Immunity to human influenza A – an overview

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Abstract

The effector immunological mechanisms that function to prevent, contain and eliminate infection by type A influenza viruses in humans are reviewed. The role of vaccination in eliciting protective immunity against influenza A, and differences between seasonal and pandemic influenza A virus strains that may be responsible for the lower immunity and greater pathogenicity observed in pandemic influenza, are outlined.

Key Words: *immunity, immunopathology, influenza A, vaccination*

Introduction

Influenza viruses belong to the family Orthomyxoviridae and are a common viral cause of human illness. Three main types of influenza viruses, viz A-C, are responsible for disease in humans [1]. Type A also infects domestic and wild animals. There are many subtypes of A due to antigenic variation and therefore it is mainly responsible for the seasonal outbreaks of influenza in winter in temperate climates and possesses the potential to cause devastating pandemics. Type B in contrast causes sporadic outbreaks of mild disease, is mainly restricted to humans, and shows less antigenic variation than Type A. Type C is a common virus that is well adapted to humans and therefore rarely causes disease [1]. The present review focuses on Type A influenza which is of the greatest interest from a human health perspective. Influenza can lead to fatal secondary infections like pneumonia, particularly in immunocompromised persons and in elderly persons where the immune system progressively become less effective.

The Type A virus has eight different negative-sense RNA strands in a virion. Each of the RNA molecules contains one or more genes coding for different viral proteins [2] as shown in Table 1.

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Table 1. Genome and Proteome of the Influenza A Virus

RNA/Gene	Protein	Function
HA	Haemagglutinin (HA). Three different antigenic types viz. H1, H2, and H3, are found in human viruses; At lease 13 others in animal flu viruses	Binding of host cell sialic acid receptors for invasion; fusion of endosomal membrane with viral membrane allowing escape of RNA and viral proteins into the host cell cytoplasm
NA	Neuraminidase (NA). Two antigenic types in human viruses and seven others in animal viruses	Hydrolysis of terminal sialic acid from virions membrane facilitating virion release and its motility in respiratory tract
NP	Nucleoprotein	Structural
M	Matrix proteins M1 and M2	M1- Structural; M2- Proton channel
NS	Non-structural proteins NS1 and NS2 found in host cell cytoplasm but not virions	Suppression of host pre-mRNA processing and innate immunity; Transport of viral ribonucleoprotein particles from nucleus to cytoplasm
Pol 1-3	Three subunits of the viral RNA-dependent RNA polymerase PA, PB1 and PB2 and a pro-apoptotic protein PB1-F2	Viral RNA replication

The influenza virus is an enveloped virus that in transmission electron micrographs shows prominent spikes extending outward from the lipid bilayer (Figure 1). These are due to surface bound neuraminidase (NA) and trimers of haemagglutinin (HA). During the infection process [2], the A-type virus binds to sialic acid residues on the host cell plasma membrane though HA molecules causing the formation of an endosome. The subsequent acidification of the endosome produces a conformation change in the HA that the causes the viral envelope to fuse with the membrane of the endosome, thereby releasing the viral RNA and protein contents of the virion into the host cell cytoplasm. The viral nucleoprotein then translocates to the nucleus where -ve strand RNA is copied to form +ve RNA strands by the

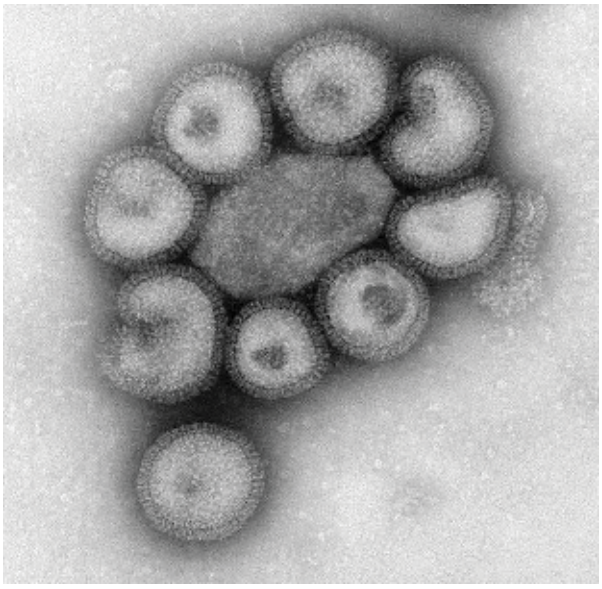

Figure 1. Influenza virus particles**.** The surface spikes are due to Neuraminidase and Haemagglutinin molecules. Courtesy of Center for Disease Control/ Dr. F. A. Murphy [via pingnews] ID#: 8432.

viral RNA polymerase. The +ve strand RNA moves out of the nucleus to serve as mRNA for the synthesis of viral proteins in the endoplasmic reticulum. Glycoproteins like HA and NA become incorporated into the host cell plasma membrane after processing by the endoplasmic reticulum and Golgi body. Extra copies of the –ve strand genome of the virus are also produced by the viral RNA polymerase and together with the relevant proteins are assembled into budding virions that are released from the infected cell with the help of the NA.

HA and NA glycoproteins, are the major target antigens for neutralising antibodies. However HA and NA accumulate mutations in critical epitopes that lead to escape from an anti-viral immune response. Typical winter epidemics involve this *Antigenic or Genetic Drift* which produces new strains of viruses within a subtype.

Antigenic or Genetic Shift results from major changes in antigenicity of HA and NA due to re-assortment with animal and bird influenza viral genomes in co-infected cells. This produces new subtypes of viruses with that differ significantly in antigenicity. The segmented RNA genome of the influenza virus facilitates this process. Such re-assortment can result in a markedly decreased human population immunity to the new strain of virus. In turn, this can lead to epidemics and pandemics, depending on the extent of antigenic difference in the new strain. After the introduction of standardized nomenclature, Influenza A is referred to by the major serotype or subtype e.g. H1N1, and strain e.g. A/Brisbane/2007. A pandemic is usually due to a change in the subtype of HA e.g. the 1968 pandemic involved the emergence of H3N2. The World Health Organization has therefore developed a network of collaborating centres to continuously monitor influenza antigenicity and produce seed material for seasonal vaccine manufacture.

Influenza A pandemics have caused considerable human mortality in the past [1]. The 1918 “Spanish” influenza, due to a H1N1 type virus, caused approximatley 20-50 million deaths worldwide. A 1957 pandemic of H2N2 resulted in 2 million deaths. The lower mortality of this pandemic is attributed to better health care and the availability of antibiotics for the treatment of secondary bacterial infections. In 1968 a pandemic of H3N2 caused 1 million deaths. In 1997, a H5N1influenza A strain that was highly virulent to birds and also able to cause severe illness or death in humans emerged. It was spread by wild birds and poultry, but because of poor human to human transmissibility of the virus no pandemic has resulted to date. In 2009 a new H1N1 strain emerged in Mexico to cause a pandemic. This strain is referred to as 2009 A(H1N1). The pandemic occurred despite the fact that H1N1strains had been circulating in human populations since 1977 and H1N1 (Strain A/ Brisbane/2007) was a component of the 2008/9 seasonal influenza vaccine. The new “swine influenza” variant has been shown to contain many genetic re-assortments with common swine influenza virus genes. Its emergence however has caused a need to rethink the nomenclature of influenza A virus typing, because although typed as H1N1, the antigenicity of its HA was significantly different from the HA of the seasonal H1N1 virus that had been circulating in human populations at that time [3].

Neutralising antibodies that are produced by vaccination or natural infection are largely directed against HA and NA and are therefore generally type and subtype specific. Also there is little immunity against antigenic drift viruses and this results in seasonal influenza epidemics. However there is

data from epidemiological and cotton rat model studies to suggest that a degree of heterosubtypic immunity can develop after natural infection or vaccination [4, 5]

2. Innate Immune Mechanisms in Immunity to Influenza

Infection with influenza virus initially activates the innate immune system through the utilization of specific receptors in human cells termed Pattern Recognition Receptors (PRR). The PRR include Toll-like receptors that recognize viral nucleic acids in endosomes as well as cytoplasmic molecules recognizing unusual structural features of viral RNA. Other viral molecules can activate inflammasomes in macrophages to release inflammatory cytokines like IL-1 and IL-18 [6]. The major mechanisms that play a role in the innate immune response are summarized in Table 2.

The innate immune response provides a measure of protection against infection until the more effective and specific adaptive immune response comes into action

Table 2. Effector Mechanisms in Innate Immunity to Influenza

Induction	Effector cell or molecule	Mechanism of action
-	Naturally occurring mucins	Bind virus
Altered surface of the virion and infected cells	Complement	Activation through the alternate or lectin pathway to promote lysis and opsonisation
PRR	Type 1 interferon (α, β)	Induction of anti-viral state in infected and neighbouring cells through inhibition of protein synthesis and mRNAdegradation. Activation of phagocytic cells and dendritic cells
PRR	Inflammasome in macrophages and dendritic cells	Formation of IL-1, IL-6 and TNFα that promote an inflammatory response in tissue, fever and the synthesis of acute phase proteins
PRR	Macrophage and dendritic cell synthesis of IL-12, IL-18	Activation of NK cells to lyse virus infected cells and enhancement of the adaptive immune response
Stress molecules expressed by infected cells	γδT cells secreting Type 2 interferon γ	Activation of NK cells, phagocytes, dendritic cells and the adaptive immune response

and also has an essential role in activating the adaptive immune response through the supply of relevant cytokines.

3. Adaptive Immune Mechanisms in Immunity to Influenza

protection against infection. Activation of the B cells to become antibody secreting plasma cells takes place in the draining lymph nodes and non - encapsulated mucosa - associated lymphoid tissue in the nasopharynx and the lung. Experiments in animal models show that the appearance of first IgA antibodies and later IgG antibodies in nasal washings correlates well with the decrease in viral titres in the same washings [7]. B cell activation requires helper T cells (T_H cells) that recognize peptides derived from viral proteins that can be membrane, matrix and nucleoproteins, displayed by class II major histocompatibility complex (MHC) molecules on antigen presenting dendritic cells.

Table 3. Effector Mechanisms in Adaptive Immunity to Influenza

Effector Molecule or cell	Mechanism of action
IgA antibodies in mucus and mucosa	Preventing virus binding to host cells through agglutination and neutralization
IgG antibodies in mucus, mucosa and blood	Preventing virus binding to host cells through agglutination and neutralization, activation of complement through the classical pathway, promoting opsonisation and phagocytosis, assisting NK cell killing through Fcγ receptors
T_H Cells	Activation of B cells, promoting immunoglobulin class switching and affinity maturation, secretion of cytokines like IFNγ that activate phagocytes and NK cells and upregulate MHC molecules.
T_c	Apoptosis of virus-infected cells through action of granzyme perforin, etc.

The major adaptive immune mechanisms that serve to prevent infection by limiting viral spread and then eliminating the virus are summarized in Table 3. These mechanisms have been extensively reviewed elsewhere [7].

Dimeric IgA antibodies secreted into the mucus of the respiratory tract provide the first line of defense by the adaptive immune system. IgG antibodies in blood and interstitial fluids have an important role in limiting the systemic spread of the virus. High affinities and levels of these primarily subtype - specific antibodies facilitate

CD8+ Cytotoxic T cells (T_C) are involved in containing and clearing an established infection. Their activation depends on presentation of viral peptides in the context of class I MHC molecules by antigen presenting dendritic cells. This occurs in the draining lymph nodes and mucosal lymphoid

tissues. Activated effector T_C kill virus infected epithelial and other cells displaying viral peptides on the surface in association with Class I MHC molecules. In mouse models, CD8+ cells have been shown to appear in the nasal mucosa 5days post-infection and peak on day 7 [7].

4. Characteristics of the Adaptive Immune Response and Vaccination Against Influenza

Both natural infection and parenteral vaccination produce good protective immunity against the vaccinating strain of virus within a subtype and this phenomenon forms the basis for the seasonal trivalent influenza vaccine that is administered parenterally. The typical influenza vaccine is formalin-inactivated virus produced in eggs. It usually incorporates three major strains in common circulation (two A strains of different subtypes and one B strain) and has a reported efficacy of 60-70%. A vaccine that is reportedly 100% effective is a trivalent cold adapted (and thereby attenuated) live vaccine produced in eggs termed FluMist™ produced by MedImmune [1]. This vaccine is nasally administered as a spray but only to 2-49y old persons who have well functioning immune systems. FluMist™ is currently available in the USA and is reported to show significant subtype specific immunity i.e. against antigenically drifted viruses within a subtype [8]. In this sense it mimics the immunity induced by natural infection better than parenteral vaccination with non-replicating virus. Three factors may be considered to be important in this context. Firstly, the generation of local immunity in the mucosa of the respiratory tract involving IgA and effector T cells is better achieved through nasal immunisation with a live attenuated virus such as FluMist™ than intramuscular injection with inactivated virus. Secondly it is not yet possible to induce effective immunisation through the intranasal route with recombinant proteins or non-replicating viruses without the use of strong adjuvants that are not licensed for this route of administration. Lastly, and from a safety point of view, nasal delivery carries a risk of viruses or vaccine components accessing the brain through the anatomical proximity of the olfactory epithelium and lobe, and of the vaccine inducing hypersensitivity reactions in the respiratory tract. The available information on the nature of protection against the different forms of the influenza virus resulting from natural infection and different forms of vaccination is summarized in Table 4.

A vaccine that is able to generate broadly neutralising antibodies against many different strains and subtypes is being sought by many researchers. A priming immunisation with a plasmid H1N1 HA – based vaccine boosted with seasonal H1N1 influenza vaccine or recombinant HA protein

Table 4. Cross-Protection with Current Vaccines and Natural Infection

Target for Immunity	Natural Infection	Parenteral Vaccine	FluMist™
Immunising or infecting strain	Very good	Very good	Very good
Different strains of a subtype	Good	Poor	Good
Different subtypes	Poor	Nil	Not Known
Different types e.g. A, B or C	Nil	Nil	Nil

was recently shown to elicit antibodies that were able to neutralize a range of HIN1 strains from 1934 to 2007 [9]. These antibodies reacted with a relatively conserved region in the stem of the haemagglutinin molecule, which is also known to be a target for broadly cross-reacting monoclonal antibodies to the haemagglutinin [2]. Because the peptide epitopes recognised by T_C in viral proteins present in the interior of the virion are less prone to antigenic drift caused by antibody selection, such proteins are also being investigated for the development of vaccines effective against diverse strains and subtypes.

Another feature of the antibody response to influenza A viruses is its ability to persist for long periods of time. Survivors of the 1918 H1N1 pandemic have been shown to possess antibodies that neutralized this strain and circulating memory B cells that were capable of becoming activated to produce specific antibodies 90 years later [10]. The antibodies produced were class switched and had undergone multiple somatic mutations to generate high binding affinity for the antigen [10]. Long lasting antibody responses are also characteristic of vaccines such as the Yellow Fever vaccine. Antigen-independent polyclonal activation of memory B cells and long-lived plasma cells may be responsible for the persistent antibody response seen in these elderly persons.

5. Immunopathology of the 2009 A (H1N1) Influenza Virus

Data from the Centre for Disease Control in the USA [11] indicates that approximately 61 million cases of infection were recorded in the USA between April 2009 when the first case was diagnosed and April 2010. There were also an estimated 274,000 hospitalizations and 12479 deaths during this period. The 2009 A(H1N1) influenza tended to affect more young people under the age of 30 than the very young or elderly which contrasts with the normal effects of seasonal influenza [12]. In the near term the virus is expected to establish itself as a seasonal influenza virus. Clinical data indicates that the 2009 A(H1N1) influenza caused somewhat more severe symptoms and lung pathology than the seasonal influenza viruses that had been circulating in the recent past [12, 13]. An unusual feature of 2009 A(H1N1) influenza infection is a greater incidence of severe viral pneumonitis, leading to acute respiratory distress syndrome. Its greater pathology compared to normal seasonal influenza strains is therefore partly attributable to infection and replication in lower respiratory tract. In this sense it resembled the 1918 H1N1 influenza strain. Experimental studies in macaques showed that the 1918 H1N1 influenza virus caused greater lung pathology compared to a conventional influenza virus control A/Kawasaki/173/01 (K173; H1N1) [14]. Studies on microarray-based analysis of gene expression in bronchial tissue showed that the 1918 H1N1 influenza virus induced an atypical innate immune response involving a reduced early Type 1 IFN response, compared to the conventional virus. It is speculated that this may be responsible to the greater disease severity associated with the 1918 H1N1 influenza virus [14]. It appears that the 2009 A(H1N1) influenza virus may also elicit an aberrant early innate immune response that results in less protection and greater disease severity [12-16].

There is evidence to suggest that differential tropism of influenza viruses in the respiratory tract may also play a role in disease severity. The upper respiratory tract epithelium of humans have mainly α 2-6 linked terminal sialic acid residues present at the ends of long glycan chains while birds have terminal α 2-3 sialic acid and α 2-6 sialic acid on shorter glycan chains that have a particular topology favouring the binding of avian influenza viral HA [17]. However human lower respiratory tract type II pneumocytes of humans contain α 2-3 as well as α 2-6 sialic acid-linked glycans. Although the 2009 A(H1N1) influenza HA preferentially binds α 2-6 sialic-linked glycans and to the trachea, it also binds to alveolar tissue [16], a property that may contribute to more prominent viral infection of the lower respiratory tract, systemic spread and consequent greater pathogenicity.

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