Microbiological Overview about Immune response to Influenza virus

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Abstract

The main site of infection for influenza viruses is the respiratory epithelium. The first line of defense is performed by the innate immune system which is rapid but lacks specificity and memory. Innate immunity is formed by physical barriers (e.g., mucus and collectins) and innate cellular immune responses. The virus infects the epithelial cells then spreads to leukocytes such as macrophages, dendritic cells (DCs), and Natural Killer (NK) cells. Infected cells recognize viral PAMPS (pathogen associated molecular patterns) through cell specific PPRs (pattern recognition receptors).There are three families of receptors that recognize viral PAMPs: TLRs (toll-like receptors), RIG-I (retinoic acid inducible gene I), and NOD-like (nucleotide oligomerization domain-like) receptors. Although host responses to infection are critical for final viral clearance and stimulation of adaptive immune responses, an exacerbated response can lead to immunopathology and severe disease. For example, high levels of neutrophil extracellular traps (chromatin-based structures released from neutrophils) can lead to lung damage and severe influenza virus infection. Therapies aimed to reduce these exacerbations which are called cytokine storm might be beneficial for patients with severe influenza. Here we would like to give an overview about Immune response to Influenza virus.

Keywords: Immune response, Influenza virus

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The main site of infection for influenza viruses is the respiratory epithelium. The first line of defense is performed by the innate immune system which is rapid but lacks specificity and memory. Innate immunity is formed by physical barriers (e.g., mucus and collectins) and innate cellular immune responses. The virus infects the epithelial cells then spreads to leukocytes such as macrophages, dendritic cells (DCs), and Natural Killer (NK) cells. Infected cells recognize viral PAMPS (pathogen associated molecular patterns) through cell specific PPRs (pattern recognition receptors).There are three families of receptors that recognize viral PAMPs: TLRs (toll-like receptors), RIG-I (retinoic acid inducible gene I), and NOD-like (nucleotide oligomerization domain-like) receptors **(1)**.

Stimulation of these receptors induces the release of Interferons, chemokines and other immune mediators. These immune mediators induce an antiviral state by inhibiting viral spread and recruitment of specific immune cells for viral clearance **(1)**.

Both type I interferons (interferon-α (IFNα) and IFNβ) and type III interferons (namely, IFNλ) can inhibit viral replication in epithelial cells **(2)**.

NK cells help in influenza viral clearance by recognizing and lysing influenza virus infected cells. Alveolar macrophages phagocytose virus infected cells for antigen presentation. Both conventional and plasmacytoid dendritic cells (DCs) have a central role in switching innate immunity to the targeted adaptive immunity during influenza virus infection **(1)**.

Conventional DCs are settled under the epithelium and lung parenchyma to continuously survey the airway environment with their dendrites. Detection of influenza virus initiates DC up-regulation of the chemokine receptor CCR7 for migration to the draining lymph node **(1)**.

Pathological innate immune response:

Although these host responses to infection are critical for final viral clearance and stimulation of adaptive immune responses, an exacerbated response can lead to immunopathology and severe disease. For example, high levels of neutrophil extracellular traps (chromatin-based structures released from neutrophils) can lead to lung damage and severe influenza virus infection. Therapies aimed to reduce these exacerbations which are called cytokine storm might be beneficial for patients with severe influenza **(3)**.

Viral evasion of innate immune response:

Host innate immunity is considered as an initial barrier that viruses need to evade to replicate and propagate in new hosts and influenza viruses allocate several viral proteins in attempts to overcome. The NS1 protein of the virus is an RNA-binding protein that prevents the activation of cytoplasmic viral RNA sensors such as retinoic acid-inducible gene I protein (RIG-I; also known as DDX58) **(4)**.

RIG-I recognizes the influenza viral RNAs on the basis of the presence of a terminal 5′-triphosphate moiety and an adjacent double-stranded RNA structure formed by the 5′ and 3′ ends of the viral RNAs that is required for viral RNA replication and transcription **(4)**.

NS1 binds to the host factors E3 ubiquitin-protein ligase TRIM21 and E3 ubiquitin-protein ligase RNF135 (also known as Riplet), which are required for RIG-I activation after viral RNA recognition, preventing a signaling cascade that leads to induction of interferon and interferon-inducible antiviral genes with antiviral activity such as *MX1*, *EIF2AK2* (more commonly known as *PKR*),*OAS1*, *IFITM* family members and *IFIT* family members among others **(5)**.

Influenza A virus NS1 can also inhibit host mRNA synthesis and processing preventing host responses to viral infection. Interferon signaling is mediated by the Janus kinase (JAK)–signal transducer and stimulation of transcription (STAT) pathway as well as the antiviral functions of several interferon-stimulated genes might also be targeted by NS1 to prevent the host antiviral response **(3)**.

Many other influenza virus proteins can reduce the interferon-mediated antiviral response. Polymerase basic protein 1-F2 (PB1–F2) which is a viral nonstructural protein generated from an alternative open reading frame present in the PB1 RNA suppresses the stimulation of mitochondrial antiviral signaling protein (MAVS) which is an adaptor molecule located downstream of RIG-I and required for interferon induction**(4)**.

PB2, a part of the viral polymerase, targets MAVS activation. PA-x, which is a newly discovered viral protein resulting from ribosomal frame shift of the viral RNA polymerase acidic protein (PA) mRNA, also inhibit host gene expression of its RNA endonuclease activity. The multiple ways in which influenza virus resists the interferon-mediated antiviral response define the importance of this host pathway **(4)**.

Adaptive immune response:

Role of cell mediated immunity:

In the lymph node, DCs present viral antigen to CD4+ T cells, CD8+ T cells, and B cells. CD4+ T cells differentiate into Th1 or Th2 cells relying on other mediating cytokines such as IL-2, IL-12 and IL-4. CD8+ T cells stimulate chemokine receptors (CXCR3 and CCR4) to home back to the lung to kill virus infected cells **(1)**.

Cytotoxic T lymphocyte (CTL) effectors play an important role in influenza virus clearance. The major antigenic components of the virus are the NP, matrix protein M1 and the viral polymerases. Infected host cells display viral peptides 8–12 amino acids in length that bind major histocompatibility complex (MHC)

class I glycoproteins to form the antigenic complex recognized by T cell receptors on influenza virus-specific CTLs **(4)**.

These peptides derived from intrinsic constituent of the virus that are less involved in antibody selected antigenic drift. So they are more conserved across distinct influenza virus strains and subtypes **(6)**.

CTL memory directed towards conserved viral peptides presented by widely prevalent human MHC glycoproteins (such as: that are encoded by *HLA-A2*) can provide protection against a newly emerging influenza A virus that passes from an avian reservoir to humans (for example,H7N9cases). Moreover, the increased influenza virus susceptibility of some indigenous populations (in Australia and Alaska) correlates with the relatively low frequency of such protective HLA types among these populations **(6)**.

Effector CTLs will likely need to be recalled from the recirculating CTL memory pool. This idea can give more rapid virus clearance than is the case in a CTL naive individual resulting in milder disease outcomes. Since naive CTL sets are lost with age, priming influenza virus-specific CTLs early is important **(7)**.

Viral evasion of cell mediated immune response:

Relatively more non-synonymous mutations are observed in the CTL epitope regions of influenza virus NP than in the rest of the protein, indicating that CTL epitopes are under selective pressure. However, mutations flanking CTL epitopes may also affect the liberation of antigenic peptides from the protein by the proteasome or transport by TAP into the ER **(8**)

Mutations at T cell receptor (TCR) contact residues can affect recognition by specific T cells, since the epitope no longer matches the specificity of the TCR **(8**)

Both types of amino acid substitutions also have been observed during the evolution of seasonal A/H3N2 influenza viruses **(8**)

Role of Humoral immunity:

Antibodies play a major role in neutralizing infection. B cell responses mainly targeting viral HA and NA and to limited extent target other viral proteins, including the ribonucleoprotein and the matrix proteins. The discrete antigenic sites on the head domain of HA seem to be the favorable target of the antibody response. **(9)**.

Viral infection can also induce low level humoral immune responses against conserved epitopes of viral proteins. Among these epitopes are the membrane proximal stalk domain of HA, the extracellular domain of the M2 protein and conserved epitopes of NA. The peak antibody titer levels are within 2 weeks of onset and then gradually diminish over the subsequent months in a variable degrees **(10)**.

Antibody to the ribonucleoprotein appears to provide little or no protection against reinfection because it is an intrinsic protein of the virus particle that cannot be identified by the circulating antibody **(10)**.

Antibody to hemagglutinin (H) is considered the most protective as it neutralizes the virus on re-exposure as it is the surface protein of the virus and easily recognized by the antibody **(11)**.

However, this immunity is relative and quantitative differences in responsiveness exist among individuals. Moreover, antigenic shifts and drifts eventually allow the virus to destabilize the antibody response on subsequent exposures **(11)**.

Neutralizing Antibodies *in vitro* are HA stalk reactive antibodies. Due to the conserved nature of the HA stalk, these antibodies are often cross reactive within and across HA subtypes. Most stalk-reactive antibodies are restricted in binding to group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, HA-like H17, HA-like H18) or group 2 (H3, H4, H7, H10, H14, H15) HAs **(12)**.

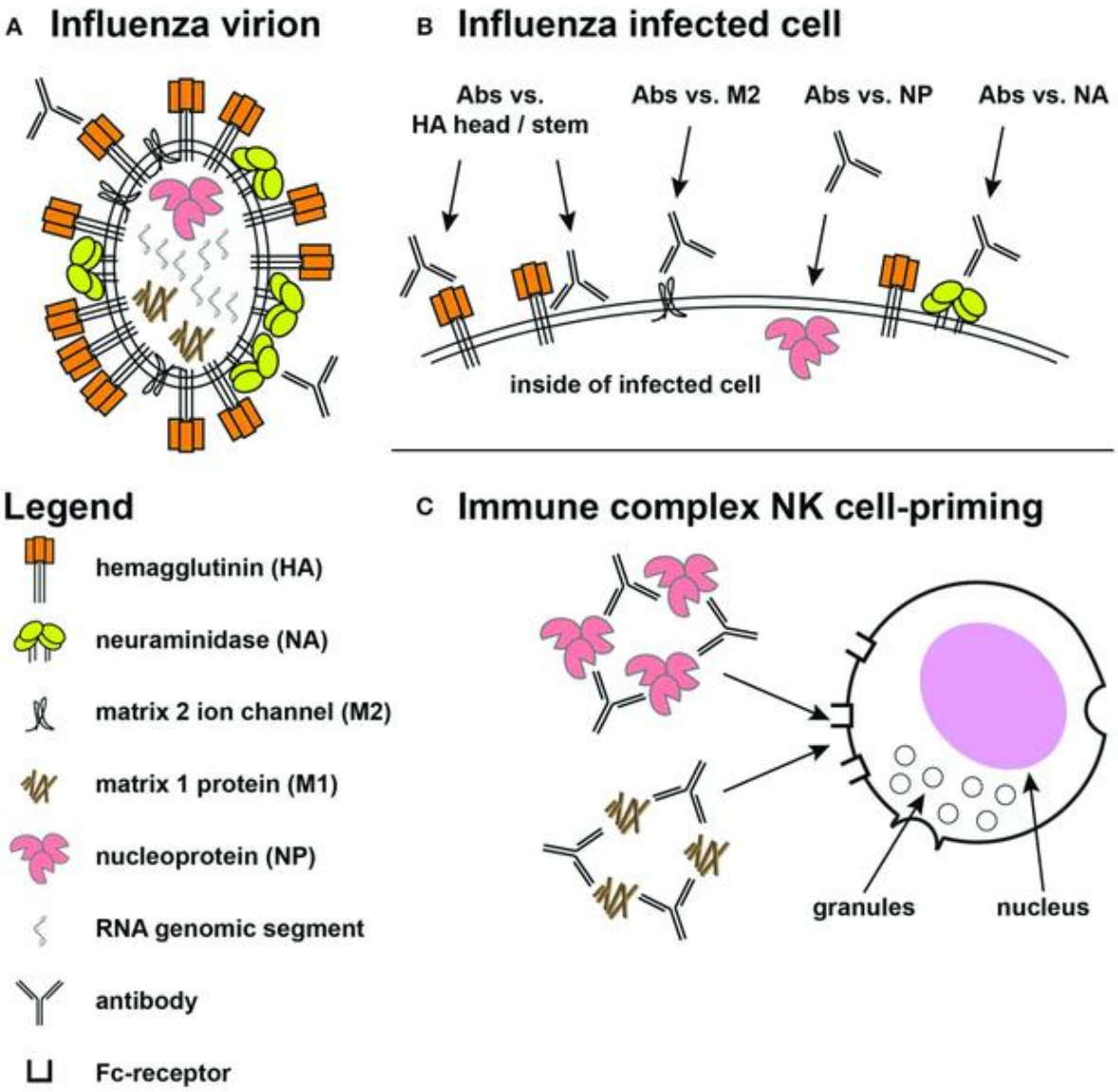

Figure (1): Targets for protective influenza responses (13).

(A) Influenza virion showing the tight packing of viral hemagglutinin (HA), neuraminidase (NA), and matrix 2 (M2) ion channels on the surface. Inside the virion are the 8 genomic segments, nucleoprotein (NP), and the structural matrix 1 (M1) protein. (B) The surface of an infected cell shows the same antigens spread out on the surface, providing access for ADCC-mediating antibodies directed at the HA head and stem, along with greater access to the M2 ion channel. Antibodies against NP have also shown ADCC activity. (C) Immune complexes of NP and M1 have been shown to prime NK cells to secrete cytokines. ***(13).***

Antibodies against the NA can block its enzymatic function: NA inhibition(NI). NI active antibodies interfere with virus release and may inhibit the effective passage of the virus through mucosal fluids and help in protection from the disease but not as hemagglutinin. NA reactive antibodies have been shown to be broadly reactive within the subtype but usually do not exhibit heterosubtypic activity **(12)**.

The broad antibody responses work through mechanisms that rely on crystallizable fragment (Fc)–Fc receptor (FcR) interactions (effector functions) or in the case of anti NA antibodies NA inhibition. However, the antibody responses induced by such conserved epitopes through infection or immunization (with current vaccines) are weak and reduce their potential contributions to protection among population **(14)**.

The mucosal surfaces of the respiratory tract are the main entry pathway for influenza viruses in humans, so the mucosal antibodies play an important role in the prevention of infection. Key experiments in the guinea pig model of influenza have shown that only mucosal immune responses (including immunoglobulin A (IgA))

but not systemic immune responses can efficiently inhibit transmission of the virus which is important to consider that influenza virus vaccines should prevent both viral disease and transmission **(4)**.
Cross reactive antibodies can also provide protection *in vivo* without being neutralizing in vitro. Several mechanisms including antibody dependent cell-mediated cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC) have been postulated to contribute to non-neutralizing cross-protection *in vivo*. ADCC has been recently shown to play a major role in the protective efficacy of HA stalk-reactive antibodies **(14)**.

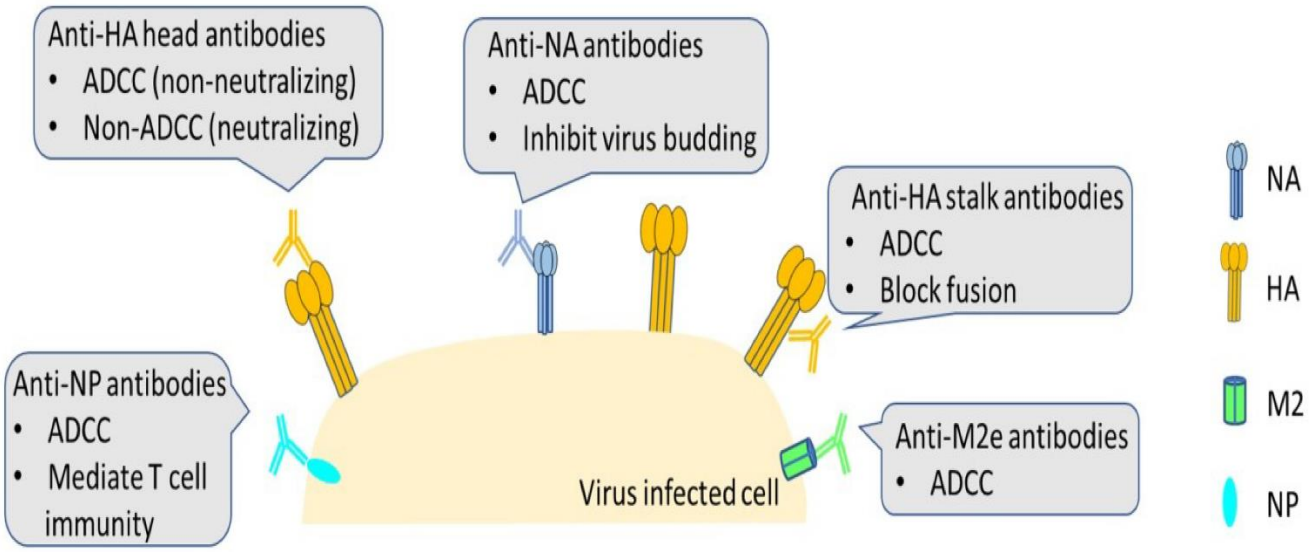

Figure (2): ADCC- mediating antibodies (ADCC-Abs) targeting different influenza antigens (15).
HA, NA and M2 are the surface-exposed proteins, and they are capable of inducing ADCC-Abs. Antibodies targeting the conserved epitopes in these proteins confer broad protection against divergent viruses. NP expressed on the surface of the influenza-infected cell serves as a promising target for ADCC-Abs.

Non neutralizing antibodies can also mediate antibody dependent phagocytosis (ADP) through CD32, CD64 and CD89 receptors ***(15).***

4.2.4. Viral evasion of humoral immune response:

Due to the lack of proofreading activity, the transcription of viral RNA by the viral RNA polymerase is error prone and results in mis-incorporation of nucleotides. As a result, quasispecies of viruses are formed with random mutations in the genome. Under the selective pressure of antibodies that are present in the human population, induced after influenza virus infections and/or vaccination, variants are positively selected from the quasi species that have accumulated amino acid substitutions in the antigenic sites of HA that are recognized by virus-neutralizing antibodies **(8**)
This phenomenon is known as antigenic drift and allows the virus to evade recognition by antibodies and to cause recurrent influenza epidemics yearly **(8**)

Role of complement system in influenza immune response:

In primary infection with influenza virus, mice deficient in component C3 showed delayed viral clearance and increased viral titers in lungs. The presence of complement permits the neutralization of influenza virus by antibodies in vitro. Complement is also known to stimulate influenza virus specific CD4 and CD8 T cell responses and to help maintaining long term memory of influenza viruses in mice **(16)**.
In addition to the neutralization of cell free virus by antibodies to HA and the interference of virus release from infected cells by antibodies to neuraminidase (NA), influenza virus specific antibodies bind to infected cells and are able to lyse the virus-infected cells through stimulation of complement (complement dependent lysis) (CDL) **(16)**.

Epidemiology:

1. Host:

Humans are the major hosts of the influenza viruses. Influenza A viruses closely related to those prevalent in humans also circulate among many mammalian and avian species. As mentioned before, some of these may undergo antigenic mutation or genetic recombination (reassortment) and emerge as new human epidemic strains **(10)**.

Susceptible host according to age group:

Children younger than 5 years old, especially younger than two, due to immaturity of their immune system have the highest susceptibility to influenza virus. Another risk group frequently susceptible to influenza virus is members of the population older than 65 years with an aging immune system. In the USA, it is estimated that around 85% of deaths and 70% of the hospital admissions related to influenza occur to individuals older than 65 years **(17)**.

Host risk factors:

Influenza can have a severe or lethal course relying on the health status of the host. Significantly, high percentage of the population globally is exposed to those risk factors associated with the increase in influenza morbidity and mortality. The comorbidities of respiratory diseases such as chronic obstructive pulmonary disease and asthma, cardiovascular diseases such as ischemic heart diseases, diabetes, obesity, neuromuscular disease, cognitive dysfunction, renal disorders and liver disease are considered risk factors. The prevalence of multiple chronic conditions is expanding worldwide **(17)**.

The role of host genetic factors in disease remains unclear, but polymorphisms and nonsense mutations in several host genes (such as HLA, *IFITM3* and *IRF7*, which encode genes involved in the interferon pathway) have been shown to predispose to severe disease. An important at risk group is pregnant women who are not always aware of the importance of the influenza vaccination**(17)**.

Pathogen related factors:

One of the factors that make influenza an uncontrollable disease is the pathogen itself and its capacity to evade from the immunization induced by the vaccine **(18)**.

Seasonal influenza A and B viruses primarily evolve to escape human humoral immunity via amino acid substitutions, insertions, or deletions coding for haemagglutinin and neuraminidase epitopes that help the viruses to evade key antibodies induced through previous infections, vaccinations or both **(18)**.

Influenza virus has a direct effect on the respiratory system. It is responsible for the weakening of the local immune defense, change in the microbiome and the activation of the inflammatory processes **(17)**.

Human influenza viruses are liable in the environment. They are sensitive to heat, acid pH, and solvents. However avian influenza viruses (H5N1 and others) retain infectivity for several weeks outside the host. The avian virus is shed in respiratory secretions and feces and the virus survives in the feces for a long period of time **(10)**.

Environmental factors:

Seasonal influenza virus outbreaks mostly occur in the winter months, when low humidity and low temperatures help transmission **(19)**.

Influenza epidemics occur annually during relatively cooler and lower humidity periods in temperate climates of the northern and southern hemispheres, whereas in tropical and subtropical climates, one or more peaks in influenza activity during higher absolute humidity or higher precipitation months and year-round influenza activity can occur. Influenza outbreaks can occur during interepidemic periods among people who are epidemiologically linked to travel to areas with influenza activity including people in congregate settings **(20).**

Viable influenza A virus is detected for up to two weeks (stainless steel), one week (cotton and microfiber), up to four days (plastics), up to one day (paper) and less than two hours (wood). Viable influenza B virus is detected for up to one day (stainless steel), six-eight hours (cotton and microfiber), up to one day (plastics) and up to eight hours (paper) (**21)**.

Influenza virus transmission:

Mode of transmission:

Direct droplet spread is the most common mode of transmission. Influenza is transmitted at a short range (1–2 meters) from person to person through large (≥5 μm) that are expelled by infected people through coughing and sneezing. The role of contact transmission and fomite spread is not well understood but is theoretically possible because animal studies have detected viral aerosolization from fomites **(22).**

Transmission patterns of Influenza virus:

The epidemiologic pattern of influenza is based on multiple factors such as the antigenic variations of the virus, range of transmission of the virus and the susceptibility of the population. The susceptibility of community is one of the most important factors in the strength of epidemics and its mortality or morbidity effects in specific. Influenza takes place worldwide and causes morbidity and mortality with pandemic, epidemic, or seasonal patterns **(23)**.

Major epidemics of influenza A usually occur at 2 to 3 year intervals and influenza B epidemics occur irregularly usually every 4 to 5 years. The typical epidemic develops over a period of 3 to 6 weeks, and can involve 10% of the population. In severe influenza A epidemics the mortality rate exceeds the number expected for that period. This is considered an indicator of severe, widespread illness. Influenza B rarely causes such severe epidemics **(10)**.

Seasonal influenza A and B outbreaks occurs annually in temperate regions occurs most commonly due to antigenic drifts with varying in dominant antigenic types and subtypes **(24).**

Pandemic influenza A occurs due to antigenic shift with a major change in HA/NA resulting in new virus and subtype. As influenza is able to undergo multiple mutations and pass through species more over the potential of avian, porcine and human strains to recombine increase the extraordinary ability to undergo more emerging pandemics **(24).**

Incidence of influenza virus infections increases during pandemic years owing to the absence of pre-existing immunity against the new virus, but the degree of severity varies depending on the pandemic virus itself. As the 1918 influenza A H1N1 pandemic was the most severe of the past 100 years **(4)**.

Viability of the virus inside the host:

Influenza virus concentrations in the upper respiratory tract are highest at illness onset or 1–2 days after illness onset and then diminish consequently within 3 days for influenza A but can remain higher for influenza B in immune-competent individuals. Young infants can shed influenza virus for more than 1 week and severely immunocompromised people can have prolonged viral shedding for weeks to months **(26).**

Zoonotic nature spill over:

Zoonotic spillover occurs when the pathogen from an infected animal host enters a human host either directly from a natural reservoir, an intermediate animal host or indirectly from virus in the environment. Avian influenza often pass to humans from an intermediate host (domestic poultry) instead of directly from the natural reservoir (waterfowl including ducks and geese). Avian Influenza is caused by influenza A. The AI viruses that infect humans are generally associated with either H5, H7 or H9 subtypes. Outbreaks around the world of H5N1, H7N9 and H9NS have been reported with a confirmed human case but no human-to-human transmission **(26)**.

Health impact and mortality rates:

The annual epidemiology of influenza shows that while the influenza virus is circulating, the incidence of acute respiratory illness increases. That is evidenced by highest hospital admissions related to influenza complicated pneumonia. In the last two decades, the circulation of influenza B virus caused severe cases and higher death rates, possibly due to the mismatch between the circulating and the vaccine strains **(26)**.

As Influenza virus mainly affects the respiratory tract it can leads to exacerbation of other underlying chronic diseases in the lower respiratory tract such as asthma, chronic obstructive pulmonary disease or infections as pneumonia. Moreover, influenza virus has an indirect effect on many other organ systems due to the systematic inflammation, triggering cardiovascular events such as acute myocardial infarction (AMI), ischemic heart disease, cerebrovascular stroke and exacerbation of renal disorders or diabetes **(4)**.

Another major complication that leads to influenza related hospitalizations and deaths is acute AMI especially with influenza B. Influenza usually has the highest attack rates among young people, while high mortality rates are reported among older adults. In addition to the elderly, mortality and morbidity are specifically high in those with definite high risk underlying medical **(27)**.

Children with underlying respiratory conditions are considered with the highest hospitalization rate complicated with pneumonia. Other influenza related complications observed in children are acute encephalopathy, mild encephalitis/encephalopathy with a reversible splenic lesion, febrile seizures, myositis and thrombocytopenia. Influenza is also associated with significant pediatric mortality, where the most frequent causes of fatality are neurological disease (33%) and pulmonary disease (26%; 16% of asthma). Between the years 2010 and 2016, half of the influenza-associated deaths were pediatric without any underlying medical condition. These children were more likely to die before hospital admission as opposed to the ones with medical conditions **(28)**.

Influenza A and influenza B viruses cause epidemic seasonal infections resulting in 500,000 deaths annually worldwide, with the most recently calculated estimates being 291,243–645,832 deaths per year during the 1999–2015 period **(29)**.

Both disease and mortality in patients with influenza virus infection are probably due to either virus induced pneumonia or secondary bacterial infection **(4)**.

Surveillance systems of influenza virus:

Routine surveillance systems allow for ongoing descriptive assessments, timely, situational, awareness, and informed response, including risk communication to clinicians and the public. Globally, the World Health Organization's Global Influenza Surveillance and Response System performs year-round influenza surveillance include two data bases: syndrome-based (aggregate influenza-like illness (ILI) case) and virologic based (circulating virus subtypes and lineages, proportion of ILI specimens tested that are influenza-positive and development of antiviral resistance). This data is carried out by week, region/country and age group **(10)**.

In Japan, influenza is monitored on the same previous basis. A nationwide network of sentinel sites provides sustained, timely influenza data under the National Epidemiological Surveillance of Infectious Diseases (NESID) system report patients diagnosed with influenza on a weekly basis. Monitoring these sentinel data on medically attended influenza patients provides useful indicators of the spread, rapid and magnitude of transmission. It clarifies the substantial morbidity attributable to seasonal influenza in Japan **(30)**

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