Acta virologica **63**: 347 – 365, 2019 doi:10.4149/av_2019_408

REVIEW

Different mechanisms of the protection against influenza A infection mediated by broadly reactive HA2-specific antibodies

K. TOMČÍKOVÁ, E.VAREČKOVÁ*

Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic

Received June 03, 2019; accepted June 12, 2019

Summary. – Influenza A viruses (IAVs) cause yearly repeating infections in humans. The current vaccination approach is based on the production of virus-neutralizing antibodies. Virus-neutralizing antibodies, however, are closely strain-specific due to the IAV variability. Therefore, antibodies produced during the previous influenza season do not provide sufficient protection against new infection, and, hence, annual revaccination is needed. The utilization of the influenza conserved stem domain of hemagglutinin (HA), the HA2 gp, led to a new vaccine design based on cross-reactive cellular and especially humoral immune responses represented by HA2-specific antibodies. The HA2-specific antibodies exhibit cross-reactivity with HA2 gp within one subtype or even among subtypes and play a role in protective immunity against influenza infection. There are several elimination mechanisms of viral replication mediated by HA2-specific antibodies. After recognition of the epitope, they prevent the conformational rearrangement of HA or the insertion of the fusion protein into the endosomal membrane and, consequently, the fusion pore formation. In this case, no release of viral genetic information into the target cell is enabled and virus cannot replicate. The HA2-specific antibodies are involved in the elimination of pathogen via the Fc fragment by activation of the cytotoxic mechanisms of innate immunity as are the antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent phagocytosis (ADP), or complement-dependent cytotoxicity (CDC), resulting in virus elimination and earlier recovery of the host from the infection. Though the protective effect of HA2-specific antibodies on the course of IAV infection was shown, few cases of worsening of IAV infection mediated by HA2-specific antibodies have been described. The identification of antigenic epitopes on HA2 gp that induce antibodies with such deteriorating effect on influenza infection can help to eliminate the unsuitable epitopes of HA2 gp as immunogens during the design of heteroprotective vaccine against influenza and can remove the side effects linked with the observations mentioned above.

Keywords: influenza A virus; HA2 stem domain of hemagglutinin; immunization strategies; HA2-specific antibodies

*Corresponding author. E-mail: viruevar@savba.sk; phone: +421-2-59302427.

Abbreviations: ADCC = antibody-dependent cellular cytotoxicity; ADE = antibody-dependent enhancement; ADP = antibody-dependent phagocytosis; Fab = fragment antigen-binding; Fc = fragment crystallizable region; FcR = Fc receptor; FcγR = FcR specific for IgG; HA = hemagglutinin; HA0 = precursor of HA; HA1 = heavy chain of HA; HA2 = light chain of HA; IAV(s) = influenza A virus(es); IgG = immunoglobulin G; ITAM = immunoreceptor tyrosine-based activation motif; MAb(s) = monoclonal antibody (ies); NA = neuraminidase; VAERD = vaccine-associated enhanced respiratory disease; WIV = whole inactivated virus

Contents:

1. Introduction

It's been a whole century since the world's biggest pandemic broke out that has claimed a large number of human lives. The pandemic emerged in the year 1918 and killed at least 50 million people worldwide. The pathogen responsible for this pandemic was influenza A virus (IAV). It has an important impact on human health until today. In humans, IAV causes a generally known disease of respiratory tract accompanied by a sudden onset of fever, headache, joint and muscle pain, cough and runny nose. The course of the disease varies from mild to severe. The symptoms usually appear on the second day after infection and ends with complete recovery. In some cases, especially in high risk group of patients, the course of the infection can be complicated and can lead to a fatal end. The infection often has a more severe course in older patients (over 65 years), children under the age of 5 years, or individuals suffering from other chronic disease or immunocompromised patients. Out of three to five million people infected by IAV during the usual influenza season, approximately 7–11% cases have lethal outcome (Saunders-Hastings and Krewski, 2016; WHO, 2018).

Due to the IAV variability, host can be infected by IAVs repeatedly, even though the effective immune response to previous infection by IAV has been established. The reason for this is the ability of influenza A viruses to avoid the immunity gained after the infection with previous epidemic strain. There are mechanisms, by which IAVs escape the pressure of the preexisting host immunity. One of the mechanisms of this antigenic variation is known as antigenic drift. It is characterized as an accumulation of minor changes in the nucleotide sequences of genes encoding the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Mutations result from the lack of proofreading activity of influenza RNA-dependent RNA polymerase. Escape mutant viruses, which are not effectively neutralized by preexisting immunity, have a growth advantage and can result in propagation of new antigenic IAV variant. The second mechanism responsible for the antigenic variation is antigenic shift. Each virion of IAV comprises eight genome segments consisting of negative-sense single-stranded RNA. These segments can be mixed and reassembled during the co-infection of a single host by two or more viral strains. Exchange of genes, esspecialy segments encoding surface glycoproteins HA and NA, during virus assembly and budding of viral particle is the key process in formation of potentially new pandemic virus in the immune naive population. The natural reservoir of IAV is considered to be the aquatic birds. However, IAVs were found also in many different animals, including ducks, chickens, whales, horses, seals (Herfst *et al*., 2014; Schrauwen and Fouchier, 2014). It is important to mention that the respiratory tract of pigs is sensitive to human as well as to avian IAVs. Therefore, pigs could serve as a vessel to mix the genetic material of two different viruses (of avian and human origin) during the co-infection and thus are important elements in interspecies IAV transmission (Briedis, 2011). Occasionally, IAVs can directly cross the interspecies barrier, fortunately without the ability of ongoing spread from human to human (Wright *et al*., 2013; Webster and Govorkova, 2014; Yoon *et al*., 2014; Joseph *et al*., 2017). The discovery of new host organisms sensitive to IAV urges to intensive study of interspecies transmission of IAV and its role in a potentially dangerous new pandemic virus creation.

2. Activation of immune mechanisms early after the infection

After confrontation of the host with IAV, the innate and adaptive immune response is activated. Anatomical and chemical barriers are the first line of defense against infection. Immediately after the virus overcomes the first barrier and enters into the host cells, the pattern recognition receptors (PRRs) localized on the surface of host epithel recognize the pathogen-associated molecular patterns (PAMPs) derived from virus. The antiviral effect of innate immune response is a result of many interactions resulting in prevention of infection dissemination. Ligand, present on virus, binds to PRRs and activates downstream signaling pathway, leading to production of pro-inflammatory cytokines, chemokines and

interferon (IFN) type I (Pang and Iwasaki, 2011; Sanders *et al*., 2011). Intracellularly, PRRs recognize IAV components (dsRNA, ssRNA) either in endosome by Toll like receptors (TLRs) 3, 7, 8 (Wang *et al*., 2008; Lee *et al*., 2013) or in the cytoplasm by retinoic inducible gene-I (RIG-I) (Loo *et al*., 2008) and nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain containing-3 (NLRP3) (Allen *et al*., 2009). PRRs are expressed on many immunocompetent cells, including monocytes, macrophages, neutrophils, basophils, eosinophils, dendritic cells (DC) and even on lymphocytes. Innate immunity is activated by the infection within few minutes or hours. The immune cells recognize small molecular motifs conserved within a class of pathogens. They are not pathogen-specific, in contrast to motifs recognized by mechanisms of adaptive immunity. Outcome of adaptive immune response is the production of IAV-specific antibodies induced after the activation of cellular components – B cells, T_H and T_C cells. Amount and the promptness of IAV-specific Abs release is increased by every following encounter with the antigen (Gerhard, 2001; van de Sandt *et al*., 2012). The protection provided by the adaptive immune response, particularly against HA and NA, is the basis of the vaccination strategy against IAV.

3. Prevention of influenza disease

Currently used seasonal vaccines require permanent attention because their efficacy is time-limited due to the continual changes in the IAV genome. Permanent monitoring of the viruses circulating in the population allows researchers to predict the vaccination virus strains for the following influenza season. At present, available seasonal influenza vaccines are trivalent or quadrivalent. Every dose of trivalent vaccine is designed to confer the protection against IAV of two subtypes (H1, H3) and influenza B virus from predicted circulating viruses. The quadrivalent vaccine is supplemented with influenza B virus from the second, antigenically distinct virus lineage i.e. it contains two influenza B viruses of Yamagata and Victoria lineages (cdc, 2018a,b; ecdc, 2018). Influenza vaccines are available in two forms: as live attenuated vaccine or as inactivated vaccine (Sridhar *et al*., 2015). The antibodies specific to HA glycoprotein have virus-neutralizing activity (Gamblin and Skehel, 2010), unlike the antibodies specific to neuraminidase (NA), which limit IAV spread by inhibition of the esterase activity of NA. Virus neutralizing antibodies recognize antigenic sites on HA near the receptor-binding site and thus hinder the virus attachment to cell receptors. Antibodies directed to these sites on HA mediate protective immunity against infection with identical virus or antigenically very closely related IAVs. High variability of this HA region (Kirkpatrick *et al*., 2018), which complicates the prediction and production of effective vaccine, has brought the researchers to the idea of looking for widely conserved parts of IAV, which could be used for the development of universally effective vaccine (Staneková and Varečková, 2010; Pica and Palese, 2013; Yamayoshi and Kawaoka, 2019).

4. The role of HA and its HA1 and HA2 subunits in viral replication cycle

The immune response elicited after the application of current vaccines is targeted to the main surface antigen, the HA. It is a glycoprotein, which is a key player during the virus replication cycle. It is encoded by the fourth IAV genome segment. The replication and transcription of IAV genome take place in the nucleus of the infected cell (Fodor, 2013; Dou *et al*., 2018) and the transcribed viral RNA is transported to the cytoplasm, where the viral proteins are synthesized. The viral life cycle is finalized after the assembly of newly replicated genomic RNA, which forms, together with the newly synthesized viral nucleoprotein and viral polymerase proteins PB1, PB2 and PA, a ribonucleoprotein complex (RNP). RNP is transported to the site of virus particle assembly at the cytoplasmic membrane. Replication cycle is finished by virus budding from the cell membrane of infected cells.

HA is synthesised on the ribosomes of rough endoplasmic reticulum (ER) as a precursor molecule HA0. It undergoes several posttranslation modifications, trimerization, glycosylation, acylation and proteolytic cleavage of HA0 into HA1 and HA2 gp (Braakman *et al*., 1991; Hebert *et al*., 1997; Skehel and Wiley, 2000; Daniels *et al*., 2003; Vigerust *et al*., 2007; Krammer *et al*., 2012; Magadán *et al*., 2013; Tate *et al*., 2014; Zhang *et al*., 2015). The cleavage of the precursor HA molecule into two subunits is an important posttranslation modification of HA0, a step essentially required for the infectivity of virus. HA0 trimers containing the multibasic sequences in the cleavage site formed by aa sequence rich in arginines and lysines (cleavage site consensus R-X-R/K-R) are proteolytically cleaved intracellularly in Golgi apparatus. They are present mainly in the HA of highly pathogenic avian viruses (HPAI) and are cleaved by ubiquitously present subtilisin-like cell proteases, such as furin or PC6. Low pathogenic avian viruses (LPAI) and the majority of human IAVs contain a monobasic cleavage site (cleavage site consensus Q/E-X-R). In this case, HA0 is cleaved extracellularly by trypsin-like serine proteases, e.g. tryptase Clara, HAT-protease or plasmin, the localization of which is restricted to the epithelial cells of the respiratory or intestinal tract (Skehel and Wiley, 2000; Böttcher-Friebertshäuser *et al*., 2014; Mair *et al*., 2014, Peitsch *et al*., 2014). In mature HA, after the cleavage, HA1 and HA2 gps remain linked together by disulfide bonds (Steinhauer, 1999; Gamblin and Skehel, 2010; Mair *et al*., 2014).

Each monomer of HA is composed of globular HA1 domain and the stem domain, which is created mainly by HA2 gp and only by a minor part of HA1 gp. Both, HA1 and HA2 subunits participate in the virus entry into the host organism and thus ensure the propagation of IAVs. The HA provides the first contact of the virus with cells at the site of entry (respiratory tract in humans and mammals). Virus is attached to the host cell receptors via the receptor binding site on HA1 gp. Studies of viral isolates revealed that the virus recognizes the host cell receptors dependending on species from which the virus originates (Rogers and Paulson, 1983). IAVs of human or mammalian origin recognize sialic acid terminally linked to the galactose of the cell surface glycoproteins or glycolipids by Sia(α-2,6)Gal glycosidic bond, while avian IAVs recognize sialic acid linked to galactose by Sia(α-2,3)Gal type bond (Sriwilaijaroen *et al*., 2009). The IAVs attached to the cell surface receptor then enter into the cells by endocytosis. The gradual decrease of pH in endosomes causes refolding of HA of endocyted virus to the fusion-active form. The intermolecular bonds between HA1 globular parts of thermodynamically unstable HA trimer become weaker, the distance among them increases and the originally closed globular domain is opened, enabling the exposure of the N-terminal end of the HA2 gp. Simultaneously, the HA2 gp undergoes a complex structural rearrangement. Complex structural changes of HA2 gp result in the release of HA2 N-terminus, until then trapped inside the HA trimer, and its insertion into the endosomal membrane of the host cell. To achieve the thermodynamically stabile HA conformation, the viral and endosomal membranes mutually approach. Then the hemi-fusion and fusion pore creation occurs and genetic material is released into the cell cytoplasm. The low pH conformational change of HA is an irreversible process and requires the low pH ranging from pH 5 to pH 6 (Jakubcová *et al*., 2016; Russell *et al*., 2018). The pH optimum of fusion is strain-specific and is predetermined by many factors, including the primary structure of HA. As this process is endothermic, it can be influenced also by the temperature, at which the fusion occurs (Wharton *et al*., 1986, Wiley and Skehel, 2000). The temperature increase from 37°C to 56°C shortens the time needed for the structural rearrangements of HA. A small fraction (from 3 to 7%) of HA trimers was detected in this low pH conformation on purified virus as well as on the newly synthesized HA of infected cell surfaces (Kostolanský *et al*., 1988; Varečková *et al*., 1993). The reason of such micro-heterogeneity can be the spontaneous conformational change due to the flexibility of HA trimer at the physiological temperature (Yewdell *et al*., 1983). These two domains of HA, HA1 and HA2 gp, have irreplaceable role in infectious cycle of IAV and simultaneously they represent the main target for the induction of protective immune response.

5. Antigenic properties of HA and its subunits

Based on the antigenic properties and the HA reactivity with virus-specific sera in hemaglutination inhibition assay, double immune-diffusion assays (Schild *et al*., 1980; WHO memorandum 1980) and amino acid sequence analysis (Fouchier *et al*., 2015), there are currently 18 defined subtypes of influenza HA. The first 16 HA subtypes comprise avian influenza viruses. The subtype H16, described in the year 2015, is, as of today, the last HA subtype of IAV isolated from aquatic birds (Fouchier, 2005). Few years later, IAV was detected in another species, in bats. Viruses found in bats were antigenically different from known IAVs and were classified as new HA subtypes H17 and H18 (Tong *et al*., 2012, Tong *et al*., 2013).

On the other hand, based on phylogenetic analyses HAs of different IAVs were divided into two groups. The first group contains IAVs with HA subtypes H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18. The second phylogenetic group comprises H3, H4, H7, H10, H14 and H15 subtypes. Both these groups comprise the highly pathogenic viruses: H1, H2, H5, H6 and H9 subtypes from the first group, and H3, H7, H10 from the second group (Medina and Garcia-Sastre, 2011; Belser *et al*., 2009, 2013; Herfst *et al*., 2014; Vachieri *et al*., 2014; Wang *et al*., 2014), which represent viruses of high risk for human health. The widespread incidence of IAV among different species and permanent mutations in IAV genome enable the generation of new influenza viruses, which can infect humans without preexisting immunity and make the universal protection against influenza disease difficult.

5.1 Antigenic structure of HA1 and HA2 gp

The globular HA1 gp of HA trimer is the immunodominant domain of HA. Therefore, HA1 gp was well characterized many years ago. There were defined five antigenic sites on HA1 gp, which are predominantly located on the loops of the amino acid (aa) chain (Caton *et al*., 1982). First, using monoclonal antibodies and escape variants of A/PR/8/34 (H1N1) virus, antigenic sites were defined and signed as site Sa, in the region of amino acid sequence comprising aa 128–167, site Sb comprising aa 156–198, site Ca1 aa 169–240, Ca2 140–225 and Cb 79–122. Antigenic sites of IAV viruses differ in length and aa composition (Caton *et al*., 1982). Later, five antigenic sites A, B, C, D and E were identified on the HA of H3 subtype (Skehel and Wiley, 2000). However, the sites B and C were subdivided on the basis of fine specificity. Namely antigenic sites A (121–146), B1 (155–163), B2 (186–197), C1 (50–57), C2 (275–279), D (207–219), and E (62–83) were defined (Wiley *et al*., 1981; Jackson, 1982; Okada *et al*., 2010; Ye *et al*., 2012; Shaw and Palese, 2013). Despite the high variability of HA globular

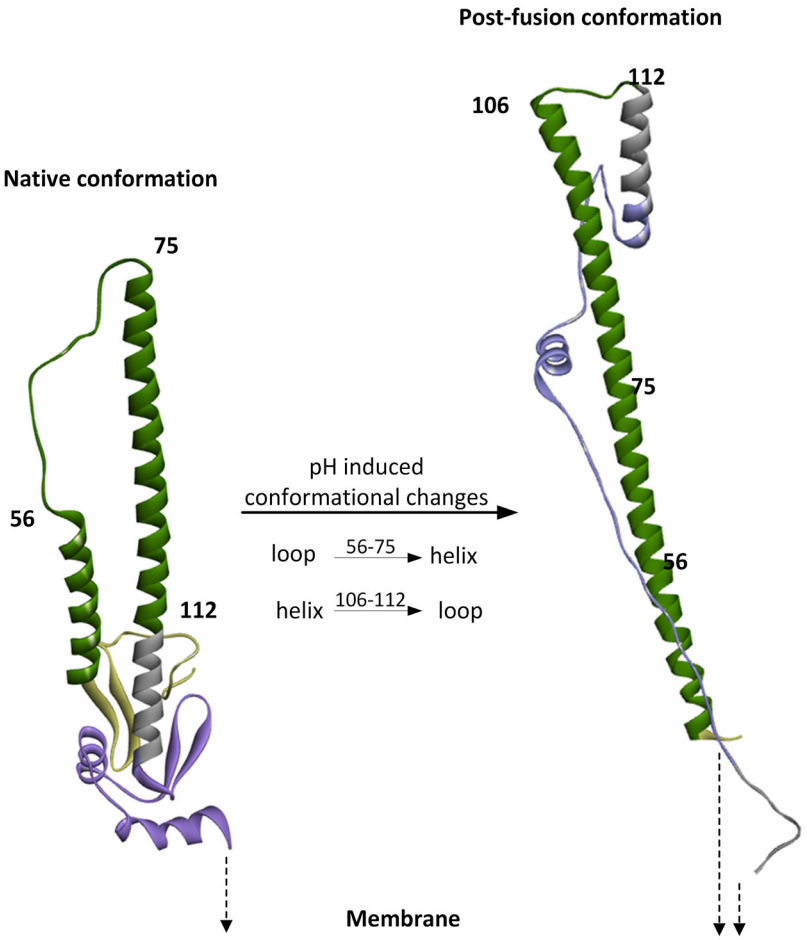

Fig. 1

Structural rearrangements of HA2 gp from native conformation to post-fusion conformation induced by decreasing pH with an effect on stem domain immunogenicity

The antigenic regions of HA2 were determined using HA2-specific monoclonal Abs. Antigenic site I is colored yellow (aa 1–38) and includes first 23 aa of fusion peptide, two different antigenic sites II and IV in the same region are colored purple (aa 125–175) and site III is shown in green (aa 38–112). The pH-induced structural changes take place at the antigenic site III. Loop (in position aa 56–75) is changed to α-helix and α-helix (in position aa 106–112) is changed to loop durin this change. (The figure was created in Discovery Studio 2019. Source: PDB 4WE4 (HA2 in native conformation), PDB 1QU1 (HA2 in post-fusion conformation) and modified from Varečková *et al.* (2003a).

domain, there are some conformational epitopes, which can be recognized by neutralizing antibodies cross-reactive with viruses of H1 phylogenetic group (Whittle *et al.*, 2011; Lee *et al.*, 2012; Tsibane *et al.*, 2012; Krause *et al.*, 2012) or H2 group (Kostolanský *et al.*, 2000)

In contrast to HA1 gp, the stem of HA, which is formed predominantly by HA2 gp, is relatively conserved. The reason is the absolute requirement of HA2 functionality for viral and endosomal membrane fusion and thus for the infectivity of the virus. As a consequence, the HA2, unlike the HA1 of IAV, is more antigenically stable with high degree of aa conservation (Nobusawa *et al.*, 1991; Varečková *et al.*, 2008, 2013; Margine *et al.*, 2013a; Jakubcová *et al.*, 2019). On HA2 gp, four antigenic sites were defined by competitive radioimmunoassay using seven HA2-specific monoclonal Abs. The antigenic site I is localized at aa position 1–38 of the N-terminal end of HA2 peptide. The highest immunogenic potential have antigenic sites II and IV, which are localized at the aa position 125–175, but they create different epitopes as it was implied by the competitive radioimmunoassay studies and western-blot analyses using HA2-specific MAbs. Antigenic site III is localized in the region of aa 38–112 (Varečková *et al.*, 2003a). All 4 antigenic sites on HA2 gp are poorly accessible in the native HA trimer and become more accessible after the low pH exposure of the virus, resulting in the conformational change of HA. This was confirmed by the increased binding of HA2-specific MAbs, recognizing all four antigenic sites on HA2 gp, to the low pH- (pH 5)

Table 1. HA2-specific Abs broadly reactive among various subtypes of influenza viruses

Name of Ab	Reactivity	References
C179	H1,H2,H5,H6,H9	Okuno *et al*., 1993
A06	H1,H5,H9	Kashyap *et al*., 2008, 2010
CR6323	H1,H5,H9	Throsby *et al*., 2008
CR6261	H1,H2,H5,H6,H8,H9,H13,H16	Throsby *et al*., 2008
FC12	H3,H4	
FE1	H3,H4	Varečková *et al*., 2008
IIF4	H2,H3,H4,H5,H6,H8,H13	
CF2	H3,H4,H7	Stropkovská *et al*., 2009
F10	H1,H2,H5,H6,H8,H9,H11,H12,H13,H16	Sui *et al*., 2009
12D1	H3	Wang *et al*., 2010
FI6	H1-H16	Corti *et al*., 2011
CR8020	H3,H4,H7,H10,H14,H15	Ekiert *et al*., 2011
CR9114	IAV, IBV	Dreyfus *et al*., 2012
05-2G02	H1,H3,H5	Li *et al*., 2012
6F12	H1	Tan *et al*., 2012
GG3	H1,H5	Heaton *et al*., 2013
KB2	H1,H5	Heaton *et al*., 2013
39.29	H1,H3,H5,H7	Nakurama *et al*., 2013
CR8043	H3,H10	Friesen *et al*., 2014
9H10	H3,H10	Tan *et al*., 2014
MAb3.1	H1,H2,H5,H6	Wyrzucki *et al*., 2014
VIS410	H1,H3,H7	Tharakaraman *et al*., 2015
CT149	H1,H3,H5,H7	Wu *et al*., 2015
MEDI8852	H1,H3,H5,H7	Kallewaard *et al*., 2016
81.39	H1-H10,H14,H15	Marjuki *et al*., 2016
CT-P27	H1,H2,H3,H5,H7,H9	Celltrion; (Sparrow *et al*., 2016)

treated viruses, without changes of their antigen-binding affinities (Fig. 1) (Varečková *et al*., 1993, 2003a,b; Staneková *et al*., 2012).

The immunogenic potential of HA2 antigenic sites described above differs in their ability to induce specific antibodies during natural influenza A infection in humans. The analysis of paired acute and convalescent sera of human patients with confirmed influenza infection showed that HA2 antigenic sites II and IV, localized in the region aa 125–175, are the most immunogenic. These two antigenic sites, together with antigenic site I could be important for the hetero-protective immunity induced during the influenza infection of humans, as antibodies recognizing these sites inhibited the fusion activity of HA as well as the replication of virus (Varečková *et al*., 2003b; Stropkovská *et al*., 2009; Staneková *et al*., 2011, 2013; Janulíková *et al*., 2012).

5.2 Immunogenic properties of HA2 gp

It was shown that HA2 is a weak inducer of humoral immune response during the natural infection (Styk *et al*., 1979; Gerhard, 2001; Kostolanský *et al*., 2002; Varečková *et al*., 2013). The reason is that HA2 is hidden inside the HA trimer and is not accessible for the immunocompetent cells due to the covering of the HA2 subunit by HA1 globular domain carrying the immunodominant antigenic sites (Angeletti *et al*., 2017). The first report about the ability of HA2 gp to induce specific antibodies was published by Styk and Russ (Russ *et al*., 1978; Styk *et al*., 1979). An important feature of antibodies produced against the HA2 domain is their intra-subtype (Graves 1983) and even inter-subtype cross-reactivity (Russ *et al*., 1987; Okuno *et al*., 1993; Varečková *et al*., 2002, 2003a,b, 2013), enabling the recognition of a wide range of influenza viruses, as has been described in the literature (Table 1). It was shown that some HA2-specific monoclonal antibodies can be protective and cross-reactive, therefore the HA2 gp was considered to be a good immunogen for induction of the broader immune protection against influenza (Gocník *et al*., 2007; Prabhu *et al*., 2009). Many approaches have been described to overcome the low immunogenicity of HA2 gp and to enhance the induction of HA2-specific antibodies to be utilized in new vaccine design with the aim to broaden the vaccine efficacy. These approaches are based on improved accessibility of HA2 gp and its effective delivery or exposure to the immune system (Krammer and Palese, 2013; Margine *et al*., 2013b).

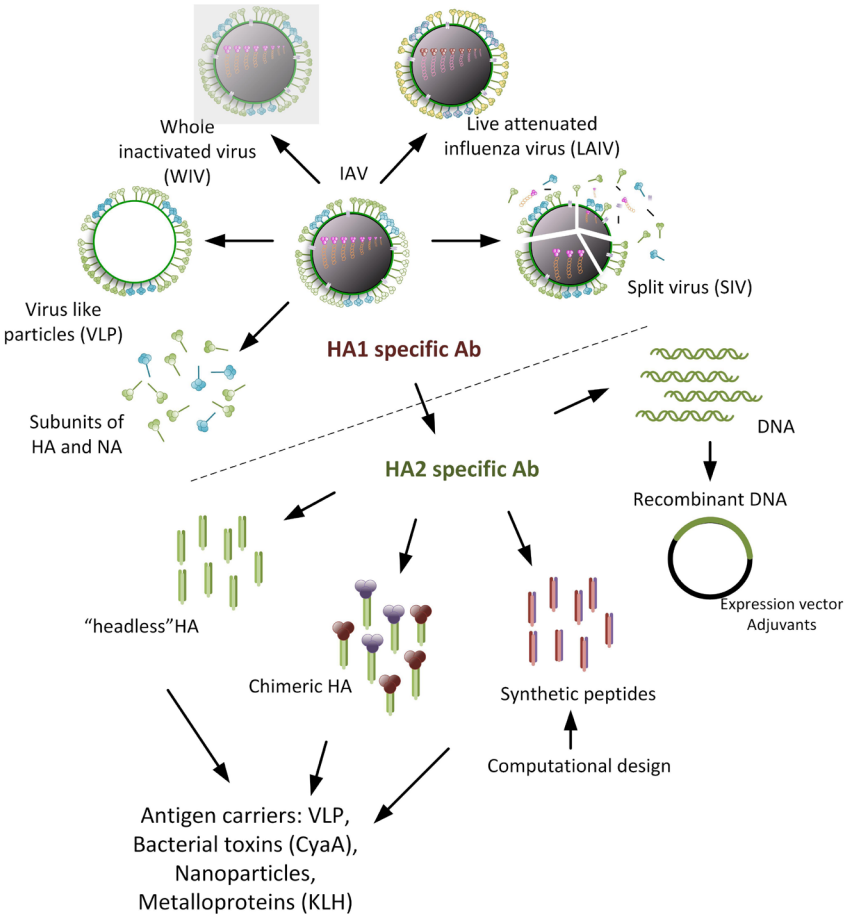

Fig. 2

Influenza virus vaccines based on induction of strain-specific or broadly reactive stalk-specific humoral immune response
Vaccines using whole hemaglutinin as an antigen are focused on the induction of virus-neutralizing antibodies targeted to immunodominant globular domain (WIV, LAIV, SIV, VLP, subunit vaccine). Stimulated Abs have narrow protective effect against close/relative influenza virus strains to vaccine strains in comparison with Abs against HA2 gp. There have been suggested modifications increasing the immunogenicity of stalk domain ("headless" HA gp lacking immunodominant globular domain, chimeric HA with exotic globular domain, computational optimized synthetic peptides and DNA-based vaccines).

New strategies increasing immunogenicity of HA2 gp based on more effective exposure or delivery of antigen for presentation to immune competent cells have been developed (Fig. 2) (Staneková and Varečková, 2010; Krammer and Palese 2013; Margine *et al.*, 2013b). Increased induction of HA2-specific antibodies was achieved using various carriers as are Keyhole limpet hemocyanin (KLH- a large metalloprotein from the giant keyhole limpet), flagellin of the Salmonella vaccine strain (a polymeric character enables to express multiple copies of the epitope), nanoparticles based on metalloproteins (Kanekiyo *et al.*, 2013; Yassine *et al.*, 2015), or virus-like particles (VLP) (Kang *et al.*, 2012, Chen *et al.*, 2015). Other approach utilized non-infectious non-replicating *Escherichia coli*-derived plasmids as DNA vaccines (Katz *et al.*, 2006), or detoxified bacterial toxin from *Bordetella pertussis* (Staneková *et al.*, 2013). KLH was used as the carrier for different peptides of HA2, for example aa sequence of highly conserved long α-helix recognized by cross-reactive Ab 12D1 (Wang *et al.*, 2010), aa sequence of fusion peptide (Staneková *et al.*, 2011) or the ectodomain of HA2 (Janulíková *et al.*, 2012). Immunization of mice with two or three immunization doses of mentioned immunogens induced significant antibody response with cross-protective potential, resulting in improved survival and morbidity of mice challenged with homologous or heterologous virus. Genetically detoxified adenylate cyclase toxin (CyaA) produced by the gram-negative bacteria *Bordetella pertussis* was used to present the ectodomain of HA2 to the immune system. The advantage of use of the adenylate-cyclase toxoid is primarily in its ability to induce cross-protection mediated by both cellular and humoral immunity, though CyaA-HA2 toxoid is a non-replicating immunogen. This is an advantage

from the point of view of safety of the immunization. In this case, a specific cellular and broadly anti-HA2 cross-reactive humoral immune response was induced, which protected mice against lethal infection with both homologous and heterologous IAV without addition of any adjuvants (Staneková *et al*., 2013).

Another approach to increase the HA2 immunogenicity is the generation of HAs lacking the HA1 subunit. In earlier experiments, the unmasking of HA2 domain was achieved by enzymatic cleavage of low pH-exposed virus named "Graves particules" (Graves, 1983). Later, genetic engineering enabled a more effective preparation of HA molecule partially or completely lacking the HA1 globular domain (Sagawa *et al*., 1996; Bommakanti *et al*., 2010; Bommakanti *et al*., 2012). A novel HA2 immunogen with deleted HA1 globular part of HA, the "headless" HA, a linker sequence preserving the proper folding of protein molecule in the native structure, and importantly, preserving its ability to express on the cell surface was constructed. This headless HA have, in contrast to enzymatically cleaved HA2, "the neutral pH conformation" (Steel *et al*., 2010). To improve antigen expression and appropriate folding of protein in order to induce robust protective antibody response, a minimized stem polypeptide was engineered that includes the epitope recognized by broadly reactive monoclonal antibodies and mimics the HA trimer in the pre-fusion conformation (Mallajosyula *et al*., 2014; Lu *et al*., 2013; Wohlbold *et al*., 2015; Valkenburg *et al*., 2016; Sutton *et al*., 2017). A similar approach was used in construction of a "mini" HA, which has also properties like native HA2 in pre-fusion trimeric conformation (Impagliazzo *et al*., 2015). The both "headless" and "mini" HAs induced broad reactive antibody response and improved mice survival after viral challenge.

The development of a plasmid reverse-genetic technique opened the new possibilities for the influenza research (Fodor *et al*., 1999; Neumann *et al*., 1999; Hoffmann *et al*., 2000). Chimeric HAs, which are composed of globular and stem domain of different virus subtypes represent a promising strategy in the development of a universal influenza vaccine (Krammer and Palese, 2014). Many variations of various chimeric HAs with exotic globular domain and the stem domain of irrelevant subtype of the same or different phylogenetic groups were described (Hai *et al*., 2012). Chimeric HAs were used as part of the whole or split inactivated virus vaccine (WIV), or live attenuated influenza virus vaccine (LAIV) (Nachbagauer *et al*., 2018; Sunwoo *et al*., 2018). HA was presented also by virus vectors as are vaccinia virus (Gocník *et al*., 2008), influenza B virus, vesicular stomatitis virus (VSV), adenovirus type 5 (Nachbagauer *et al*., 2016). Another method of boosting stalk-reactive antibodies was achieved by repeated immunization refocusing the immune response to conserved HA2 domain, thus eliciting humoral and cellular HA2-specific immune response (Margine *et al*., 2013a,b; Nachbagauer *et al*., 2014). Protective antibodies have also been obtained after the immunization with recombinant HAs comprising the HA2 conserved sequence derived from the H1 subtype inserted into the globular domain of H3 subtype (Klausberger *et al*., 2016).

Manipulation with the glycosylation sites on HA1 gp refocuses the immune system to the epitopes on HA2 gp. It was found that the number of glycosylation sites on the HA surface can differ over time (Medina *et al*., 2013). Changes in the glycosylation rate help the HA to escape from its recognition by neutralizing antibodies. Modifications of the globular domain by introducing seven new N-glycosylation sites into this immunogenic region of HA of the influenza virus A/PR/8/34 (H1N1), naturally containing only a small amount of bound saccharide residues, were described. After intramuscular administration of three doses of antigen, produced HA2-specific antibodies were able to reduce morbidity and mortality of mice infected with A/PR/8 reassortant virus with the head domain of H9 subtype compared to wild-type globular domain (Eggink *et al*., 2014).

The approaches mentioned above have been shown as promising vaccine strategy leading to the production of broadly reactive antibodies, because as it was shown, HA2 antigen in variously modified forms can be good immunogen. Therefore, it could be an excellent player in the protection from IAV infections.

6. Protective mechanisms mediated by HA2-specific antibodies

Vaccine strategy is based on immunological memory. When the immunization is followed by an infection, the memory cells are stimulated faster and the organism is protected more efficiently. The production of HA2-specific antibodies during the natural IAV infection is limited. However, due to the conserved character of HA2 gp, the subsequent infection by several antigenically different IAVs, or immunization by selected epitopes of HA2 led to a stronger HA2-specific antibody response (Kostolanský, 2002; Gocník *et al*., 2008; Sui *et al*. 2009). Significantly increased level of anti-HA2 antibodies positively contributes to the efficient protection against lethal infection with homologous or heterologous virus in mice (Gocník *et al*., 2008; Staneková *et al*., 2011, 2013; Janulíková *et al*., 2012). The presence of these antibodies influences the infection, which becomes milder and the recovery from the disease is faster. Moreover, HA2-specific antibodies with fusion-inhibition activity, when administered intravenously before the infection, improve the survival of infected individuals and accelerate the clearance of the virus (Gocník *et al*., 2007). The antibodies targeted

to HA2 domain influence the course of IAV infection at several levels. Intracellularly, anti-HA2 antibodies block the conformational HA rearrangements after binding to/or near the fusion epitope, or they block the insertion of HA2 fusion peptide into the endosomal membrane and thus inhibit fusion pore formation and consequently the viral replication (Ekiert *et al*., 2009; Varečková *et al*., 2003a, 2013). Moreover, cross-reactive HA2-specific antibodies can prevent the intracellular or extracellular proteolytical cleavage of HA0 (Ekiert *et al*., 2011; Brandenburg *et al*., 2013).

On the other hand, there are reports considering the negative effect of HA2-specific antibodies on the course of infection (Gocník *et al*., 2007; Khurana *et al*., 2013; Gauger *et al*., 2014). The first report about deteriorating effect of an antibody on IAV infection was described by Gocník and colleagues (Gocník *et al*., 2007). Passive immunization of mice with anti-HA2 antibody without fusion inhibition activity, recognizing the antigenic site III (aa 38–112 of HA2 gp), and their subsequent infection with lethal dose (1LD50) of homologous influenza A virus caused more severe infection in comparison to the control, non-immunized mice. In contrast, infected mice passively immunized with three other MAbs with fusion-inhibition activity were protected from the lethal IAV infection (Gocník *et al*., 2007). Negative impact of vaccination mediated by induced cross-reactive antibodies has been described later, during the circulation of IAV with pandemic potential p(H1N1) in human population (Janjua *et al*., 2010; Skowronski *et al*., 2010; Tsuchihashi *et al*., 2012). The phenomenon was designated as Vaccine-associated enhanced respiratory disease (VAERD) and its mechanism is not yet completely understood. There are several other reports describing the possible role of cross-reactive antibodies in VAERD.

The experimental vaccination with H1N2 whole inactivated vaccine (WIV) followed by infection with p(H1N1) in swine model was accompanied by prolonged course of the disease. It induced high level of cross-reactive HA2-specific antibodies, but worsened the clinical symptoms (Khurana *et al*., 2013; Gauger *et al*., 2014). Similar course of infection was observed also in a ferret model (Skowronski *et al*., 2014). There are reported some other data ascribing the contributions to development of VAERD, as are the low levels or absence of virus-neutralizing antibodies (Cox *et al*., 2009), increased avidity of virus non-neutralizing antibodies (To *et al*., 2012), deficiency of neuraminidase-specific antibodies (Rajão *et al*., 2016), or route of vaccine administration (Bernelin-Cottet *et al*., 2016). In spite of these reports, the vaccination still remains the only prevention against IAV. However, these results underline the need for better understanding of the mechanisms of action of HA2-specific antibodies and their antigen-binding or effector function, as well as the role of HA2 gp as an immunogen.

7. Different contribution of Fab and Fc fragments of HA2-specific antibodies to antiviral immunity

7.1 Characterization of antibodies and their fragments

Antibodies play a key role in the antiviral immunity, depending on their localization and on their structure. They are present in the organism in two forms. The first form of antibody is bound to the membranes of B-cells, bearing the function of the B-cell receptors (BCR). Another population of antibodies is present in a soluble, free form in the blood. The strucutre of both forms of these molecules is identical, except for the short hydrophobic aa region enabling the anchoring of the BCR into the membrane of B-cells. This small aa region is not present in the soluble form of the antibody molecule (Valentine and Green, 1967; Ribatti, 2015) (Fig. 3).

The structure of antibody molecule was decribed by Nobel prize winners Porter and Edelman, (Edelman, 1959; Porter, 1959). They estimated the molecular weight of IgG molecules by ultracentrifugation as 150 kDa and, based on the cleavage of IgG molecule with proteolytic enzymes, defined three functional fragments. Two fragments with antigen-binding activity were of identical structure and were named as Frag-

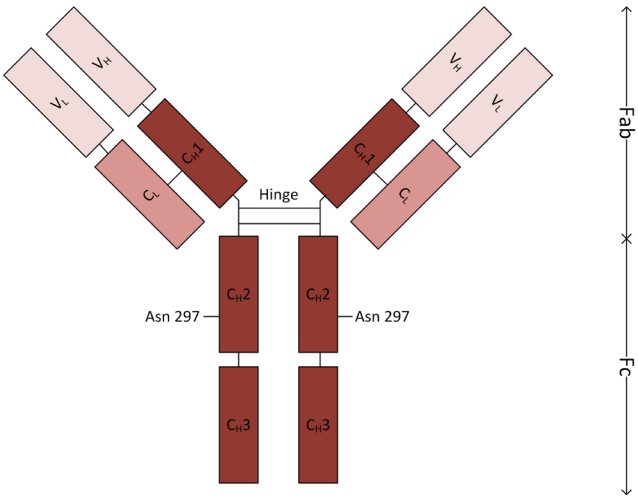

Fig. 3

Antibody structure

The immunoglobulin G (IgG) molecule is made up of two identical heavy (H) and two identical light (L) chains. Each H chain is linked to one L chain and both H chains are held together with disulphide bonds, forming Y-shaped structure. L chains contain one variable region (V_L) and one constant region (C_L), while H chains contain one variable region (V_H) and three constant regions (C_H1-C_H3). Antigen-binding fragments (Fab) are heterodimers composed of H and L chains (V_L-C_L and V_H-C_H1), while the Fc fragment contains only conserved domains of H chains (C_H2-$C_H3)_2$. Fab and Fc fragments of H chains are connected by amino acid sequence creating the flexible hinge region.

ment Antigen-Binding-Fab. The third fragment was able to spontaneously crystalize, therefore it was named as Fragment Crystallizable (Fc).

The IgG molecule is Y-like shaped, composed of two identical heterodimers consisting of two heavy (H) and two light (L) chains interconnected by disulphide bonds (Fig. 3). Light chains have two domains, one variable (V_L) and one constant (C_L), and heavy chains have four domains, one variable (V_H) and three constant (C_H1-C_H3). Antigen-binding domains (Fab) are heterodimers composed of H and L chains (V_L-C_L and V_H-C_H1), while the Fc domain is formed by homodimers of conserved domains of H chains only (C_H2-$C_H3)_2$, which are connected by interchain disulphide bonds (Schroeder and Cavacini, 2010; Vidarsson *et al*., 2014; Ribatti, 2015). The number and localization of these bonds differ depending on the isotype of the antibody.

Fab fragment of IgG molecule is responsible for antigen recognition and for the affinity of antibody binding to the antigen. In binding of the antibody to the epitope are involved the hypervariable complementarity-determining (CDR) regions created by loops of β-sheets of variable domains. These are composed of less variable framework regions (FR), which create the basic frame (skeleton) of the V domain. By approaching the CDR regions of H and L chains during the formation of quarternary structure of variable V chain, one hypervariable site, named paratop of the antibody, is formed (Schroeder and Cavacini, 2010; Sondermann *et al*., 2013; Murphy and Weaver, 2017).

The Fab and Fc domains of heavy chains are linked by amino acid sequence creating „hinge region", the flexibility of which influences the antigen binding activity of the antibody and effector functions of the IgG molecule, i.e. its interaction with C1q component of complement and the recognition of the Fc receptor (Valentine and Green, 1967). The hinge area is proline and cystein rich, which enables its flexibility. The length and the extent of the flexibility of the hinge area differs among isotypes. The longer is the hinge, the higher is the flexibility of the antibody molecule (Vidarsson *et al*., 2014).

The role of the Fc domain is to activate the reaction stimulating the destruction of pathogens. Antibodies differ in N-glycosidically bound carbohydrates not only on the Fab arm, but also on the Fc domain. Glycosylation highly influences the effector function of immunoglobulines (Arnold *et al*., 2007; Anthony *et al*., 2012; Lobner *et al*., 2016). The best studied is the human IgG glycosylation. Human IgG have the conserved glycosylation site at the position Asn 297 of each heavy chain (C_H2 domain). The binding of carbohydrate to this part contributes to the creation of quarternary structure and the stability of the Fc domain (Butler *et al*., 2003). There were described two conformations of the Fc domain. In the closed form of Fc, the interaction with Fc receptor is localized at the interface (borderline) between C_H2-C_H3 domains. This form of the Fc region interacts with type II FcR. In the open conformation, the interaction with FcR shifts towards the hinge region. The open conformation of antibody results in the activation of type I FcR and together with an immunoreceptor tyrosine-based activation motiv (ITAM) it triggers the proinflammatory processes and activates immunomodulating components with pleiotropic effect participating on the antibody mediated protective immune response (Graziano and Guyre, 2006; Arnold *et al*., 2007; Bruhns, 2012; Sondermann *et al*., 2013; Moldt and Hessell, 2014; Yu *et al*., 2014; Bournazos and Ravetch, 2015; Quast *et al*., 2017).

7.2 Antibody-dependent cell-mediated cytotoxicity mechanisms (ADCC)

Antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the important mechanisms of pathogen desctruction, where the secretory antibodies participate (Fig. 4). In humans, two isotypes, IgG1 and IgG3, are involved in this mechanism. In mice have this function mainly antibodies of IgG2a and IgG2b isotype (Jegaskanda *et al*., 2014). The typical cells participating in ADCC mechanism are natural killer (NK) cells with receptors FcγRIII (CD16). The stimulation signal triggering ADCC is the interaction of the Fc fragment of an antibody linked with antigen (antibody-coated target cell) and FcγR expressed on effector cells of the immune system. The signalization cascade continues by phosphorylation of the C-terminus of ITAM resulting in the release of granzyme B and perforin granules, mediators that induce the apoptosis of infected cells (Graziano and Guyre, 2006; Jegaskanda *et al*., 2014). The contribution of this mechanism to the defense against influenza infection has not been yet completely established. The research studies point to the fact that the virus-neutralizing antibodies do not participate in the ADCC mechanisms, in contrast to the antibodies targeted to the conserved virus proteins, which are non-neutralizing. Results show that antibodies mediating ADCC mechanism, are higly cross-reactive among influenza virus subtypes (Jegaskanda *et al*., 2013, 2014; Srivastava *et al*., 2013; DiLillo *et al*., 2014; Terajima *et al*., 2015; DiLillo *et al*., 2016; Vanderven *et al*., 2016; de Vries 2017a). Leon and colleagues showed in their study that two contacts are required for the activation of ADCC mechanism: the first is the interaction between Fc fragment of an antibody bound to HA with FcR on effector cell and second is the interaction between HA on the infected cell and sialic acid on the effector cells (Leon *et al*., 2016). ADCC is a complex mechanism, which can be influenced by several factors. These are: the presence of neutralizing antibodies, with which HA2-specific antibodies compete for HA binding on the epitope, the accessibility of the epitope and affinity of paratope binding to the antigen, as well as on interaction between Fc fragment and FcR (Ferrara *et al*., 2011; Thomann *et al*., 2015; He *et al*., 2016; Wang *et*

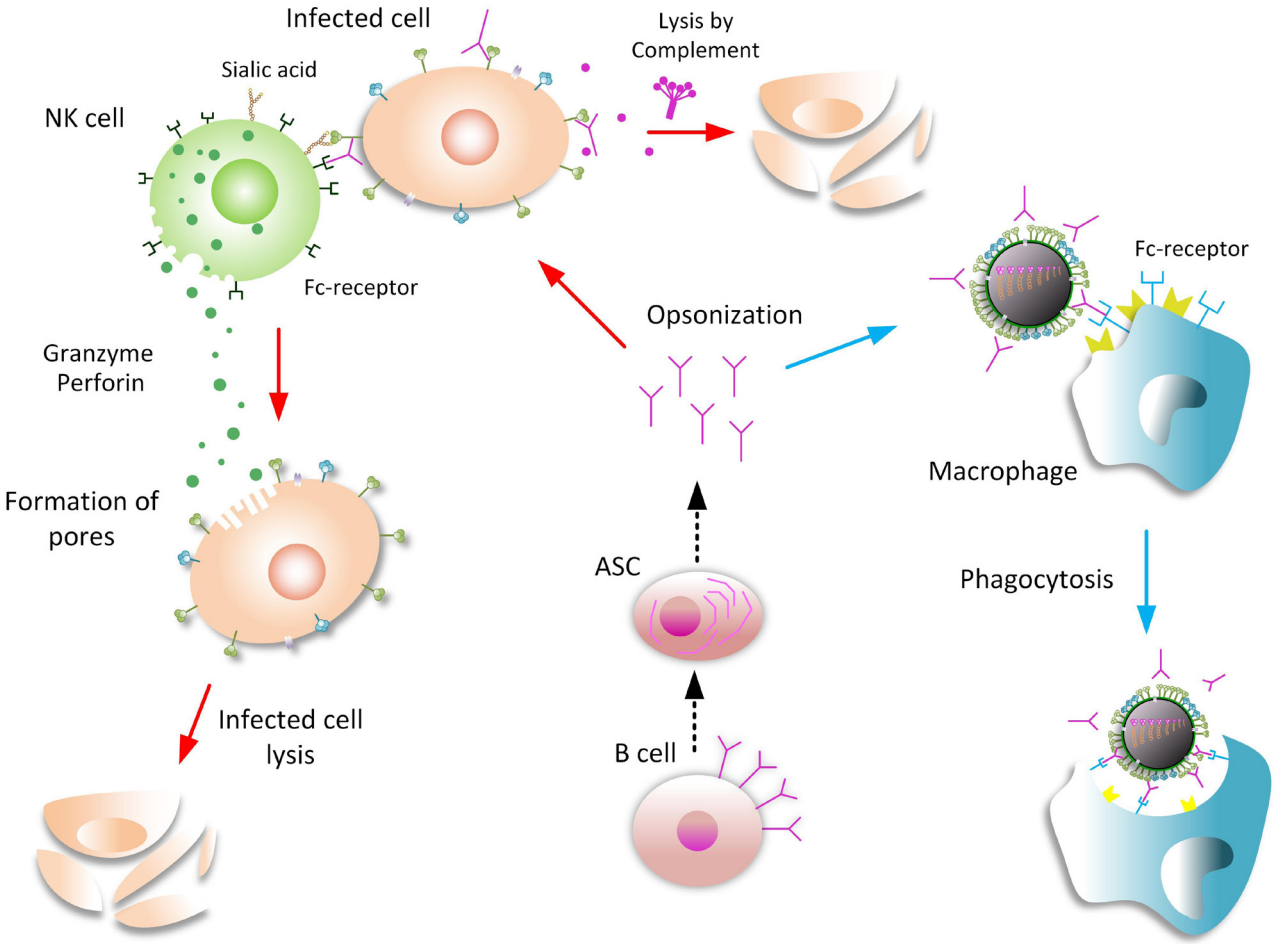

Fig. 4

Protection against influenza virus activated via Fc fragment of anti-HA2 Abs

During primary IAV infection, B-cells produce Abs against antigen. A portion of the B-cells differentiate into antibody-secreting plasma cells (ASCs), which respond more rapidly to infection with the same or similar antigen. The produced HA2-specific antibodies act as the opsonins and tag the virus or the IAV infected cells for effector cells of innate immunity. The interaction between the Fc fragment of the antibody and the Fc receptor on effector cells provides an activation signal for the elimination mechanism leading to destruction of infected cell. The red line schematically depicts the antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. In order to activate ADCC, another conection between hemagglutinin (presented on the infected cell) and the sialic acid of the natural killer cells (NK cell) is necessary. The activation of the IAV destruction mechanism by the interaction of the Fc fragment of HA2-specific antibody and the C1 component of complement is represented by an orange arrow. The blue arrows lead through the phagocytosis of viral particles of influenza virus mediated by HA2-specific Abs and phagocytic cells (adapted from Staneková and Varečková, 2010).

al., 2017). Currently, there is growing evidence supporting the role of broadly reactive anti-HA antibodies mediating the ADCC as a potencial defense against influenza during the natural infection. The knowledge of the mechanisms of heterosubtypic immunity against influenza and their understanding are important for the proper efficacy and safety of newly designed influenza vaccines (de Vries *et al.*, 2017b).

7.3 The role of complement in anti-influenza immunity

Complement is a system of more than 30 proteins co-operating in a cascade manner to assemble the membrane attack complex. Activation of complement may be achieved in several ways, but antibodies are necessary particularly during the classical pathway of complement activation (Fig. 4). The activation of complement is triggered after the antibody binding to the surface of the pathogen (Rattan *et al.*, 2017). The complement-mediated protection is involved at the early stage of infection. In addition to IgM, also IgG1 and IgG3 isotypes are able to participate in activation and, to a lower extent, also IgG2. All these isotypes are able to bind to the C1 component of complement via their Fc fragment. During the IAV infection, complement contributes to a more rapid virus elimination, lowering of virus titer

in lungs and, in cooperation with antibodies, it improves the *in vitro* neutralization of influenza virus. Experimental studies pointed to the increased virus-neutralizing and hemagglutinin-inhibiting activity of anti-HA antibodies in the presence of C1. The activity of complement is influenced by antibody isotype and by epitope-specificity of antibody (Feng *et al*., 2002; Jayasekera *et al*., 2007). It was shown that monoclonal antibodies activating the antibody-dependent cell lysis, which can have neutralizing effect, are targeted to the conserved area of IAV as is the stem domain of HA trimer with cross-reactive potential (Terajima *et al*., 2011, 2015). It is supposed, that HA-specific immune response, particularly production of HA2-specific antibodies, can be influenced by complement (Kopf *et al*., 2002; Rattan *et al*., 2017). The effect of complement can be minimalized by the M1 protein. N-terminal domain of M1 binds to C1q part of the C1 molecule and prevents the interaction of virus with antibodies and consequently the activation of complement. Thus, in this way the M1 protein helps the IAV to avoid the the immune system of the host (Zhang *et al*., 2009).

7.4 Antibody-dependent phagocytosis (ADP)

Cells capable of phagocytosis represent the first defense barrier of the immune system. These are myeloid cells comprising the monocytes, macrophages, neutrophils, eosinophils, basophils and dendritic cells. On their surfaces are expressed FcγRs, which are responsible for the effective recognition of pathogen by these cells, resulting in engulfment of the pathogen opsonized by antibodies and its destruction by phagocyting cells (Fig. 4). Based on the results obtained in the mouse model, when IAV infection elicited the production of antibodies involved in the ADP, it was suggested that Fc receptor mediating phagocytosis plays an important role in the elimination of respiratory viral infections (Huber *et al*., 2001). Ana-Sosa-Batiz and colleagues studied the role of anti-influenza antibodies in ADP and concluded that in antiviral protection mediated by phagocytes also cross-reactive anti-HA antibodies participate, though to a lower extent. Engulfing of IAV stimulated by antibodies lowered the ability of the pathogen to establish *in vitro* infection (Ana-Sosa-Batiz *et al*., 2016). In comparison to other studies, focusing on cooperation of HA antibodies and monocytes, results of *in vitro* experiments with neutrophils indicate that HA2-specific antibodies play an important role during the elimination of IAV, particularly by binding of their Fc domain with FcR on neutrophils. The engulfed IAV particle is eliminated by reactive form of oxygen (ROS), the production of which is induced by creating the bond between Fc and FcR. In contrast, antibodies targeted to globular domain of HA do not have this ability (Mullarkey *et al*., 2016).

7.5 Antibody-dependent enhancement of viral infection (ADE)

Antibodies represent the effector molecules, which play important role in the immune response against pathogens. They prevent the pathogen entry into the cells, or they participate in elimination of infected cells. However, under certain conditions, antibodies can support the spread of the virus infection by mediation of virus particle transfer into the cell, interpreted as indirect virus entry into the cell (Halstead, 1994). In this case, antibody causes the enhancement of virus infection, the process called as antibody-dependent enhancement or ADE mechanism of infection increase (Takada and Kawaoka, 2003). This mechanism was for the first time described in the sixties of the last century (Hawkes, 1964).

Since that time ADE phenomenon was observed in connection with several viruses (Taylor *et al*., 2015). In the context of influenza A virus infection, the ADE mechanism was described for the first time in the year 1988. There was observed a higher internalization of A/NWS virus (H1N1) in macrophage-like cell line P388D1 treated with neuraminidase. The highest transfer of virus particles into P388D1 cells was observed in virus, which was preexposed to optimal concentration of subneutralizing antiviral IgG antibodies (Ochiai and Kurokawa, 988). Several years later Ochiai *et al*. (1990) described the cross-reactivity of antibodies as a factor supporting ADE mechanism of virus enhancement. It was hypothesized that the entry of the complex Ab-IAV into the cell is mediated by the Fc receptor (Ochiai and Kurokawa, 988; Ochiai *et al*., 1990). Based on the experimental data it was conluded that ADE mechanism requires the presence of cells expressing Fc receptors and the optimal concentration of antibodies (Ochiai *et al*., 1992). Antibodies specific to HA or NA, mainly cross-reactive and non-neutralizing were shown to be candidates included in the ADE (Tamura *et al*., 1991). Besides *in vitro*, the ADE mechanism was also described *in vivo*. These studies showed that natural infection and vaccination by atenuated influenza virus enhanced the recognition and capturing of homologous virus by antigen presenting cells with expressed FcR on their surfaces (Gotoff *et al*., 1994).

The unambiguous connection of ADE mechanism with IAV and a worse course of infection in humans hasn't been described yet (Chan-Hui and Swiderek, 2016). The first HA2-specific antibody with a worsening effect on the course of infection was identified by Gocník *et al*. (2007) during the study of the effect of HA2-specific MAbs recognizing different antigenic epitopes of HA2 on the course of influenza infection in the mouse model. Passive immunization with three of the HA2-specific Abs of interest contributed to protection from infection with the homologous IAV virus. One of the studied HA2-specific Abs, which, unlike the other

MAbs, did not have fusion-inhibitory activity, contributed to the deterioration of the course of infection. Compared to the other studied antibodies, the delayed elimination of virus from lungs and higher mortality were observed in mice immunized with this Ab (Gocník *et al*., 2007). The vaccination with conserved IAV glycoproteins, which can result in more severe symptoms of infection, was described also in pigs (Gauger *et al*., 2011). ADE is mentioned in connection with viral infections, caused mainly by Dengue, HIV, but also by respiratory viruses (Takada and Kawaoka, 2003; Tirado and Yoon, 2003; Taylor *et al*., 2015; Ramakrishnan *et al*., 2016).

8. Conclusion. Immunoprotective or immunopathological character of HA2-specific antibodies?

The data described above suggest that the contact of Fc domain of antibody and FcR triggers mechanisms, which can have a beneficial as well as immunopathological impact on the host. Besides the known age dependence and the evolution status of the immune system of an individuum, experiments on mice showed that also the level of infectious dose has an impact on the course of immune response, mediated predominantly by ADCC mechanism and by complement, when the protective potential can be redirected towards an immunopathological process (Terajima *et al*., 2015).

HA2-specific antibodies represent only one subpopulation of antibodies participating in the complex defense against IAV (DiLillo *et al*., 2014, 2016; Vanderven *et al*., 2016). Antibodies are important players in immune response as they cooperate with NK cells. Thus, the innate immunity can modulate the adaptive immune response just as the IAV is able to influence the course of the immune response. The mechanism of protection mediated by HA2-specific antibodies is a result of their cooperation with other immune cells and molecules. It must be stressed that their protective potential is dependent on the epitope-specificity and antigen-binding affinity. By detailed studies and understanding of the relations between the particular variables of this triangle, we can get closer to the universal vaccine formulation.

Acknowledgments. Authors are thankful to Miriam Mikušová, Mária Vozárová and Katarína Briestenská for help with preparation of documentation and to Dr. F. Kostolanský for reading the manuscript. This work was supported by grants from Scientific Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences: 2/0048/19 (EV), 2/0106/17 (FK) and from the Slovak Research and Development Agency grant APVV-17-0445 (EV).

References

Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, Guthrie EH, Pickles RJ, Ting JP, Immunity 30(4), 556–565, 2009. https://doi.org/10.1016/j.immuni.2009.02.005

Ana-Sosa-Batiz F, Vanderven H, Jegaskanda S, Johnston A, Rockman S, Laurie K, Barr I, Reading P, Lichtfuss M, Kent SJ, PLoS One 11(4), 1–18, 2016. https://doi.org/10.1371/journal.pone.0154461

Angeletti D, Gibbs JS, Angel M, Kosik I, Hickman HD, Frank GM, Das SR, Wheatley AK, Prabhakaran M, Leggat DJ, Mcdermott AB, Yewdell JW, Nat. Immunol. 18(4), 456–463, 2017. https://doi.org/10.1038/ni.3680

Anthony RM, Wermeling F, Ravetch JV, Ann. N Y Acad. Sc. 1253(1), 170-180, ISSN 0077-8923, 2012. https://doi.org/10.1111/j.1749-6632.2011.06305.x

Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA, Annu. Rev. Immunol. 25, 21–50, 2007. https://doi.org/10.1146/annurev.immunol.25.022106.141702

Belser JA, Bridges CB, Katz JM, Tumpey TM, Emerg. Infect. Dis. 15(6), 859–865, 2009. https://doi.org/10.3201/eid1506.090072

Belser JA, Gustin KM, Pearce MB, Maines TR, Zeng H, Pappas C, Sun X, Carney PJ, Villanueva JM, Stevens J, Katz JM, Tumpey TM, Nature 501(7468), 556–559, 2013. https://doi.org/10.1038/nature12391

Bernelin-Cottet C, Deloizy C, Stanek O, Barc C, Bouguyon E, Urien C, Boulesteix O, Pezant J, Richard CA, Moudjou M, Da Costa B, Jouneau L, Chevalier C, Leclerc C, Sebo P, Bertho N, Schwartz-Cornil I, Front. Immunol. 7, 641, 2016. https://doi.org/10.3389/fimmu.2016.00641

Bommakanti G, Citron MP, Hepler RW, Callahan C, Heidecker GJ, Najar TA, Lu X, Joyce JG, Shiver JW, Casimiro DR, ter Meulen J, Liang X, Varadarajan R, Proc. Natl. Acad. Sci. USA 107(31), 13701–13706, 2010. https://doi.org/10.1073/pnas.1007465107

Bommakanti G, Lu X, Citron MP, Najar TA, Heidecker GJ, ter Meulen J, Varadarajan R, Liang X, J. Virol. 86(24), 13434–13444, 2012. https://doi.org/10.1128/JVI.01429-12

Böttcher-Friebertshäuser E, Garten W, Matrosovich M, Klenk HD, Curr. Top. Microbiol. Immunol. 385, 3–34, 2014. https://doi.org/10.1007/82_2014_384

Bournazos S, Ravetch JV, Immunol. Rev. 268(1), 88–103, 2015. https://doi.org/10.1111/imr.12343

Braakman I, Hoover-Litty H, Wagner KR, Helenius A, J. Cell Biol. 114(3), 401–411, 1991. https://doi.org/10.1083/jcb.114.3.401

Brandenburg B, Koudstaal W, Goudsmit J, Klaren V, Tang C, Bujny MV, Korse HJWM, Kwaks T, Otterstrom JJ, Juraszek J, van Oijen AM, Vogels R, Friesen RHE, PLoS One 8(12), e80034, 2013. https://doi.org/10.1371/journal.pone.0080034

Briedis DJ (2011): Influenza Viruses. In Acheson NH (Eds.): Fundamentals of Molecular Virology. 2nd ed. John Wiley & Sons. Ch. 18., pp. 210-224. ISBN : 978-0-470-90059-8.

Bruhns P, Blood 119(24), 5640-5650, 2012. https://doi.org/10.1182/blood-2012-01-380121

Butler M, Quelhas D, Critchley AJ, Carchon H, Hebestreit HF, Hibbert RG, Vilarinho L, Teles E, Matthijs G, Schollen E, Argibay P, Harvey DJ, Dwek RA, Jaeken J, Rudd PM, Glycobiology 13(9), 601–622, 2003.

Caton AJ, Brownlee GG, Yewdell JW, Gerhard W, Cell 31(2), 417–427, 1982. https://doi.org/10.1016/0092-8674(82)90135-0

Chan-Hui PY, Swiderek KM, Hum. Vaccin. Immunother. 12(2), 474–477, 2016. https://doi.org/10.1080/21645515.2015.1079676

Chen S, Zheng D, Li C, Zhang W, Xu W, Liu X, Fang F, Chen Z, Biomed. Res. Int. ID 901817, 12, 2015. https://doi.org/10.1155/2015/901817

Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, Vachieri SG, Pinna D, Minola A, Vanzetta F, Silacci C, Fernandez-Rodriguez BM, Agatic G, Bianchi S, Giacchetto-Sasselli I, Calder L, Sallusto F, Collins P, Haire LF, Temperton N, Langedijk JP, Skehel JJ, Lanzavecchia A, Science 333, 850-856, 2011. https://doi.org/10.1126/science.1205669

Cox RJ, Madhun AS, Hauge S, Sjursen H, Major D, Kuhne M, Höschler K, Saville M, Vogel FR, Barclay W, Donatelli I, Zambon M, Wood J, Haaheim LR, Vaccine 27(13), 1889–1897, 2009. https://doi.org/10.1016/j.vaccine.2009.01.116

Daniels R, Kurowski B, Johnson AE, Hebert DN, Mol. Cell. 11(1), 79–90, 2003. https://doi.org/10.1016/S1097-2765(02)00821-3

de Vries RD, Nieuwkoop NJ, van der Klis FRM, Koopmans MPG, Krammer F, Rimmelzwaan GF, J. Infect. Dis. 217(1), 3–11, 2017a. https://doi.org/10.1093/infdis/jix546

de Vries RD, Nieuwkoop NJ, Pronk M, de Bruin E, Leroux-Roels G, Huijskens EGW, van Binnendijk RS, Krammer F, Koopmans MPG, Rimmelzwaan GF, Vaccine 35(2), 238–247, 2017b. https://doi.org/10.1016/j.vaccine.2016.11.082

DiLillo DJ, Palese P, Wilson PC, Ravetch JV, J. Clin. Invest. 126(2), 605–610, 2016. https://doi.org/10.1172/JCI84428

DiLillo DJ, Tan GS, Palese P, Ravetch JV, Nat. Med. 20(2), 143–151, 2014. https://doi.org/10.1038/nm.3443

Dou D, Revol R, Östbye H, Wang H, Daniels R, Front. Immunol. 9, 17, 2018. https://doi.org/10.3389/fimmu.2018.01581

Dreyfus C, Laursen NS, Kwaks T, Zuijdgeest D, Khayat R, Ekiert DC, Lee JH, Metlagel Z, Bujny MV, Jongeneelen M, van der Vlugt R, Lamrani M, Korse HJ, Geelen E, Sahin Ö, Sieuwerts M, Brakenhoff JP, Vogels R, Li OT, Poon LL, Peiris M, Koudstaal W, Ward AB, Wilson IA, Goudsmit J, Friesen RH, Science 337(6100), 1343–1348, 2012. https://doi.org/10.1126/science.1222908

Edelman GM, J. Am. Chem. Soc. 81(12), 3155–3156, 1959. https://doi.org/10.1021/ja01521a071

Eggink D, Goff PH, Palese P, J. Virol. 88(1), 699–704, 2014. https://doi.org/10.1128/JVI.02608-13

Ekiert D, Frisen R, Bhabha G, Kwaks T, Jongeneelen M, Yu W, Ophorst C, Cox F, Korse H, Brandenburg B, Vogels R, Brakenhoff JPJ, Kompier R, Koldijk MH, Cornelissen LHM, Poon LLM, Peiris M, Koudstaal W, Wilson I, Goudsmit J, Science 333, 843–850, 2011. https://doi.org/10.1126/science.1204839

Ekiert DC, Bhabha G, Elsliger MA, Friesen RH, Jongeneelen M, Throsby M, Goudsmit J, Wilson IA, Science 324(5924), 246–251, 2009. https://doi.org/10.1126/science.1171491

Feng JQ, Mozdzanowska K, Gerhard W, J. Virol. 76(3), 1369–1378, 2002. https://doi.org/10.1128/JVI.76.3.1369-1378.2002

Ferrara C, Grau S, Jäger C, Sondermann P, Brünker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, Umaña P, Benz J, Proc. Natl. Acad. Sci. USA 108(31), 12669–12674, 2011. https://doi.org/10.1073/pnas.1108455108

Fodor E, Devenish L, Engelhardt OG, Palese P, Brownlee GG, García-Sastre A, J. Virol. 73 (11), 9679–9682, 1999.

Fodor E, Acta Virol. 57(2), 113–122, 2013. https://doi.org/10.4149/av_2013_02_113

Fouchier RA, Munster V, Wallensten A, Besterbroer TH, Herfst S, Smith D, Rimmelzwaan GF, Olsen B, Osterhaus AD, J. Virol. 79(5), 2814–2822, 2005. https://doi.org/10.1128/JVI.79.5.2814-2822.2005

Friesen RHE, Lee PS, Stoop EJM, Hoffman, RMB, Ekiert DC, Bhabha G, Yu W, Juraszek J, Koudstaal W, Jongeneelen M, Korse HJWM, Ophorst C, Brinkman-van der Linden ECM, Throsby M, Kwakkenbos MJ, Bakker AQ, Beaumont T, Spits H, Kwaks T, Vogels R, Ward AB, Goudsmit J, Wilson IA, Proc. Natl. Acad. Sci. USA 111(1), 445–50, 2014. https://doi.org/10.1073/pnas.1319058110

Gamblin SJ, Skehel JJ, J. Biol. Chem. 285(37), 28403–28409, 2010. https://doi.org/10.1074/jbc.R110.129809

Gauger PC, Loving CL, Khurana S, Lorusso A, Perez DR, Kehrli ME, Roth JA, Golding H, Vincent AL, Virology 471–473; 93–104, 2014. https://doi.org/10.1016/j.virol.2014.10.003

Gauger PC, Vincent AL, Loving CL, Lager KM, Janke BH, Kehrli ME Jr, Roth JA, Vaccine 29(15), 2712–2719, 2011. https://doi.org/10.1016/j.vaccine.2011.01.082

Gerhard W, Curr. Top. Microbiol. Immunol. 260, 171–190, 2001. https://doi.org/10.1007/978-3-662-05783-4_9

Gocník M, Fislová T, Sládková T, Mucha V, Kostolanský F, Varečková E, J. Gen. Virol. 88, 951–955, 2007. https://doi.org/10.1099/vir.0.82563-0

Gocník M, Fislová T, Mucha V, Sládková T, Russ G, Kostolanský F, Varečková E, J. Gen. Virol. 89, 958–967, 2008. https://doi.org/10.1099/vir.0.83524-0

Gotoff R, Tamura M, Janus J, Thompson J, Wright P, Ennis FA, J. Infect. Dis. 169(1), 200-203, 1994. https://doi.org/10.1093/infdis/169.1.200

Graves PN, Schulman JL, Young JF, Palese P, Virology 126(1), 106–116, 1983. https://doi.org/10.1016/0042-6822(83)90465-8

Graziano RF, Guyre PM, Antibody-dependent Cell-mediated Cytotoxicity (ADCC). Encyclopedia of life sciences, eLS, 2006. https://doi.org/10.1038/npg.els.0000498

Hai R, Krammer F, Tan GS, Pica N, Eggink D, Maamary J, Margine I, Albrecht RA, Palese P, J. Virol. 86(10), 5774–5781, 2012. https://doi.org/10.1128/JVI.00137-12

Halstead SB (1994): Antibody-Dependent Enhancement of Infection: A Mechanism for Indirect Virus Entry into Cells. Cellular Receptors for Animal Viruses. Cold Spring Harbor Laboratory Press, pp. 493–516, ISBN : 0-87969-429-7.

Hawkes RA, Aust. J. Exp. Biol. Med. Sci. 42, 465–482, 1964. https://doi.org/10.1038/icb.1964.44

He W, Tan GS, Mullarkey CE, Lee AJ, Lam MMW, Krammer F, Henry C, Wilson PC, Ashkar AA, Palese P, Miller MS, Proc. Natl. Acad. Sci. USA 113(42), 11931–11936, 2016. https://doi.org/10.1073/pnas.1609316113

Heaton NS, Leyva-Grado VH, Tan GS, Eggink D, Hai R, Palese P, J. Virol. 87(15), 8272–8281, 2013. https://doi.org/10.1128/JVI.00969-13

Hebert DN, Zhang JX, Chen W, Foellmer B, Helenius A, J. Cell Biol. 139(3), 613–623, 1997. https://doi.org/10.1083/jcb.139.3.613

Herfst S, Imai M, Kawaoka Y, Fouchier RAM, Curr. Top. Microbiol. Immunol. 385, 135–55, 2014.

Hoffmann E, Neumann G, Kawaoka Y, Hobom G, Webster RG, Proc. Natl. Acad. Sci. USA 97(11), 6108–6113, 2000. https://doi.org/10.1073/pnas.100133697

Huber VC, Lynch JM, Bucher DJ, Le J, Metzger DW, J. Immunol. 166(12), 7381–7388, 2001. https://doi.org/10.4049/jimmunol.166.12.7381

Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, Van Meersbergen R, Huizingh J, Wanningen P, Verspuij J, De Man M, Ding Z, Apetri A, Kükrer B, Sneekes-Vriese E, Tomkiewicz D, Laursen NS, Lee PS, Zakrzewska A, Dekking L, Tolboom J, Tettero L, Van Meerten S, Yu W, Koudstaal W, Goudsmit J, Ward AB, Meijberg W, Wilson IA, Radošević K, Science 349(6254), 1301–1306, 2015. https://doi.org/10.1126/science.aac7263

Jackson DC, Murray JM, White DO, Gerhard WU, Infect. Immun. 37(3), 912–918, 1982.

Jakubcová L, Hollý J, Varečková E, Acta Virol. 60, 121–135, 2016. https://doi.org/10.4149/av_2016_02_121

Jakubcová L, Vozárová M, Hollý J, Tomčíková K, Fogelová M, Polčicová K, Kostolanský F, Fodor E, Varečková E, J. Gen. Virol. 100(9), 1282–1292, 2019. https://doi.org/10.1099/jgv.0.001305

Janjua NZ, Skowronski DM, Hottes TS, Osei W, Adams E, Petric M, Sabaiduc S, Chan T, Mak A, Lem M, Tang P, Patrick DM, De Serres G, Bowering D, Clin. Infect. Dis. 51(9), 1017–1027, 2010. https://doi.org/10.1086/656586

Janulíková J, Staneková Z, Mucha V, Kostolanský F, Varečková E, Acta Virol. 56(3), 169–176, 2012. https://doi.org/10.4149/av_2012_03_169

Jayasekera JP, Moseman EA, Carroll MC, J. Virol. 81(7), 3487–3494, 2007. https://doi.org/10.1128/JVI.02128-06

Jegaskanda S, Job ER, Kramski M, Laurie K, Isitman G, De Rose R, Winnall WR, Stratov I, Brooks AG, Reading PC, Kent SJ, J. Immunol. 190(4), 1837–1848, 2013. https://doi.org/10.4049/jimmunol.1201574

Jegaskanda S, Reading PC, Kent SJ, J. Immunol. 193(2), 469–475, 2014. https://doi.org/10.4049/jimmunol.1400432

Joseph U, Su YC, Vijaykrishna D, Smith GJ, Influenza Other Respir. Viruses 11(1), 74–84, 2017. https://doi.org/10.1111/irv.12412

Kallewaard NL, Corti D, Collins PJ, Neu U, Mcauliffe JM, Benjamin E, Wachter-Rosati L, Palmer-Hill FJ, Yuan AQ, Walker PA, Vorlaender MK, Bianchi S, Guarino B, De Marco A, Vanzetta F, Agatic G, Foglierini M, Pinna D, Fernandez-Rodriguez B, Fruehwirth A, Silacci C, Ogrodowicz RW, Martin SR, Sallusto F, Suzich JA, Lanzavecchia A, Zhu Q, Gamblin SJ, Skehel JJ, Cell 166(3), 596–608, 2016. https://doi.org/10.1016/j.cell.2016.05.073

Kanekiyo M, Wei CJ, Yassine HM, Mctamney PM, Boyington JC, Whittle JRR, Rao SS, Kong WP, Wang L, Nabel GJ, Nature 499(7456), 102–106, 2013. https://doi.org/10.1038/nature12202

Kang SM, Kim MC, Compans RW, Expert Rev. Vaccines. 11(8), 995–1007, 2012. https://doi.org/10.1586/erv.12.70

Kashyap AK, Steel J, Rubrum A, Estelles A, Briante R, Ilyushina NA, Xu L, Swale RE, Faynboym AM, Foreman PK, Horowitz M, Horowitz L, Webby R, Palese P, Lerner RA, Bhatt RR, PLoS Pathog. 6(7), e1000990, 2010. https://doi.org/10.1371/journal.ppat.1000990

Kashyap AK, Steel J, Oner AF, Dillon MA, Swale RE, Wall KM, Perry KJ, Faynboym A, Ilhan M, Horowitz M, Horowitz L, Palese P, Bhatt RR, Lerner RA, Proc. Natl. Acad. Sci. USA 105(16), 5986–5891, 2008. https://doi.org/10.1073/pnas.0801367105

Katz JM, Garg S, Sambhara S (2006): Influenza Vaccines: Current and Future Strategies. In Kawaoka Y (Ed.): Influenza Virology: Current Topics. Caister Academic Press, U.K. pp. 203–228. ISBN : 978-1-904455-06-6.

Khurana S, Loving CL, Manischewitz J, King LR, Gauger PC, Henningson J, Vincent AL, Golding H, Sci. Transl. Med. 5(200), 200ra114, 2013. https://doi.org/10.1126/scitranslmed.3006366

Kirkpatrick E, Qiu X, Wilson PC, Bahl J, Krammer F, Sci. Rep. 8(1), 10432, 2018. https://doi.org/10.1038/s41598-018-28706-1

Klausberger M, Tscheliessnig R, Neff S, Nachbagauer R, Wohlbold TJ, Wilde M, Palmberger D, Krammer F, Jungbauer A, Grabherr R, PLoS One 11(4), 1–21, 2016. https://doi.org/10.1371/journal.pone.0153579

Kopf M, Brombacher F, Bachmann MF, Eur. J. Immunol. 32(8), 2229–2236, 2002. https://doi.org/10.1002/1521-4141(200208)32:8<2229::AID-IMMU2229>3.0.CO;2-T

Kostolanský F, Mucha V, Slováková R, Varečková E, Acta Virol. 46, 229–236, 2002.

Kostolanský F, Russ G, Mucha V, Styk B, Arch. Virol. 101, 13–24, 1988. https://doi.org/10.1007/BF01314648

Kostolanský F, Varečková E, Betáková T, Mucha V, Russ G, Wharton S, J. Gen. Virol. 81, 1727–1735, 2000. https://doi.org/10.1099/0022-1317-81-7-1727

Krammer F, Margine I, Tan GS, Pica N, Krause JC, Palese P, PLoS One 7(8), e43603, 2012. https://doi.org/10.1371/journal.pone.0043603

Krammer F, Palese P, Curr. Opin. Virol. 3, 1–10, 2013. https://doi.org/10.1038/ni.2761

Krammer F, Palese P, Nat. Immunol. 15, 3–5, 2014. https://doi.org/10.1038/ni.2761

Krause JC, Tsibane T, Tumpey TM, Huffman CJ, Albrecht R, Blum DL, Ramos I, Fernandez-Sesma A, Edwards KM, García-Sastre A, Basler CF, Crowe JE Jr, J. Virol. 86(11), 6334–6340, 2012. https://doi.org/10.1128/JVI.07158-11

Lee N, Wong CK, Hui DS, Lee SK, Wong RY, Ngai KL, Chan MC, Chu YJ, Ho AW, Lui GC, Wong BC, Wong SH, Yip SP, Chan PK, Influenza Other Respir. Viruses 7(5), 666–675, 2013. https://doi.org/10.1111/irv.12109

Lee PS, Yoshida R, Ekiert DC, Sakai N, Suzuki Y, Takada A, Wilson IA, Proc. Natl. Acad. Sci. USA 109(42), 17040-17045, 2012. https://doi.org/10.1073/pnas.1212371109

Leon PE, He W, Mullarkey CE, Bailey MJ, Miller MS, Krammer F, Palese P, Tan GS, Proc. Natl. Acad. Sci. USA 113(40), E5944–E5951, 2016. https://doi.org/10.1073/pnas.1613225113

Li GM, Chiu C, Wrammert J, McCausland M, Andrews SF, Zheng NY, Lee JH, Huang M, Qu X, Edupuganti S, Mulligan M, Das SR, Yewdell JW, Mehta AK, Wilson PC, Ahmed R, Proc. Natl. Acad. Sci. USA 109(23), 9047–9052, 2012. https://doi.org/10.1073/pnas.1118979109

Lobner E, Traxlmayr MW, Obinger C, Hasenhindl C, Immunol. Rev. 270(1), 113–131, 2016. https://doi.org/10.1111/imr.12385

Loo YM, Fornek J, Crochet N, Bajwa G, Perwitasari O, Martinez-Sobrido L, Akira S, Gill MA, García-Sastre A, Katze MG, Gale M Jr, J. Virol. 82(1), 335–345, 2008. https://doi.org/10.1128/JVI.01080-07

Lu Y, Welsh JP, Swartz JR, Proc. Natl. Acad. Sci. U. S. A. 111(1), 125–130, 2013.

Magadán JG, Khurana S, Das SR, Frank GM, Stevens J, Golding H, Bennink JR, Yewdell JW, J. Virol. 87(17), 9742–53, 2013. https://doi.org/10.1128/JVI.00471-13

Mair C, Ludwig K, Herrmann A, Sieben C, Biochim. Biophys. Acta 1838(4), 1153–1168, 2014. https://doi.org/10.1016/j.bbamem.2013.10.004

Mallajosyula VV, Citron M, Ferrara F, Lu X, Callahan C, Heidecker GJ, Sarma SP, Flynn JA, Temperton NJ, Liang X, Varadarajan R, Proc. Natl. Acad. Sci. USA 111(25), E2514–23, 2014. https://doi.org/10.1073/pnas.1402766111

Margine I, Krammer F, Hai R, Heaton NS, Tan GS, Andrews SA, Runstadler JA, Wilson PC, Albrecht RA, García – Sastre A, Palese P, J. Virol. 87(19), 10435–10446, 2013a. https://doi.org/10.1128/JVI.01715-13

Margine I, Hai R, Albrecht RA, Obermoser G, Harrod AC, Banchereau J, Palucka K, García-Sastre A, Palese P, Treanor JJ, Krammer F, J. Virol. 87(8), 4728–37, 2013b. https://doi.org/10.1128/JVI.03509-12

Marjuki H, Mishin VP, Chai N, Tan M-W, Newton EM, Tegeris J, Erlandson K, Willis M, Jones J, Davis T, Stevens J, Gubareva LV, J. Virol. 90(23), 10446–10458, 2016. https://doi.org/10.1128/JVI.01284-16

Medina RA, García-Sastre A, Nat. Rev. Microbiol. 9(8), 590-603, 2011. https://doi.org/10.1038/nrmicro2613

Medina RA, Stertz S, Manicassamy B, Zimmermann P, Sun X, Albrecht RA, Uusi-Kerttula H, Zagordi O, Belshe RB, Frey SE, Tumpey TM, García-Sastre A, Sci. Transl. Med. 5(187), 187ra70, 2013. https://doi.org/10.1126/scitranslmed.3005996

Moldt B, Hessell AJ (2014): FcγRs Across Species. In Antibody Fc: Linking Adaptive and Innate Immunity. Elsevier Inc. pp. 145–157. ISBN : 978-0-12-394802-1. https://doi.org/10.1016/B978-0-12-394802-1.00008-X

Mullarkey CE, Bailey MJ, Golubeva DA, Tan GS, Nachbagauer R, He W, Novakowski KE, Bowdish DM, Miller MS, Palese P, MBio. 7(5), e01624-16, 2016. https://doi.org/10.1128/mBio.01624-16

Murphy K, Weaver C (2017): Janeway's immunology. 9th ed. Garland Science, Taylor & Francis Group, LLC, p. 928. ISBN : 978-0-8153-4505-3.

Nachbagauer R, Krammer F, Albrecht RA, Vaccines 6(3), 47, 2018. https://doi.org/10.3390/vaccines6030047

Nachbagauer R, Wohlbold TJ, Hirsh A, Hai R, Sjursen H, Palese P, Cox RJ, Krammer F, J. Virol. 88(22), 13260-13268, 2014. https://doi.org/10.1128/JVI.02133-14

Nachagauer R, Miller MS, Hai R, Ryder AB, Rose JK, Palese P, García-Sastre A, Krammer F, Albrecht RA, J. Virol. 90(6), 3268–3273, 2016. https://doi.org/10.1128/JVI.02481-15

Nakurama G, Chai N, Park S, Chiang N, Lin Z, Chiu H, Fong R, Yan D, Kin J, Zhang J, Lee WP, Estevez A, Coons M, Xu M, Lupardus P, Balazs M, Swem LR, Cell 14(1), 93–103, 2013. https://doi.org/10.1016/j.chom.2013.06.004

Neumann G, Watanabe T, Ito H, Watanebe S, Goto H, Gao P, Hughes M, Perez DR, Donis R, Hoffmann E, Hobom G, Kawaoka Y, Proc. Natl. Acad. Sci. USA 96(16), 9345–9350, 1999. https://doi.org/10.1073/pnas.96.16.9345

Nobusawa E, Aoyama T, Kato H, Suzuki Y, Tateno Y, Nakajima K, Virology 182, 475–485, 1991. https://doi.org/10.1016/0042-6822(91)90588-3

Ochiai H, Kurokawa M, J. Virol. 62(1), 20-26, 1988.

Ochiai H, Kurokawa M, Kuroki Y, Niwayama S, J. Med. Virol. 30(4), 258–265, 1990. https://doi.org/10.1002/jmv.1890300406

Ochiai H, Kurokawa M, Matsui S, Yamamoto T, Kuroki Y, Kishimoto C, Shiraki K, J. Med. Virol. 36(3), 217–221, 1992. https://doi.org/10.1002/jmv.1890360312

Okada J, Ohshima N, Kubuta-Koketsu R, Ota S, Takase W, Azuma M, Iba Y, Nakagawa N, Yoshikawa T, Nakajima Y, Ishikawa T, Asano Y, Okuno Y, Kurosawa Y, Virology 397(2), 322–330, 2010. https://doi.org/10.1016/j.virol.2009.11.025

Okuno Y, Isegawa Y, Sasao F, Ueda S, J. Virol. 67, 2552–2558, 1993.

Pang I, Iwasaki A, Trends Immunol. 32(1), 34–41, 2011. https://doi.org/10.1016/j.it.2010.11.004

Peitsch C, Klenk H-D, Garten W, Böttcher-Friebertshäuser E, J. Virol. 88(1), 282–291, 2014. https://doi.org/10.1128/JVI.01635-13

Pica N, Palese P, Annu. Rev. Med. 64, 189–202, 2013. https://doi.org/10.1146/annurev-med-120611-145115

Porter RR, Biochem. J. 73(1), 119–126, 1959. https://doi.org/10.1042/bj0730119

Prabhu N, Prabakaran M, Ho HT, Velumani S, Qiang J, Goutama M, Kwang J, J. Virol. 83(6), 2553–2562, 2009. https://doi.org/10.1128/JVI.02165-08

Quast I, Peschke B, Lünemann JD, Cell. Mol. Life Sci. 74(5), 837–847, 2017. https://doi.org/10.1007/s00018-016-2366-z

Rajão DS, Chen H, Perez DR, Sandbulte MR, Gauger PC, Loving CL, Shanks GD, Vincent A, J. Gen. Virol. 97(7), 1489–1499, 2016. https://doi.org/10.1099/jgv.0.000468

Ramakrishnan B, Viswanathan K, Tharakaraman K, Dančík V, Raman R, Babcock GJ, Shriver Z, Sasisekharan R, Trends Microbiol. 24(12), 933–943, 2016. https://doi.org/10.1016/j.tim.2016.09.003

Rattan A, Pawar SD, Nawadkar R, Kulkarni N, Lal G, Mullick J, Sahu A, PLoS Pathog. 13(3), e1006248, 2017. https://doi.org/10.1371/journal.ppat.1006248

Ribatti D, Immunol. Lett. 164(2), 72–75, 2015. https://doi.org/10.1016/j.imlet.2015.02.005

Rogers GN, Paulson JC, Virology 127(2), 361–373, 1983. https://doi.org/10.1016/0042-6822(83)90150-2

Russ G, Styk B, Poláková K, Acta Virol. 22(5), 371–382, 1978. https://doi.org/10.1016/0040-6031(78)85106-5

Russ G, Poláková K, Kostolanský F, Styk B, Vancíková M, Acta Virol. 31(5), 374–386, 1987.

Russell CJ, Hu M, Okda FA, Trends Microbiol. 26(10), 841–853, 2018. https://doi.org/10.1016/j.tim.2018.03.005

Sagawa H, Ohshima A, Kato I, Okuno Y, Isegawa Y, J. Gen. Virol. 77, 1483–1487, 1996. https://doi.org/10.1099/0022-1317-77-7-1483

Sanders CJ, Doherty PC, Thomas PG, Cell Tissue Res. 343(1), 13–21, 2011. https://doi.org/10.1007/s00441-010-1043-z

Saunders-Hastings PR, Krewski D, Pathogens 5(4), pii: E66, 2016. https://doi.org/10.3390/pathogens5040066

Shaw, ML, Palese, P (2013): Orthomyxoviridae. In Knipe DM, Howley PM (Eds.): Fields Virology. (6th ed.). Loppincott Williams & Wilkins a Welters Kluwer business : Philadelphia 40, pp. 1151–1185. ISBN-10 : 1-4511-0563-0.

Schild GC, Newman RW, Webster RG, Major D, Hinshaw VS, Arch. Virol. 63(3–4), 171–184, 1980. https://doi.org/10.1007/BF01315024

Schrauwen EJ, Fouchier RA, Emerg. Microbes Infect. 3(2), e9, 2014. https://doi.org/10.1038/emi.2014.9

Schroeder HW, Cavacini L, J. Allergy Clin. Immunol. 125(2), 41–51, 2010. https://doi.org/10.1016/j.jaci.2009.09.046

Skehel JJ, Wiley DC, Annu. Rev. Biochem. 69, 531–569, 2000. https://doi.org/10.1146/annurev.biochem.69.1.531

Skowronski DM, De Serres G, Crowcroft NS, Janjua NZ, Boulianne N, Hottes TS, Rosella LC, Dickinson JA, Gilca R, Sethi P, Ouhoummane N, Willison DJ, Rouleau I, Petric M, Fonseca K, Drews SJ, Rebbapragada A, Charest H, Hamelin ME, Boivin G, Gardy JL, Li Y, Kwindt TL, Patrick DM, Brunham RC; Canadian SAVOIR Team, PLoS Med. 7(4), e1000258, 2010. https://doi.org/10.1371/journal.pmed.1000258

Skowronski DM, Hamelin ME, De Serres G, Janjua NZ, Li G, Sabaiduc S, Bouhy X, Couture C, Leung A, Kobasa D, Embury-Hyatt C, de Bruin E, Balshaw R, Lavigne S, Petric M, Koopmans M, Boivin G, PLoS One 9(1), e86555, 2014. https://doi.org/10.1371/journal.pone.0086555

Sondermann P, Pincetic A, Maamary J, Lammens K, Ravetch JV, Proc. Natl. Acad. Sci. USA 110(24), 9868–9872, 2013. https://doi.org/10.1073/pnas.1307864110

Sparrow E, Friede M, Sheikh M, Torvaldsen S, Newall AT, Vaccine 34(45), 5442–5448, 2016. https://doi.org/10.1016/j.vaccine.2016.08.057

Sridhar S, Brokstad KA, Cox RJ, Vaccines 3(2), 373–389, 2015. https://doi.org/10.3390/vaccines3020373

Srivastava V, Yang Z,Hung IFN, Xu J, Zheng B, Zhang M-Y, J. Virol. 87(10), 5831–5840, 2013. https://doi.org/10.1128/JVI.00273-13

Sriwilaijaroen N, Suzuki Y, Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci. 88(6), 226–49, 2012. https://doi.org/10.2183/pjab.88.226

Staneková Z, Varečková E, Virol. J. 7, 351, 2010. https://doi.org/10.1186/1743-422X-7-351

Staneková Z, Adkins I, Kosová M, Januliková J, Šebo P, Varečková E, Antiviral Res. 97, 24–35, 2013. https://doi.org/10.1016/j.antiviral.2012.09.008

Staneková Z, Mucha V, Sládková T, Blaškovičová H, Kostolanský F, Varečková E, Influenza Other Respir. Viruses 6(6), 389–395, 2012. https://doi.org/10.1111/j.1750-2659.2011.00328.x

Staneková Z, Király J, Stropkovská A, Mikušková T, Mucha V, Kostolanský F, Varečková E, Acta Virol. 55(1), 61–67, 2011. https://doi.org/10.4149/av_2011_01_61

Steel J, Lowen AC, Wang TT, Yondola M, Gao Q, Haye K, García-Sastre A, Palese P, MBio 1(1), pii: e00018-10, 2010. https://doi.org/10.1128/mBio.00018-10

Steinhauer DA, Virology 258, 1–20, 1999. https://doi.org/10.1006/viro.1999.9716

Stropkovská A, Mucha V, Fislová T, Gocník M, Kostolanský F, Varecková E, Acta Virol. 53, 15–20, 2009. https://doi.org/10.4149/av_2009_01_15

Styk B, Russ G, Poláková K, Acta Virol. 23, 1–8, 1979.

Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, Santelli E, Stec B, Cadwell G, Ali M, Wan H, Murakami A, Yammanuru A, Han T, Cox NJ, Bankston LA, Donis RO, Liddington RC, Marasco WA, Nat. Struct. Mol. Biol. 16(3), 265–273, 2009. https://doi.org/10.1038/nsmb.1566

Sunwoo SY, Schotsaert M, Morozov I, Davis AS, Li Y, Lee J, McDowell C, Meade P, Nachbagauer R, García-Sastre A, Ma W, Krammer F, Richt JA, Vaccines 6(3), pii: E64, 2018. https://doi.org/10.3390/vaccines6030064

Sutton TC, Chakraborty S, Mallajosyula VVA, Lamirande EW, Ganti K, Bock KW, Moore IN, Varadarajan R, Subbarao K, NPJ Vaccines 2, 35, 2017. https://doi.org/10.1038/s41541-017-0036-2

Takada A, Kawaoka Y, Rev. Med. Virol. 13(6), 387–398, 2003. https://doi.org/10.1002/rmv.405

Tamura M, Webster RG, Ennis FA, Virology 182(1), 211–219, 1991. https://doi.org/10.1016/0042-6822(91)90664-W

Tan GS, Krammer F, Eggink D, Kongchanagul A, Moran TM, Palese P, J. Virol. 86, 6179–6188, 2012. https://doi.org/10.1128/JVI.00469-12

Tan GS, Lee PS, Hoffman RM, Mazel-Sanchez B, Krammer F, Leon PE, Ward AB, Wilson IA, Palese P, J. Virol. 88(23), 13580-13592, 2014. https://doi.org/10.1128/JVI.02289-14

Tate MD, Job ER, Deng YM, Gunalan V, Maurer-Stroh S, Reading PC, Viruses 6(3), 1294–1316, 2014. https://doi.org/10.3390/v6031294

Taylor A, Foo SS, Bruzzone R, Vu Dinh L, King NJC, Mahalingam S, Immunol. Rev. 268(1), 340-364, 2015. https://doi.org/10.1111/imr.12367

Terajima M, Co MD, Cruz J, Ennis FA, J. Infect. Dis. 212(7), 1052–1060, 2015. https://doi.org/10.1093/infdis/jiv181

Terajima M, Cruz J, Co MDT, Lee J-H, Kaur K, Wilson PC, Ennis FA, J. Virol. 85(24), 13463–13467, 2011. https://doi.org/10.1128/JVI.05193-11

Tharakaraman K, Subramanian V, Viswanathan K, Sloan S, Yen HL, Barnard DL, Leung YH, Szretter KJ, Koch TJ, Delaney JC, Babcock GJ, Wogan GN, Sasisekharan R, Shriver Z, Proc. Natl. Acad. Sci. USA 112(35), 10890-10895, 2015. https://doi.org/10.1073/pnas.1502374112

Thomann M, Schlothauer T, Dashivets T, Malik S, Avenal C, Bulau P, Rüger P, Reusch D, PLoS One 10(8), e0134949, 2015. https://doi.org/10.1371/journal.pone.0134949

Throsby M, Van Den Brink E, Jongeneelen M, Poon LL, Alard P, Cornelissen L, Bakker A, Cox F, Van Deventer E, Guan Y, Cinatl J, Ter Muelen J, Lasters I, Carsetti R, Peiris M, De Kruif J, Goudsmit J, PLoS One. 3(12), e3942, 2008. https://doi.org/10.1371/journal.pone.0003942

Tirado SM, Yoon KJ, Viral Immunol. 16(1), 69–86, 2003. https://doi.org/10.1089/088282403763635465

To KK, Zhang AJ, Hung IF, Xu T, Ip WC, Wong RT, Ng JC, Chan JF, Chan KH, Yuen KY, Clin. Vaccine Immunol. 19(7):1012–1018, 2012. https://doi.org/10.1128/CVI.00081-12

Tong S, Li Y, Rivailler P, Conrardy C, Alvarez DA, Chen L-M, Rogers S, Shi M, Tao Y, Weil M R, Tang K, Rowe LA, Sammons S, Xu X, Proc. Natl. Acad. Sci. USA 109(11), 4269–4274, 2012. https://doi.org/10.1073/pnas.1116200109

Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J, Chen L-M, Johnson A, Tao Y, Dreyfus C, Yu W, Mcbride R, Carney PJ, Gilbert AT, Chang J, Guo Z, Davis CT, Paulson JC, Stevens J, Ruprecht CE, Holmes EC, Wilson IA, Donis RO, PLoS Pathog. 9(10), e1003657, 2013. https://doi.org/10.1371/journal.ppat.1003657

Tsibane T, Ekiert DC, Krause JC, Martinez O, Crowe JE Jr, Wilson IA, Basler CF, PLoS Pathog. 8(12), e1003067, 2012. https://doi.org/10.1371/journal.ppat.1003067

Tsuchihashi Y, Sunagawa T, Yahata Y, Takahashi H, Toyokawa T, Odaira F, Ohyama T, Taniguchi K, Okabe N, Clin. Infect. Dis. 54(3), 381–383, 2012. https://doi.org/10.1093/cid/cir787

Vachieri SG, Xiong X, Collins PJ, Walker PA, Martin SR, Haire LF, Zhang Y, Mccauley JW, Gamblin SJ, Skehel JJ, Nature 511(7510), 475–477, 2014. https://doi.org/10.1038/nature13443

Valentine RC, Green NM, J. Mol. Biol. 27(3), 615–617, 1967. https://doi.org/10.1016/0022-2836(67)90063-0

Valkenburg SA, Mallajosyula VV, Li OT, Chin AW, Carnell G, Temperton N, Varadarajan R, Poon LL, Sci. Rep. 6, 22666, 2016. https://doi.org/10.1038/srep22666

Van De Sandt CE, Kreijtz JHCM, Rimmelzwaan GF, Viruses 4(9), 1438–1476, 2012. https://doi.org/10.3390/v4091438

Vanderven HA, Ana-Sosa-Batiz F, Jegaskanda S, Rockman S, Laurie K, Barr I, Chen W, Wines B, Hogarth PM, Lambe T, Gilbert SC, Parsons MS, Kent SJ, EBioMedicine 8, 277–290, 2016. https://doi.org/10.1016/j.ebiom.2016.04.029

Varečková E, Cox N, Klimov A, J. Clin. Microbiol. 40(6), 2220–2223, 2002. https://doi.org/10.1128/JCM.40.6.2220-2223.2002

Varečková E, Mucha V, Čiampor F, Betáková T, Russ G, Arch. Virol. 130(1–2), 45–56, 1993. https://doi.org/10.1007/BF01318995

Varečková E, Mucha V, Kostolanský F, Acta Virol. 57(2), 247–256, 2013. https://doi.org/10.4149/av_2013_02_247

Varečková E, Mucha V, Kostolanský F, Gubareva LV, Klimov A, Virus Res. 132(1–2), 181–186, 2008. https://doi.org/10.1016/j.virusres.2007.10.004

Varečková E, Mucha V, Wharton SA, Kostolanský F, Arch. Virol. 148(3), 469–486, 2003a. https://doi.org/10.1007/s00705-002-0932-1

Varečková E, Wharton SA, Mucha V, Gocník M, Kostolanský F, Acta Virol. 47(4), 229–236, 2003b.

Vidarsson G, Dekkers G, Rispens T, Front. Immunol. 5, 1–17, 2014. https://doi.org/10.3389/fimmu.2014.00520

Vigerust DJ, Ulett KB, Boyd KL, Madsen J, Hawgood S, Mccullers JA, J. Virol. 81(16), 8593–600, 2007. https://doi.org/10.1128/JVI.00769-07

Wang G, Deng G, Shi J, Luo W, Zhang G, Zhang Q, Liu L, Jiang Y, Li C, Sriwilaijaroen N, Hiramatsu H, Suzuki Y, Kawaoka Y, Chen H, J. Virol. 88(8), 3953–3964, 2014. https://doi.org/10.1128/JVI.03292-13

Wang JP, Bowen GN, Padden C, Cerny A, Finberg RW, Newburger PE, Kurt-Jones EA, Blood 112(5), 2028–2034, 2008. https://doi.org/10.1182/blood-2008-01-132860

Wang S, Ren H, Jiang W, Chen H, Hu H, Chen Z, Zhou P, J. Virol. 91(11), pii: e02065-16, 2017. https://doi.org/10.1128/JVI.02065-16

Wang TT, Tan GS, Hai R, Pica N, Petersen E, Moran TM, Palese P, PLoS Pathog. 6(2), e1000796, 2010a. https://doi.org/10.1371/journal.ppat.1000796

Wang TT, Tan GS, Hai R, Pica N, Ngai L, Ekiert DC, Wilson IA, García-Sastre A, Moran TM, Palese P, Proc. Natl. Acad. USA 107(44), 18979–18984, 2010b. https://doi.org/10.1073/pnas.1013387107

Webster RG, Govorkova EA, Ann. N. Y. Acad. Sci. 1323, 115–139, 2014. https://doi.org/10.1111/nyas.12462

Wharton SA, Skehel JJ, Wiley DC, Virology 149, 27–35, 1986. https://doi.org/10.1016/0042-6822(86)90083-8

Whittle JR, Zhang R, Khurana S, King LR, Manischewitz J, Golding H, Dormitzer PR, Haynes BF, Walter EB, Moody MA, Kepler TB, Liao H, Harrison SC, Proc. Natl. Acad. Sci. U. S. A. 108(34), 14216–14221, 2011. https://doi.org/10.1073/pnas.1111497108

Wiley DC, Wilson IA, Skehel JJ, Nature 289(5796), 373–378, 1981. https://doi.org/10.1038/289373a0

Wohlbold TJ, Nachbagauer R, Margine I, Tan GS, Hirsh A, Krammer F, Vaccine 33(29), 3314–3321, 2015. https://doi.org/10.1016/j.vaccine.2015.05.038

Wright PF, Neumann G, Kawaoka Y (2013): Orthomyxoviruses. In FIELDS Virology, D.M. Knipe DM, Howley PM (Eds.): Fields Virology. (6th ed.). Lipincott Williams & Wilkins, a Wilters Kluwer business: Philadelphia 40, pp. 1186–1243. ISBN-10 : 1-4511-0563-0

Wu Y, Cho M, Shore D, Song M, Choi J, Jiang T, Deng YQ, Bourgeois M, Almli L, Yang H, Chen L M, Shi Y, Qi J, Li A, Yi KS, Chang M, Bae JS, Lee H, Shin J, Stevens J, Hong S, Qin CF, Gao GF, Chang SJ, Donis RO, Nat. Commun. 6, 7708, 2015. https://doi.org/10.1038/ncomms8708

Wyrzucki A, Dreyfus C, Kohler I, Steck M, Wilson IA, Hangartner L, J. Virol. 88(12), 7083–7092, 2014. https://doi.org/10.1128/JVI.00178-14

Yamayoshi S, Kawaoka Y, Nat. Med. 25(2), 212–220, 2019. https://doi.org/10.1038/s41591-018-0340-z

Yassine HM, Boyington JC, Mctamney PM, Wei CJ, Kanekiyo M, Kong WP, Gallagher JR, Wang L, Zhang Y, Joyce MG, Lingwood D, Moin SM, Andersen H, Okuno Y, Rao SS, Harris AK, Kwong PD, Mascola JR, Nabel GJ, Graham BS, Nat. Med. 21(9), 1065–1070, 2015. https://doi.org/10.1038/nm.3927

Ye J, Shao H, Perez DR, Immunotherapy 4(2), 175–186, 2012. https://doi.org/10.2217/imt.11.167

Yewdell JW, Gerhard E, Bächi T, J. Virol. 48(1), 239–248, 1983.

Yoon SW, Webby RJ, Webster RG, Curr. Top. Microbiol. Immunol. 385,359–375, 2014. https://doi.org/10.1007/82_2014_396

Yu X, Baruah K, Scanlan CN, Crispin M (2014): Antibody glycosylation. In mena editorov??? Antibody Fc: Linking Adaptive and Innate Immunity. Academic Press, Elsevier Inc. pp. 179–194. ISBN : 978-0-12-394802-1. https://doi.org/10.1016/B978-0-12-394802-1.00010-8

Zhang J, Li G, Liu X, Wang Z, Liu W, Ye X, J. Gen. Virol. 90(Pt11), 2751–2758, 2009. https://doi.org/10.1099/vir.0.014316-0

Zhang X, Chen S, Jiang Y, Huang K, Huang J, Yang D, Zhu J, Zhu Y, Shi S, Peng D, Liu X, Vet. Microbiol. 175(2–4), 244–256, 2015. https://doi.org/10.1016/j.vetmic.2014.12.011

A revision of the system of nomenclature for influenza viruses: a WHO memorandum (1980): Bulletin of the World Health Organization 58(4), 585–591.

http://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)

https://ecdc.europa.eu/en/seasonal-influenza/prevention-and-control/vaccines/types-of-seasonal-influenza-vaccine (ecdc, 2018)

https://www.cdc.gov/flu/protect/vaccine/quadrivalent.htm,Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD) (cdc,2018a)

https://www.cdc.gov/flu/protect/vaccine/vaccines.htm september 5,2018 (cdc, 2018b)