UNIVERSITÉ DE GENÈVE

Archive ouverte UNIGE
https://archive-ouverte.unige.ch

Article scientifique | Article | 2010

Published version | Open Access

This is the published version of the publication, made available in accordance with the publisher's policy.

Evasion of innate and adaptive immune responses by influenza A virus

Schmolke, Mirco; García-Sastre, Adolfo

How to cite

SCHMOLKE, Mirco, GARCÍA-SASTRE, Adolfo. Evasion of innate and adaptive immune responses by influenza A virus. In: Cellular microbiology, 2010, vol. 12, n° 7, p. 873–880. doi: 10.1111/j.1462-5822.2010.01475.x

This publication URL: https://archive-ouverte.unige.ch/unige:75380
Publication DOI: 10.1111/j.1462-5822.2010.01475.x

© This document is protected by copyright. Please refer to copyright holder(s) for terms of use.

NIH Public Access
Author Manuscript
Cell Microbiol. Author manuscript; available in PMC 2011 July 1.

Published in final edited form as:
Cell Microbiol. 2010 July ; 12(7): 873–880. doi:10.1111/j.1462-5822.2010.01475.x.

Evasion of Innate and Adaptive Immune Responses by Influenza A Virus

Mirco Schmolke[1] and **Adolfo García-Sastre**[2,3,4,*]

Mirco Schmolke: mirco.schmolke@mssm.edu

[1] Department of Microbiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY, 10029, USA

[2] Department of Microbiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY, 10029, USA

[3] Department of Medicine, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY, 10029, USA

[4] Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY, 10029, USA

Summary

Host organisms have developed sophisticated antiviral responses in order to defeat emerging influenza A viruses (IAV). At the same time IAV have evolved immune evasion strategies. The immune system of mammals provides several lines of defense to neutralize invading pathogens or limit their replication. Here, we summarize the mammalian innate and adaptive immune mechanisms involved in host defense against viral infection and review strategies by which IAV avoid, circumvent or subvert these mechanisms. We highlight well-characterized, as well as recently described features of this intriguing virus-host molecular battle.

Introduction

Influenza A viruses (IAV) belong to the family *Orthomyxoviridae* (reviewed in (Palese, 2007)). They are sub-typed according to the surface antigens hemagglutinin (HA or H) and neuraminidase (NA or N). So far 16 subtypes of HA and nine types of NA have been described in birds, where the majority of the IAV strains are found. IAV are characterized by a segmented RNA genome, organized into eight ribonucleoprotein (RNP) units per virion, that encodes for up to eleven proteins. In mammals, IAV primarily infect lung epithelial cells of the upper and lower respiratory tract.

Innate immune sensors and antiviral signaling

The innate immune system is the first and oldest line of defense against invading pathogens. It recognizes pathogen associated molecular patterns (PAMP), as well as endogenous danger signals (e.g. microbial nucleic acids or components, bacterial cell walls, extracellular ATP), by different families of germ line encoded pattern recognition receptors (PRR) and creates a fast and broadly reactive response that changes the infected tissue into an alerted state. Possible consequences of this alerted state are: secretion of cytokines (among them type I and type III interferons (IFNs)) to upregulate anti microbial gene products in neighboring cells; secretion of chemokines to attract and activate cytotoxic effector cells as well as

*corresponding author: Tel: 212-241-7769, Fax: 212-534-1684, adolfo.garcia-sastre@mssm.edu.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

antigen presenting cells (APC); and apoptosis of infected cells. Consequently the subsequent adaptive immune response is shaped by the quality of the initial innate response. Several families of PRRs have been described (Mogensen, 2009), among them: Toll-like receptors (TLR), nucleotide oligomerization domain (NOD)-like receptors (NLR) and retinoic acic induced gene I (RIG-I)-like receptors (RLR).

The TLR pathway—The best-characterized family of PRRs is the toll like receptor (TLR) family (reviewed in (Akira *et al.*, 2001, Iwasaki *et al.*, 2004)). TLRs can be expressed either on the cell surface at the plasma membrane (TLR 1, 2, 4, 5, 6, 10 and 11) or on the inside of endosomes (TLR3, 7, 8 and 9) and can bind to a variety of viral and bacterial molecular patterns. Surface expressed TLRs recognize surface structures of microbes, while endosomal TLRs bind to microbe associated nucleic acids. For influenza virus infection TLR3 (recognizes double stranded RNA species) (Le Goffic *et al.*, 2006) and TLR7/8 (recognizes single stranded RNA species) have been described to be involved in the recognition of viral RNA species (Diebold *et al.*, 2004, Lund *et al.*, 2004). Activation of TLR3 ultimately leads to the activation of the transcription factors IFN regulatory factor (IRF) 3, activator protein 1 (AP1) and p50/p65 (NFκB) (Kawai *et al.*, 2007). These factors form the IFNβ enhanceosome and initiate transcription of IFNβ (Kim *et al.*, 1997). In addition, NFκB and AP1 are also involved in stimulating expression of pro-inflammatory cytokines.

TLR deficient mice show enhanced mortality, accompanied by a reduced inflammatory response upon influenza A virus infection (Le Goffic *et al.*, 2007). In consequence these mice also showed a profound decrease in the adaptive response to virus infection. A case report from 2006 correlated severe symptoms during IAV infection in humans with TLR3 dependent production of IFNβ and proinflammatory cytokines and chemokines. The investigators found a TLR3 miss-sense mutation, leading to a loss of function, in one patient with IAV associated encephalopathy (Hidaka *et al.*, 2006).

IAV infection in vivo results in robust expression of type I IFN, mainly produced by plasmacytoid dendritic cells (pDC). In contrast to myeloid DCs or fibroblasts, which predominantly recognize IAV infection via retinoic acid gene I (RIG-I), pDCs recognize IAV RNA via TLR7 and PKR (Barchet *et al.*, 2005, Diebold *et al.*, 2004). TLR7 signaling initiates activation of AP1, NFκB and IRF7. IRF7 is expressed in response to type-I IFN signaling and can bind to type I IFN promoters. Interestingly, no direct viral mechanism antagonizing TLR signaling has been described for IAV so far. However, a recent study demonstrated that PBMCs isolated from patients with severe IAV infection respond less to stimulation with TLR ligands compared to PBMCs from patients with mild or asymptomatic influenza. The underlying mechanism is not understood (Heltzer *et al.*, 2009).

The NLR pathway—NLR signaling has been extensively studied in the context of bacterial infection (review by (Franchi *et al.*, 2009). Recently three independent studies demonstrated an involvement of inflammasome signaling in influenza A virus immunity. Ichinohe and colleagues used mice deficient in expression of inflammasome components such as ASC or caspase-1 to investigate the importance of this signaling pathway for the CD4 and CD8 response, as well as IgA and IgG production against IAV, using low dose viral infection (Ichinohe *et al.*, 2009). The authors conclude an essential role of inflammasome signaling for a protective adaptive response against IAV. Interestingly, they did not see these effects in NLRP3 deficient mice. In contrast, two studies demonstrated that NLRP3−/−mice show defects in innate immunity against influenza A virus infection, while having no effect on the adaptive immune response (Allen *et al.*, 2009, Thomas *et al.*, 2009). Despite these contradictions, the importance of the NLR pro-inflammatory response is undisputable. IAV infection in IL-1β or IL-18 deficient mice results in higher mortality and morbidity (Kozak *et al.*, 1995) (Liu *et al.*, 2004) and IL-1R−/− mice show a reduced

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

inflammatory response, impaired neutrophil infiltration to the lung, deficiencies in CD4+ T-cell activation and recruitment as well as a decreased IgM response against IAV infection (Schmitz *et al.*, 2005). Interestingly, mutant IAV with deletions in the NS1 provoke enhanced induction of caspase-1 activity upon infection of macrophages and accordingly enhanced induction of IL-1β and IL18 levels (Stasakova *et al.*, 2005) implicating an antagonizing function of NS1.

The RLR pathway—In recent years a multitude of studies have broadened our understanding of RLR activation and signaling. RIG-I and the melanoma differentiation associated protein (Mda) 5 are the best-characterized members of this PRR family (reviewed in (Yoneyama *et al.*, 2009)). RIG-I is believed to be activated by cytoplasmic single strand 5′-triphosphate RNA (Hornung *et al.*, 2006), while Mda-5 preferentially binds long dsRNA species. In case of influenza virus replication, accumulating viral genomic 5-triphosphate (−) RNA is recognized by the cytosolic helicase RIG-I but not Mda5. Rehwinkel and colleagues recently demonstrated that neither non-genomic viral transcripts nor short replication intermediates act as a RIG-I agonist under physiological conditions. Moreover they exclude cleaved self-RNAs to have RIG-activating function, as proposed earlier (Rehwinkel *et al.*, 2010). It is presently unclear if RIG-I binds vRNA in intact RNPs, in which the RNA is in a complex with NP molecules, or if free or partially complexed vRNA occurs as a byproduct of viral replication and is recognized as a PAMP. In its inactive conformation the repressor domain (RD) of RIG-I is in close proximity to the two caspase-recruitment domains (CARD). Upon binding of the RD to viral RNA the conformation opens up and RIG-I interacts with the mitochondrial antiviral signaling protein MAVS via CARD-CARD interactions. MAVS subsequently activates IRF3 as well as IRF7 and NFκB. The activated transcription factors initiate the expression of type I IFN and inflammatory cytokines.

Type I IFN signaling—Upon secretion type I IFN can act in an autocrine or paracrine fashion, by binding to the type I IFN receptor IFNAR on the infected cell or neighboring cells, respectively (Randall *et al.*, 2008). IFNAR signaling activates transcription from IFN sensitive regulatory element (ISRE) containing promoters. By definition these genes are called IFN-stimulated genes (ISG). In recent years several hundred ISGs have been identified. Interestingly, most gene products involved in type I IFN expression are ISGs, among them RIG-I, Mda5, IRF7, TLR3/7, as well as STAT1, implicating positive feedback mechanisms in the antiviral response. ISGs are involved in a variety of cellular processes, among them regulation of host cell transcription/translation, attraction of immune cells, regulation of apoptosis or recognition of PAMPs. The expression of ISGs converts the infected cell, as well as neighboring cells into an alerted, antimicrobial state, that limits microbial replication and prevents spread of the invaded pathogen.

For some ISGs a direct antiviral function has been described. The myxovirus resistance gene A (MxA) protein was shown to bind to cytoplasmic RNPs of orthomyxoviruses and block nuclear import. Expression of Mx1 (the mouse homolog of human MxA) is a major determinant of the antiviral response against IAV. Interestingly not all IAV isolates respond equally to the inhibitory effects of MxA, implying viral escape mechanisms/mutations to the effects of this ISG (Dittmann *et al.*, 2008).

IAV infection also leads to up-regulation of negative host regulators of the type I IFN response. Recently, IAV induced SOCS3 dependent limitation of STAT1 signaling was proposed (Pauli *et al.*, 2008) (Fig. 1C). It is, however unclear, if this mechanism is actively induced by the virus or whether regular feedback signaling events of the host cell act to self-limit possible harmful effects caused by an exuberant antiviral response.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Antagonism of the innate immune response by IAV—IAV antagonize the innate host response of infected cells mainly by expression of the non-structural protein 1 (NS1). This multifunctional virulence factors efficiently limits expression of type I IFN at different levels (reviewed in (Hale *et al.*, 2008)). IAV with large deletions in the NS1 segment are potent inducers of type I IFN and show massively reduced morbidity and mortality in animal models, compared to wild type viruses.

NS1 was shown to interfere with RIG-I dependent activation of IRF3 and NFκB signaling by forming a complex with RIG-I and potentially viral RNA. Additionally NS-1 interferes with TRIM-25 oligomerization, thus blocking TRIM25 mediated ubiquitinylation of the RIG-I CARD, which is a required modification for RIG-I signaling (Gack *et al.*, 2009) (Fig. 1A).

The NS1 of most (but not all) IAV isolates can also block host gene expression by interfering with the 3′-polyadenylation of newly synthesized host pre-mRNAs. This occurs through interaction of NS1 with the cleavage and polyadenylation specific factor (CPSF) 30 (Fig. 1B)(Nemeroff *et al.*, 1998). Host gene expression is also limited by IAV polymerase mediated 5′-m7G-cap snatching of newly synthesized mRNAs. IAV uses these host derived 5′-m7G-capped oligonucleotides to prime the synthesis of viral mRNA. Binding of the polymerase complex to 5′-m7G-caps of host mRNAs is mediated by the PB2 subunit, while PA harbors the necessary endonuclease function to cleave the mRNA 10–13 nucleotides downstream of the 5′-m7G-cap (Fig. 1B)(Dias *et al.*, 2009). Early studies revealed that IAV infection reduces the transcription of transfected plasmid DNA by 80% already 1h after infection (Katze *et al.*, 1984). Since both mechanisms, interference with CPSF30 and 5′-m7G-cap-snatching, exclusively affects newly synthesized mRNA it has a major impact on those gene products that have a high turnover rate of mRNA or are induced by viral infection, like type I IFN and pro-inflammatory cytokines and chemokines.

Additionally NS1 was shown to directly interfere with the function of different ISGs. Different studies demonstrate a direct inhibitory effect of NS1 on the function of the double stranded RNA activated protein kinase (PKR) and the 2–5-oligoadenylate synthetase (OAS) (Hale *et al.*, 2008) (Fig. 1D). Interestingly, not all IAV strains contain all the described functions in their NS1 protein albeit replicating well in immune competent hosts. This indicates host (presumably species) specific requirement for these functions.

The innate-adaptive immune response interphase

The quality of the initial innate response against IAV has profound consequences for the following adaptive response. Triggering the adaptive immune response against IAV requires professional antigen presentating cells (APC) like DCs. Depletion of lung resident DCs was shown to result in reduced recruitment of pDCs (areviewed in(McGill *et al.*, 2009a)), which are the main producers of type I IFN, and CD8+ cells upon IAV infection.

Residential alveolar macrophages and dendritic cells are among the first cells activated by influenza A virus infection. Depending on the virus strain a significant proportion of infected cells are alveolar macrophages (Nicholls *et al.*, 2007). Upon infection with IAV alveolar macrophages produce significant amounts of inflammatory cytokines like IL-6 and TNFα, and become highly phagocytic. Additionally, infected lung epithelial cells secrete high levels of monocyte chemoattractant protein 1 (MCP-1), recruiting monocytes, which can differentiate into monocyte derived DCs and inflammatory macrophages (Herold *et al.*, 2006).

Upon activation, DCs undergo maturation and migrate to the draining lymph nodes of the lung where they present peptide antigens to IAV specific naïve T-cells. This results in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

proliferation and differentiation of these T-cells into effector cells. As shown for other cell types, NS1 limits the production of type I IFN as well as that of cytokines and chemokines upon IAV infection (Fernandez-Sesma *et al.*, 2006, Haye *et al.*, 2009).

Moreover IAV is capable of interfering with the maturation of DCs. Infected DCs show reduced upregulation of MHC class II and costimulatory receptors like CD80 and CD86, essential for T-cell priming. Young and colleagues demonstrated that infection of mature DCs by influenza virus interferes with the presentation of an endogenous viral antigen and can thus impact the adaptive immune response (Young *et al.*, 2007).

Up to 48h post infection no significant host response is detectable in mice infected with influenza A/PR/8/34, despite a profound virus load in the lung of infected animals after 24h (Moltedo *et al.*, 2009). In this postulated “stealth phase” of infection, the host is unable to prime the adaptive immune response, by induction of a robust innate and inflammatory response. The efficient inhibition of the innate antiviral host response by NS1 in directly infected APC could contribute to this delayed response. IAV infection was shown to delay the induction of antibodies against an unrelated antigen, as long as this is given within 48h post infection (Brimnes *et al.*, 2003).

In summary, IAV are capable of limiting and delaying the innate host response, mainly by expression of NS1, which inhibits the cascade of events leading to a robust innate response by reducing the initial production of type I IFN. Probably, this opens a window for replication and spreading of the virus to other hosts and also has profound consequences on the priming and the quality of the adaptive response.

Adaptive immune response to IAV infection

The humoral or antibody based response against IAV is essential to prevent infection of the host. In contrast the cellular response is important for viral clearance in late stages of infection.

IAV are antigenically highly variable. Two major mechanisms determine this antigenic variability of IAV, antigenic drift and antigenic shift (Fig. 2). The error prone RNA-dependent RNA-polymerase facilitates the generation of escape-mutants against neutralizing antibodies (antigenic drift), the CTL response or antiviral drugs. Moreover it allows the establishment of mutant viruses that can pass species barriers, by overcoming certain host specific restriction factors.

Antigenic shift occurs by re-assortment of viral gene segments of two different virus strains during double infection of one host cell. This leads to a change of viral surface protein composition and can lead to the generation of pandemic IAV strains. The sudden introduction of completely new antigenic features can allow these viruses to spread rapidly in a naïve population, without preexisting immunity to former related isolates.

Humoral response—The humoral response against IAV has been extensively studied (reviewed in (Gerhard, 2001, Martinez *et al.*, 2009)). Using B cell deficient μMT mice several studies could show the essential impact of the antibody response to lethal doses of IAV. In concordance with this, passive transfer of influenza HA specific antibodies to severe combined immunodeficiency (SCID) mice protects these animals from a lethal IAV challenge. Neutralizing antibodies are mainly directed against the viral exposed surface proteins HA (and to a lesser extend to NA) or to the membrane embedded ion channel M2 (Nayak *et al.*, 2009). Systematic analysis of H1 and H3 neutralizing antibody binding sites has led to the establishment of antigenic maps, showing the distribution of antigenic sites on the HA surface (reviewed in (Martinez *et al.*, 2009)). Antibody mediated immunity can last

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

for several months up to a lifetime. Survivors of the Spanish influenza in 1918/19 had a population of HA specific B cells in their blood almost 90 years after the last exposure.

The RNA dependent RNA polymerase of IAV has a calculated error rate of approximately 10^{-5} (Parvin *et al.*, 1986). The resulting high genetic variability of the virus is the main reason for annual updates of the seasonal vaccine due to antigenic drift and the establishment of resistant viral strains as described for M2 blockers and neuraminidase inhibitors.

Antigenic drift is mostly driven by the selective pressure of neutralizing antibodies, predominantly against the IAV HA. In the years from 1968 to 1999 antigenic drift caused an accumulated rate of amino acid substitutions per year of 3.5 in the HA of H3 viruses derived from the 1968 Hong Kong pandemic (Bean *et al.*, 1992). A recent sequence analysis of several hundred HA sequences of human H1N1 from 1918 to 2008 confirmed positive host selection pressure on the evolution of HA and also highlighted the evolutionary trends on mutations in the HA_1 residues 190 and 225, both involved in receptor binding (Shen *et al.*, 2009). While epidemic H1N1 favor changes in residue 190, 1918 and swine HAs favor position 225, suggesting interplay of antigenic adaptation and receptor binding in HA evolution. In agreement with this, it was suggested that HA receptor avidity is a major determinant driving antigenic drift in IAV infection (Hensley *et al.*, 2009). Besides changes in the amino acid sequence, posttranscriptional modifications, mainly N-glycosylation, change the structure, charge and accessibility, as well as the function of viral surface proteins. Many human pathogenic viruses, among them IAV, HIV and West Nile virus, use N-glycosylation to evade the human immune system (Vigerust *et al.*, 2007). Both IAV HA and NA are glycosylated. These posttranslational modifications are important for receptor binding, viral infectivity, and virus release. The degree of glycosylation of HA varies from isolate to isolate, ranging from five to eleven glycosylations. Interestingly, the degree of glycosylation in H3N2 isolates has increased over the last 30 years, implicating a selective pressure that favors higher glycosylated forms. It is believed that glycosylation of the globular head region shields potential neutralizing epitopes from antibody recognition. Recently, Wang and colleagues demonstrated that antibodies raised against IAV HA-derived antigens with a reduced number of structurally nonessential glycans showed enhanced neutralizing capacity compared to those raised against fully glycosylated antigens (Wang *et al.*, 2009). On the other hand, addition of glycosylation sites in close proximity to the protease cleavage site or the receptor-binding site can reduce viral pathogenicity. Overall the glycosylation of viral surface proteins needs to be balanced, to allow proper folding and function, as well as proper shielding against host antibodies.

Cytotoxic T-cell response—The virus specific cytotoxic T-cell response is essential to eliminate virus-infected cells, which present virus derived peptides by MHC class I molecules. Consequently, mice lacking CD8+ T cells show delayed virus clearance (Bender *et al.*, 1992). Upon primary infection of B6 mice the peak of predominant CD8+ T cells and viral clearance occur on day ten post inoculation. Memory T cell pools were shown to be stable for at least 500 days (Kedzierska *et al.*, 2006). Priming of a CD8+ T cell response against IAV infection requires the interaction of CD8+ T cells with professional antigen presenting DCs (McGill *et al.*, 2009a, McGill *et al.*, 2009b).

CTL epitopes of IAV were extensively characterized in different mouse models (reviewed in (Stambas *et al.*, 2008)). In general the predominance of certain CTL epitopes is broader in humans due to the variation of HLA haplotypes. The predominance of certain CTL epitopes depends on host factors (e.g. HLA haplotype, availability of CTLs, efficiency of antigen processing and presentation by APC) as well as on viral factors (affinity of viral peptides to the present MHC class I, abundance of the antigen) (Yewdell *et al.*, 1999). Escape mutations

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

in CTL epitopes seem to occur less often than in epitopes of neutralizing antibodies and can either lead to loss of presentation by a certain HLA haplotype, by amino acid substitutions in the anchor residue of the presented peptide, or a decrease in the avidity to the T cell antigen receptor (TCR), by mutations in the interacting residues.

For H3N2 viruses, isolated over a period of ten years, variations in nucleoprotein derived CTL epitopes were described recently (Rimmelzwaan *et al.*, 2009). Amino acid substitutions in the anchor residue resulted in a loss of binding to the respective MHC class I molecule. The selective advantage of these mutant viruses led to replacement of the ancestor strain within one season. Interestingly, the escape mutant viruses had to compensate the acquired resistance by co-mutations to reestablish viral fitness, implicating functional constraints associated with the escape mutation. Moreover, mutations in the TCR interface occurred that changed the avidity of specific CD8+ T for these epitopes. Interestingly, IAV CTLs can also be highly conserved as shown for the immunodominant $M1_{58–66}$ presented by HLA-A*0201, a HLA allele found in more than 50% of the human population. Mutational analysis by reverse genetics revealed that this epitope is under highly functional constraints.

In summary, high genetic variability of IAV, due to its error prone replication machinery, allows the virus to adapt fast to selective pressure by the host immune response, but also by antiviral drugs. However this variability underlies functional constraints, which can limit the establishment or require additional balancing mutations.

Concluding Remarks and Future Perspectives

During host-virus co-evolution IAV have developed remarkable strategies to avoid the host immune response. In infected cells, the virus mainly limits initial steps of PAMP detection by expression of NS1 and shutting down the host gene expression. Additionally the high genetic variability allows escape from preexisting immunity against other IAV strains. It has to be pointed out that functional constrains limit the extent of mutations that are tolerated without affecting the viral replication.

Acknowledgments

Research on influenza viruses in the AG-S lab is supported by an NIAID Center for Research on Influenza Pathogenesis (CRIP, HHSN266200700010C), and by NIAID grants R01AI046954, P01AI058113, U01AI070469, U01AI074539, U01AI082970 and U19AI083025. We thank Dr. Hale and Dr. Albrecht for their suggestions on this manuscript.

References

Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001; 2:675–680. [PubMed: 11477402]

Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009; 30:556–565. [PubMed: 19362020]

Barchet W, Krug A, Cella M, Newby C, Fischer JA, Dzionek A, et al. Dendritic cells respond to influenza virus through TLR7- and PKR- independent pathways. Eur J Immunol. 2005; 35:236–242. [PubMed: 15593126]

Bean WJ, Schell M, Katz J, Kawaoka Y, Naeve C, Gorman O, Webster RG. Evolution of the H3 influenza virus hemagglutinin from human and nonhuman hosts. J Virol. 1992; 66:1129–1138. [PubMed: 1731092]

Bender BS, Croghan T, Zhang L, Small PA Jr. Transgenic mice lacking class I major histocompatibility complex-restricted T cells have delayed viral clearance and increased mortality after influenza virus challenge. J Exp Med. 1992; 175:1143–1145. [PubMed: 1552285]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Brimnes MK, Bonifaz L, Steinman RM, Moran TM. Influenza virus-induced dendritic cell maturation is associated with the induction of strong T cell immunity to a coadministered, normally nonimmunogenic protein. J Exp Med. 2003; 198:133–144. [PubMed: 12847140]

Dias A, Bouvier D, Crepin T, McCarthy AA, Hart DJ, Baudin F, et al. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature. 2009; 458:914–918. [PubMed: 19194459]

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004; 303:1529–1531. [PubMed: 14976261]

Dittmann J, Stertz S, Grimm D, Steel J, Garcia-Sastre A, Haller O, Kochs G. Influenza A virus strains differ in sensitivity to the antiviral action of Mx-GTPase. J Virol. 2008; 82:3624–3631. [PubMed: 18199636]

Fernandez-Sesma A, Marukian S, Ebersole BJ, Kaminski D, Park MS, Yuen T, et al. Influenza virus evades innate and adaptive immunity via the NS1 protein. J Virol. 2006; 80:6295–6304. [PubMed: 16775317]

Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol. 2009; 10:241–247. [PubMed: 19221555]

Gack MU, Albrecht RA, Urano T, Inn KS, Huang IC, Carnero E, et al. Influenza A virus NS1 targets the ubiquitin ligase TRIM25 to evade recognition by the host viral RNA sensor RIG-I. Cell Host Microbe. 2009; 5:439–449. [PubMed: 19454348]

Gerhard W. The role of the antibody response in influenza virus infection. Curr Top Microbiol Immunol. 2001; 260:171–190. [PubMed: 11443873]

Hale BG, Randall RE, Ortin J, Jackson D. The multifunctional NS1 protein of influenza A viruses. J Gen Virol. 2008; 89:2359–2376. [PubMed: 18796704]

Haye K, Burmakina S, Moran T, Garcia-Sastre A, Fernandez-Sesma A. The NS1 protein of a human influenza virus inhibits type I interferon production and the induction of antiviral responses in primary human dendritic and respiratory epithelial cells. J Virol. 2009; 83:6849–6862. [PubMed: 19403682]

Heltzer ML, Coffin SE, Maurer K, Bagashev A, Zhang Z, Orange JS, Sullivan KE. Immune dysregulation in severe influenza. J Leukoc Biol. 2009; 85:1036–1043. [PubMed: 19276177]

Hensley SE, Das SR, Bailey AL, Schmidt LM, Hickman HD, Jayaraman A, et al. Hemagglutinin receptor binding avidity drives influenza A virus antigenic drift. Science. 2009; 326:734–736. [PubMed: 19900932]

Herold S, von Wulffen W, Steinmueller M, Pleschka S, Kuziel WA, Mack M, et al. Alveolar epithelial cells direct monocyte transepithelial migration upon influenza virus infection: impact of chemokines and adhesion molecules. J Immunol. 2006; 177:1817–1824. [PubMed: 16849492]

Hidaka F, Matsuo S, Muta T, Takeshige K, Mizukami T, Nunoi H. A missense mutation of the Toll-like receptor 3 gene in a patient with influenza-associated encephalopathy. Clin Immunol. 2006; 119:188–194. [PubMed: 16517210]

Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, et al. 5′-Triphosphate RNA is the ligand for RIG-I. Science. 2006; 314:994–997. [PubMed: 17038590]

Ichinohe T, Lee HK, Ogura Y, Flavell R, Iwasaki A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. J Exp Med. 2009; 206:79–87. [PubMed: 19139171]

Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004; 5:987–995. [PubMed: 15454922]

Katze MG, Krug RM. Metabolism and expression of RNA polymerase II transcripts in influenza virus-infected cells. Mol Cell Biol. 1984; 4:2198–2206. [PubMed: 6095046]

Kawai T, Akira S. Antiviral signaling through pattern recognition receptors. J Biochem. 2007; 141:137–145. [PubMed: 17190786]

Kedzierska K, Venturi V, Field K, Davenport MP, Turner SJ, Doherty PC. Early establishment of diverse T cell receptor profiles for influenza-specific CD8(+)CD62L(hi) memory T cells. Proc Natl Acad Sci U S A. 2006; 103:9184–9189. [PubMed: 16754852]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Kim TK, Maniatis T. The mechanism of transcriptional synergy of an in vitro assembledinterferon-beta enhanceosome. Mol Cell. 1997; 1:119–129. [PubMed: 9659909]

Kozak W, Zheng H, Conn CA, Soszynski D, van der Ploeg LH, Kluger MJ. Thermal and behavioral effects of lipopolysaccharide and influenza in interleukin-1 beta-deficient mice. Am J Physiol. 1995; 269:R969–977. [PubMed: 7503324]

Le Goffic R, Balloy V, Lagranderie M, Alexopoulou L, Escriou N, Flavell R, et al. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog. 2006; 2:e53. [PubMed: 16789835]

Le Goffic R, Pothlichet J, Vitour D, Fujita T, Meurs E, Chignard M, Si-Tahar M. Cutting Edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. J Immunol. 2007; 178:3368–3372. [PubMed: 17339430]

Liu B, Mori I, Hossain MJ, Dong L, Takeda K, Kimura Y. Interleukin-18 improves the early defence system against influenza virus infection by augmenting natural killer cell-mediated cytotoxicity. J Gen Virol. 2004; 85:423–428. [PubMed: 14769900]

Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, et al. Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc Natl Acad Sci U S A. 2004; 101:5598–5603. [PubMed: 15034168]

Martinez O, Tsibane T, Basler CF. Neutralizing anti-influenza virus monoclonal antibodies: therapeutics and tools for discovery. Int Rev Immunol. 2009; 28:69–92. [PubMed: 19241254]

McGill J, Heusel JW, Legge KL. Innate immune control and regulation of influenza virus infections. J Leukoc Biol. 2009a; 86:803–812. [PubMed: 19643736]

McGill J, Legge KL. Cutting edge: contribution of lung-resident T cell proliferation to the overall magnitude of the antigen-specific CD8 T cell response in the lungs following murine influenza virus infection. J Immunol. 2009b; 183:4177–4181. [PubMed: 19767567]

Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009; 22:240–273. Table of Contents. [PubMed: 19366914]

Moltedo B, Lopez CB, Pazos M, Becker MI, Hermesh T, Moran TM. Cutting edge: stealth influenza virus replication precedes the initiation of adaptive immunity. J Immunol. 2009; 183:3569–3573. [PubMed: 19717515]

Nayak B, Kumar S, Dinapoli JM, Paldurai A, Perez DR, Collins PL, Samal SK. Contributions of the Avian Influenza Virus HA, NA and M2 Surface Proteins to the Induction of Neutralizing Antibodies and Protective Immunity. J Virol. 2009

Nemeroff ME, Barabino SM, Li Y, Keller W, Krug RM. Influenza virus NS1 protein interacts with the cellular 30 kDa subunit of CPSF and inhibits 3′end formation of cellular pre-mRNAs. Mol Cell. 1998; 1:991–1000. [PubMed: 9651582]

Nicholls JM, Chan MC, Chan WY, Wong HK, Cheung CY, Kwong DL, et al. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. Nat Med. 2007; 13:147–149. [PubMed: 17206149]

Palese, PaSML. Orthomyxoviridae: the viruses and their replication. In: Knipe, DaHPM., editor. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1647-1689.

Parvin JD, Moscona A, Pan WT, Leider JM, Palese P. Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. J Virol. 1986; 59:377–383. [PubMed: 3016304]

Pauli EK, Schmolke M, Wolff T, Viemann D, Roth J, Bode JG, Ludwig S. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. PLoS Pathog. 2008; 4:e1000196. [PubMed: 18989459]

Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol. 2008; 89:1–47. [PubMed: 18089727]

Rehwinkel J, Tan CP, Goubau D, Schulz O, Pichlmair A, Bier K, et al. RIG-I Detects Viral Genomic RNA during Negative-Strand RNA Virus Infection. Cell. 2010; 140:397–408. [PubMed: 20144762]

Rimmelzwaan GF, Kreijtz JH, Bodewes R, Fouchier RA, Osterhaus AD. Influenza virus CTL epitopes, remarkably conserved and remarkably variable. Vaccine. 2009; 27:6363–6365. [PubMed: 19840674]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Schmitz N, Kurrer M, Bachmann MF, Kopf M. Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. J Virol. 2005; 79:6441–6448. [PubMed: 15858027]

Shen J, Ma J, Wang Q. Evolutionary trends of A(H1N1) influenza virus hemagglutinin since 1918. PLoS One. 2009; 4:e7789. [PubMed: 19924230]

Stambas J, Guillonneau C, Kedzierska K, Mintern JD, Doherty PC, La Gruta NL. Killer T cells in influenza. Pharmacol Ther. 2008; 120:186–196. [PubMed: 18801385]

Stasakova J, Ferko B, Kittel C, Sereinig S, Romanova J, Katinger H, Egorov A. Influenza A mutant viruses with altered NS1 protein function provoke caspase-1 activation in primary human macrophages, resulting in fast apoptosis and release of high levels of interleukins 1beta and 18. J Gen Virol. 2005; 86:185–195. [PubMed: 15604446]

Thomas PG, Dash P, Aldridge JR Jr, Ellebedy AH, Reynolds C, Funk AJ, et al. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity. 2009; 30:566–575. [PubMed: 19362023]

Vigerust DJ, Shepherd VL. Virus glycosylation: role in virulence and immune interactions. Trends Microbiol. 2007; 15:211–218. [PubMed: 17398101]

Wang CC, Chen JR, Tseng YC, Hsu CH, Hung YF, Chen SW, et al. Glycans on influenza hemagglutinin affect receptor binding and immune response. Proc Natl Acad Sci US A. 2009; 106:18137–18142.

Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu Rev Immunol. 1999; 17:51–88. [PubMed: 10358753]

Yoneyama M, Fujita T. RNA recognition and signal transduction by RIG- I-like receptors. Immunol Rev. 2009; 227:54–65. [PubMed: 19120475]

Young LJ, Wilson NS, Schnorrer P, Mount A, Lundie RJ, La Gruta NL, et al. Dendritic cell preactivation impairs MHC class II presentation of vaccines and endogenous viral antigens. Proc Natl Acad Sci U S A. 2007; 104:17753–17758. [PubMed: 17978177]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

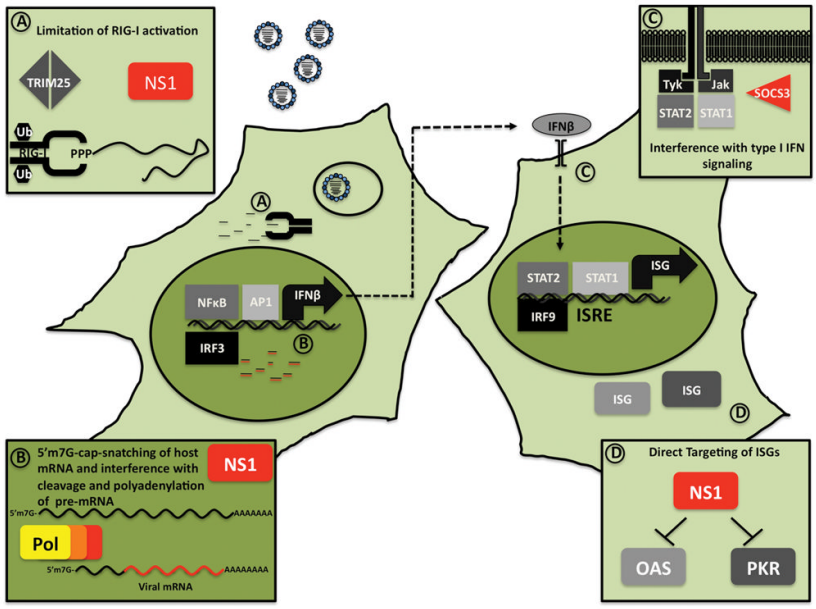

Figure 1. Viral evasion strategies in infected and bystander cells

(A) IAV NS1 limits RIG-I activation by interference with TRIM25. (B) 5′m7G-cap snatching by the IAV polymerase complex and interference of NS1 with CPSF30 result in host protein synthesis shut down. (C) Upregulation of SOCS3 limits IFN type I signaling in host cells. (D) IAV NS1 directly antagonizes ISGs.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

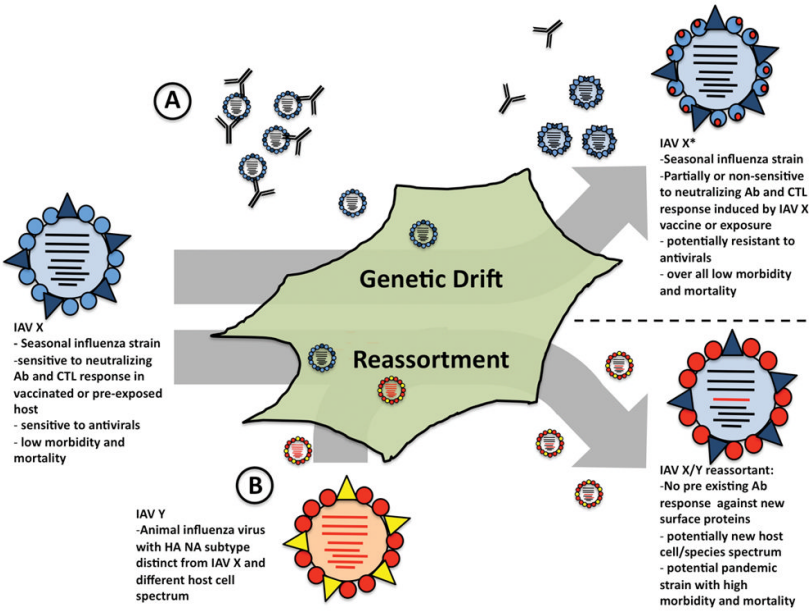

Figure 2. Genetic variability of IAV allows efficient escape from the host immune system

(A) Antigenic drift: The high mutation rate of IAV (strain X) results in the establishment of antigenically new IAV (strain X*, mutations indicated by red circles) and allows the virus to escape from the adaptive host response and antiviral drugs. (B) Antigenic shift: Genetic re-assortment in double infected cells creates IAVs with a completely new surface structure. These viruses can have pandemic potential due to a lack of preexisting immunity in the host population.