Treatment with type I IFN has also been shown to induce the secretion of cytokines from pulmonary immune cells, further amplifying immune responses and lung tissue destruction (80).

Reports have also suggested a role of IAV NS1 in inhibiting the expression of type I IFNs (96, 102). Through binding to IRF3, IAV NS1 limits its ability to transcribe type I IFNs, thus reducing IFN responses in virally-infected cells. Given that NS1 can restrict apoptosis during the early stages of viral infection, it is conceivable that this process may be mediated, in part, by its inhibition of type I IFN expression. As such, reduced IFN signaling via IFNAR results in decreased apoptosis, allowing sufficient time for virus maturation.

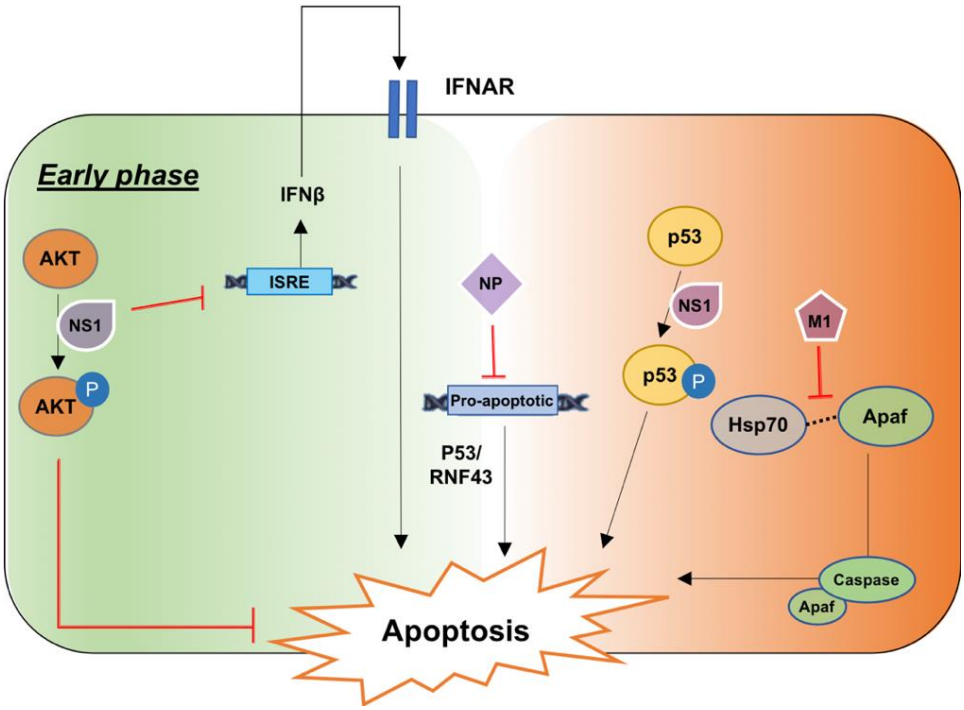

Figure 4 | Schematic representation of IAV proteins and their apoptotic effects. During the early phase of viral infection, the IAV NS1 protein upregulates phosphorylation of AKT (an anti-apoptotic protein) to prevent cellular induction of apoptosis, allowing sufficient time for the virus to undergo replication. NS1 may also inhibit type I IFN-induced apoptosis by repression of IFNβ induction, enabling infectious virions to mature. During the later phase of IAV infection, NS1 upregulates p53 to induce apoptosis, permitting the spread of mature viral particles to neighboring cells. Viral NP stabilizes p53 and induces apoptosis by repressing RNF43 expression. M1 increases APAF-1 availability, allowing for caspase activation by reducing interaction with HSP70.
AKT, protein kinase B; APAF, apoptosis protease activating factor 1; IFN, interferon; IFNAR, IFN-αβ receptor; ISRE, interferon-stimulated response element; HSP70, heat shock protein 70; M1, matrix protein 1; NP, nucleoprotein; NS1, non-structural protein 1; P, phosphorylation; RNF43, ring finger protein 43.

1.7. CONCLUSION

Considerable progress has been made to understand how IAV modulates host apoptosis. Of interest is the finding that IAV can impact host apoptosis by either facilitating or inhibiting the apoptotic process at differing stages of the viral lifecycle. Indeed, most evidence suggests that IAV favors inhibition of apoptosis during the early phase of viral replication to allow for proper maturation of the viral particle. After maturation, the virus induces host cell apoptosis to enable the propagation of viral

particles to neighboring uninfected cells, where the virus may exploit cellular proteins to form yet further progeny. Despite current understanding, more studies are required to further elucidate those factors which govern viral induction of apoptosis and which stage(s) of the viral replication cycle involve apoptotic pathways. This knowledge will allow future research to harness innate apoptotic defense mechanisms to limit viral infection and replication.

Table 1: Viral proteins and their effects on apoptosis

Viral protein	Apoptotic effect	Mechanism/evidence
Nucleoprotein (NP)	↑ apoptosis	1. Represses the expression of RNF43, improving stability of p53 [69,70] 2. Interacts with clusterin, reducing its association with BAX, allowing for BAX mitochondrial translocation [73] 3. Interacts with API5, making E2F1 available to mediate apoptosis [71]
Non-structural protein 1 (NS1)	↓ apoptosis ↑ apoptosis	1. Mutant IAV (lacking the NS1 gene) induced apoptosis more rapidly than wildtype virus [45,76]. 2. Expression of NS1 gene in MDCK or HeLa cells was sufficient to induce apoptosis [82]
Neuraminidase (NA)	↑ apoptosis	1. An IAV clone with high NA activity increased apoptosis compared to a NA-attenuated strain [83]
PB1-F2	↑ apoptosis	1. PB1-F2 expression in human monocytes induces apoptosis [86] 2. Sensitizes cells to pro-apoptotic signals such as TNFα [87]
Matrix protein 1	↑ apoptosis	1. Interacts with HSP70 thereby reducing its interaction with APAF-1, enabling caspase activation [89]
PA-X	↑ apoptosis	1. Enhances the inflammatory response

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