An ideal influenza vaccine: what does it look like and what components play a role in inducing cross-immune reactivity

Author: Sharda van Willigen
Studentnumber: 182498
Institution: Rijksuniversiteit Groningen
First assessor: Maaike Stoel
Second assessor: prof. dr. Anke Huckriede
Date: 29-06-2012

Summary

Influenza is a very contagious disease that causes serious illness and death every year. Antigenic drifting and antigenic shifting of the virus leads to the emergence of new viral strains which can evade the human immune system and thus cause epidemics and pandemics.
Vaccination is the primary strategy to protect the human population from infections with influenza viruses but the current vaccines do not provide sufficient protection against new strains. Predicting the next pandemic is impossible and therefore it is important to find new vaccines that can induce cross-immune reactivity. The innate immune system targets invading viruses first and activates the adaptive immune response which consists of a humoral and a cellular part. The humoral response can block viral infection with antibodies that can bind viral proteins and the cellular response leads to clearance of the virus with T-cells when a host has been infected. The hemagglutinin surface protein of the virus is currently used as a target for vaccination but because this protein changes rapidly vaccinating against hemagglutinin is ineffective in inducing cross-immune reactivity. The M2 surface proteins, the internal nucleoprotein and the hemagglutinin stem provide more promising targets for vaccination when a broad immune response is required. The development of a universal influenza vaccine would lead to prevention of pandemic outbreaks. Of all the possible vaccines that are in use or in development DNA vaccines against the nucleoprotein, the M2 protein or the HA stem, possibly with the addition of an adjuvant, seem like an ideal candidate for a universal vaccine.

Table of contents

1. Introduction

Influenza is a common virus infection and can lead to illness with symptoms such as fever, sore throat, coughing, headache and muscle pains but can also cause severe complications like pneumonia and especially older people or people with underlying chronic diseases are at risk of getting more serious complication [1][2][3]. Throughout history, several epidemic and pandemic influenza viruses have appeared, some of the pandemic strains had a catastrophic outcome. The "Spanish flu" for example, a H1N1 influenza virus from 1918, was the cause of over 50 million deaths worldwide [4].Also, pandemic influenza outbreaks seem to appear more frequently throughout history. Two reasons for this are the increase of global travel and the growing use of land [5][6]. The emergence of new viral strains can occur through two different processes called antigenic drift and antigenic shift, which respectively are slight changes in the hemagluttinin (HA)protein on the surface of the virus and complete replacement of the HA protein and therewith a change in influenza subtype [6]. There are two ways in which a new influenza virus can emerge. Influenza viruses that already infect humans can undergo changes in their molecular structure or transmission can occur between different species [1]. Besides humans, several species of animals can get infected by influenza, including pigs, horses, birds and sea mammals [5][6] [7]. In Asia, the close adjacency of humans and animals, particularly swine and birds, often leads to viral reassortment and thus the emergence of newly mutated influenza strains [8]. The current fear lays on the reassortment of an avian influenza strain with a human influenza virus that might produce a possible pandemic influenza strain [8].

Vaccination is a good way to prevent infection with influenza but the current vaccines do not provide protection against drifted or shifted strains. The continuing viral alterations lead to evasions of the immune system and thereby to a constant threat for new pandemics [5]. Because of this, the current influenza vaccine development is focused on creating an immune response against different strains of influenza [5]. Throughout the year, emergence of new strains is being watched in case the current seasonal vaccine would offer limited protection against these strains and a new vaccine composition would have to be developed [9]. Current research on the field of influenza vaccination is seeking for a way of inducing cross-immune reactivity against newly emerging strains. Cross-immune reactivity, also called heterologous immunity or heterosubtypic immunity, is immunity against a previously encountered pathogen that has an effect on a different, not known pathogen [10].

Until now, vaccination is the key to protecting the human population from infections with influenza viruses but the current vaccines do not provide protection against new strains [6]. It is impossible to predict when the next pandemic will arise and by which strain it will be caused [4]. Therefore it is important to find new vaccines that induce cross-immune reactivity[4]. With this thesis I will investigate what an ideal influenza vaccine would look like and what role vaccine composition plays in inducing cross-immune reactivity against influenza viruses.

2. The influenza virus

Influenza belongs to the family of Orthomyxoviridae which can be divided into 5 sub-families of which influenza A, B and sometimes C are the common [5][11]. Influenza A is the major cause of infections in humans because it leads to more serious illness and so far was the cause of 4 pandemic strains, the Spanish flu in 1918 (H1N1), H2N2 in 1957, H3N2 in 1968 and in 2009 there was an outbreak of H1N1 [5][11]. Because influenza A is the most common and most dangerous type of influenza, further discussion on the virus will be about influenza A.

2.1 Basic structure of influenza A

As mentioned before, influenza has two proteins on its surface, hemaglutinin and neuraminidase (NA). Until now, 9 different NA glycoproteins have been discovered and 17 HAs, the last of which, H17, was recently found in bats[11] [12]. Hemagglutinin (HA) and neuraminidase (NA) are glycoproteins on the surface on influenza A viruses and subdivision of Influenza A is based on these proteins [11]. Hemagglutinin is a membrane protein that is involved in binding the virus with sialic acid on the surface of a host cell and plays a role in uncoating the virus through fusion with the endosomal membranes . Neuraminidase is also a membrane glycoprotein and has a function in releasing a new virus particle by cleaving the sialic acid from a host cell [6]. The influenza A genome is a negative-single-stranded RNA virus characterized by 8 segments that together can produce 11 proteins of which hemagglutinin and neuraminidase are two [6]. The virus has a lipid membrane originated from a host cell membrane and has, besides HA and NA, one other membrane protein, namely the matrix 2 (M2) protein. These M2 proteins are settled through the lipid layer and play a role as ion channel [6]. The lipid membrane protects the virus core which is covered by matrix 1 (M1) proteins and is associated with the ribonucleoprotein (RNP) complex. RNP's are viral RNA's surrounded by nucleoprotein. Inside the M1 layer are 8 RNA molecules protected by several proteins, namely nucleoprotein (NP) and three RNA dependant RNA polymerases (PB1, PB2 and PA) (see figure 1) [6]. Because the genome of influenza consists of different segments, this can lead to reassortement between different influenza A subtypes which is the cause of new virus strains with epidemic or pandemic potential [6].

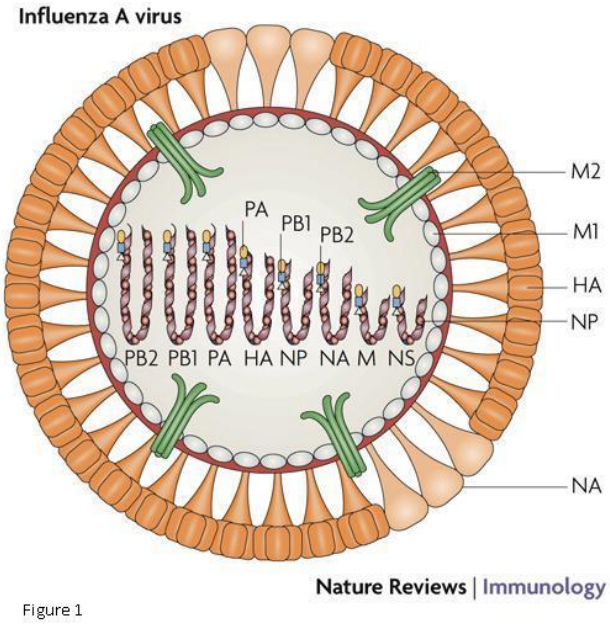


Figure 1

Source: nature reviews immunology (2007) volume 7

2.2 Viral entry of the host cell

The influenza viruses can bind to host cells attachment to the siliac acid that is expressed on the surface of the cell. Siliac acid has a carbon-2 terminal which can bind to either the carbon-3 of galactose or the carbon-6 forming two different linkages. Human influenza viruses prefer to bind α-2,6 linkage siliac acid while avian influenza viruses prefer a α-2,3 linkage[11]. Humans have an overbalance of α-2,6 linked siliac acid but the small amount of α-2,3 linkage is found in the lower respiratory tract and when a virus infects the lungs it can lead to serious complications and fast progressing pneumonia [11].

After attachment of HA to the host cell´s siliac acid, the virus is taken into the cell by endocytosis. The endosomal environment is acidic which is important for uncoating of the influenza virus. The low pH

will cause a change in the HA structure which will expose the stem of the HA protein and this will lead to the fusion of the outer layer of the virus with the endosomal membrane [13]. After fusion of the virus, RNP's from the viral core are released through a pore in the membrane. Also, a low pH ensures hydrogen ions being pumped into the virus by the M2 proteins and this also contributes to the release of RNP's [11].

2.3 How the virus replicates and spreads

Once free, the RNP's are transported to the nucleus of the host cell and a part of the RNP's, the viral RNA dependent RNA polymerase , synthesizes two different positive-sense RNA's from the negative sense viral RNA [11]. One of the RNA's forms messenger RNA templates of which virus proteins can be made and the other strand is complementary RNA to make more copies of the negative-sense viral RNA. The mRNA can get synthesized because viral proteins PB1 and PB2 steal capped primers from the host. After the cap is placed the mRNA can be translated [11]. Not much is known about the synthesis of the viral proteins, only about the three surface proteins. HA, NA and M2 are synthesized from viral mRNA and folded in the endoplasmatic reticulum before getting transported to the Golgi apparatus to undergo the last alterations. The three proteins are equipped with signals to bring them to the membrane of the cell [11]. All eight RNA segments need to be transported towards the cell surface and after everything is assembled to form a new virus particle, the virus can start budding. With this process the virus particle is pushed towards the outside of the cell and forms its own cell envelope from the host cell membrane [11]. All the while the HA of the virus will remain attached to the host cell and only NA can release it from its bond to siliac acid. Now, new virus particles are free to infect other host cells.

2.4 Antigenic drift and antigenic shift

The HA and NA genes of influenza viruses can mutate and this leads to changes in its amino acids and which leads to an antigenic drift [6]. These slight changes in the antigenic site make previously effective antibodies no longer able to protect the host [3]. This new virus can still be recognized by the immune system but is more capable of infecting the host because the immune response, gained by natural infection or vaccination, is less specific [11]. The seasonal vaccines against influenza are based on these small changes that occur in the virus and this leads to a frequent update of the composition of the vaccines [2].

While antigenic drift can result in influenza strains that may lead to epidemics, a process called antigenic shifting can lead to pandemic strains [1]. An antigenic shift is a completely new HA subtype that emerges in the human population to which no previous immunity exists [4]. This can take place because the genome of influenza consists of different segments and this can lead to reassortment between different influenza A subtypes[4]. The parts that shift are the HA and sometimes the NA and it can take place in hosts infected with different virus subtypes. This may lead to a set of new antigenic proteins to which there is no preexisting immunity [11]. A newly reassorted strain might be able to infect other species than it originally could and if the human population is susceptible to the new virus it can be the cause of a pandemic[5][11].

As said before, a drifted or shifted virus can't be recognized by the adaptive immune response of the host and thus can produce offspring to infect other hosts [5]. The reason why these processes occur is that natural immunity leads to selection pressure on the virus and thus to rapid changes in the viral structure and immunity due to vaccination might speed up this process [9].

3. Immunity against influenza

A human body has several defense mechanisms to prevent illness or defeating an infection. The goal of immunity is to prevent or fight an infection and to give the host a long-lasting protection against a pathogen that is antigenically similar. Below, the different defense mechanisms of the human body will be discussed.

3.1 The role of the innate immune system

When the influenza virus tries to enter the body through the upper respiratory tract it will have to pass different barriers. The entry site, the upper respiratory tract, contains a thick layer of mucus, secreted by goblet cells, to form a physical barrier to prevent pathogens from entering [14]. This site also contains cilia which are tiny hairs that move mucus and invading substances toward the outside of the respiratory tract. When past this first line of defense, the virus will try to attach itself to the epithelial cells and enter the host [4]. As a second barrier, the virus will be recognized by the innate immune system. Certain pathogenic structures, pathogen-associated molecular patterns (PAMPs), are recognized by pattern recognition receptors (PRRs) of the innate immune system [15]. There are four types of PRRs known, namely Toll-like receptors (TLRs), retinoic acid-inducible gene-I-like receptors (RLRs), NOD-like receptors (NLRs) and C-type lectin receptors (CLRs) [4]. Viral RNA gets recognized by TLR3 and TLR7 in the endosomes or lysosomes and this results in the production of INFα and INFβ [4]. This also leads to the production of several pro-inflammatory cytokines [4]. Viral replication, which takes place in the cytosol of the infected host, can get detected by RLRs. The two most characterized RLTs are retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) [4]. RIG-I mainly detects single-stranded RNA and short double-stranded RNA and induces type-I INF production upon stimulation [4]. The NLRs lead to the production of interleukin-1β and interleukin-18 which recruit two other cell types of the innate immune system, namely monocytes and neutrophils [4][15]. CLRs can initiate Dendritic cells or macrophages to phagocytose pathogens [4]. The innate immune system uses antigen presenting cells (APCs), like dendritic cells and marcophages to present antigens to cells of the adaptive immune system. Proinflammatory cytokines and antiviral cytokines are produced as a result of the activation of the innate immune response [15]. It is thought that the proinflammatory cytokines recruit effector cells that help clear the virus infection and motivate the adaptive immune response. The antiviral cytokine response is responsible for inducing the recruiting of interferons, especially interferon-I gets triggered by single-stranded and double stranded RNA [4]. Several host defense molecules, also known as antimicrobial peptides (AMPs), participate in the innate immune system [16]. They are reactive against different kinds of pathogens [16]. There are several AMPs that are active against viruses which include defensins and Cathelicidins. These AMPs can block infection by attacking the virus itself or by acting on the infected cell [17].

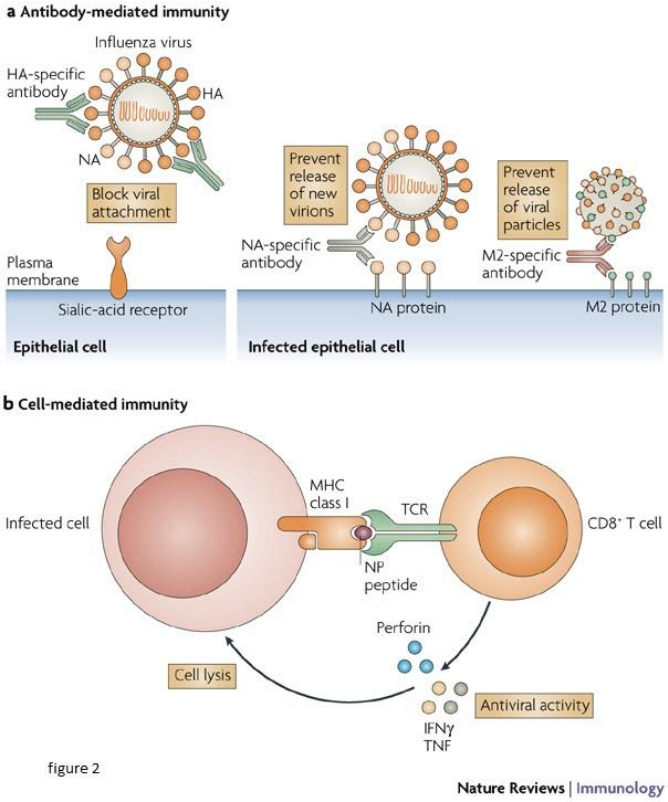
3.2 The role of the adaptive immune system

There are different parts of the adaptive immune response that play a role in clearing an influenza infection; the humoral immune response with the production of antibodies and the cellular immune response with, CD4+ and CD8+ T-lymfocytes. Below, these different aspects will be described in more detail.

3.2.1 The humoral immune response

The humoral immune response towards influenza virus consists of a mucosal- and serum antibody response [18]. There are three different types of mucosal and serum antibodies, the HA specific, the NA specific and M2 specific antibodies that play a role in preventing an infection with influenza [6]. The HA antibodies can effectively prevent infection while the NA and M2 antibodies can only reduce the release of new virus particles [18]. The immunoglobulins (Igs) A and M are the most important antibodies to prevent virus entry and replication [18]. Antibodies, or immunoglobulins (Igs), have different isotypes of which IgG is the most important antibody of the serum antibody response and

and IgA important for the mucosal immune response. Also, IgG provides a measurement for the amount of protection against influenza in a hemaglutination inhibition titer [18]. The antibodies against HA prevent attachment of the virus to a siliac acid receptor while NA antibodies and M2 antibodies both prevent the release of new viral particles (see figure 2a)[4]. Antibody mediated immunity is mostly directed against the surface proteins of influenza and therefore only effective against homosubtypic immunity [19]. This is important to prevent reinfection with a similar subtype but because the virus mutates rapidly the antibodies offer insufficient protection against influenza viruses of a different subtype. In the case of an epidemic or pandemic outbreak these antibodies would provide no protection and this is the reason why these outbreaks lead to fatality more often.



Source: nature reviews immunology (2007) volume 7

3.2.2 Cellular immune response

While the humoral response against influenza provides protection and prevention of infection, the cellular response leads to total clearance of the virus [6]. T-cells can internal proteins of the virus and thus are more effective in heterosubtypic immunity because these proteins are more conserved [19]. CD4+ T helper cells can secrete cytokines to interact with other immune cells after being presented viral proteins by the major histocompatibility complex (MHC) class II. The CD8+ gets expressed on the cytotoxic T-lymphocytes and it recognizes viral peptides on APCs with MHC class I. When a cell is infected it can initiate apoptosis to prevent the virus from releasing new particle [19]. The CD8+ T-cell can produce cytokines, such as INFγ tumor-necrosis factor (TNF), and perforins, granzymes and granulysin that lead to lysis of the host cell (see figure 2b) [6][20].

Vaccines

Until now, vaccination is the most important tool in controlling influenza viruses [1]. Vaccination is not always efficient enough to protect against illness, especially for people with a chronic illness and older people [1]. The protection vaccines offer depends not only on the antigenic match with the circulating viruses but also on the age and health of the person getting infected [1]. Currently, seasonal vaccines consist of two influenza A viruses, namely a H3N2 and a H1N1, and an influenza B strain. The exact viral composition of new seasonal vaccines is based on the currently circulating strains [1].The goal of current influenza vaccines mainly is to induce an antibody response against HA [5]. It is still impossible to predict when the next pandemic will arise or what HA virus subtype it will be so it would be beneficial to develop a vaccine that induces an immune response against circulating and future influenza viruses, a universal influenza vaccine [5]. However, no one has yet been able to find a vaccine that could induce an immune response against the different subtypes of influenza or newly emerging strains [5].

Currently, the effectiveness of influenza vaccines is tested with a hemagglutination-inhibition assay [4]. This assay is used to measure the concentration of antibodies, which prevent binding of the virus to erythrocytes [6].
There are different ways to administer a vaccine and this also plays a role in what kind of immune response is induced but it will not be discussed in this thesis.
Because the currently used vaccines do not provide sufficient cross-protection against a possible pandemic influenza strain, many vaccines are in development. Several of the currently used vaccines and vaccines in development are discussed below.

4.1 Different types of vaccines

Vaccines currently in use:

1. Whole inactivated virus vaccines: These vaccines consist of complete viruses that have been inactivated using heat or chemical agents such as formaldehyde or formalin [4][21][22]. They are no longer infectious but an immune response can still be formed against the virus [23]. Whole virus vaccines can give rise to side effects because they are more immunogenic than split or subunit vaccines [18].
2. Subunit vaccines: These vaccines contain only surface proteins HA en NA and therefore are not infectious. This vaccine is known to effectively induce humoral immunity [23]. In naïve individuals the subunit vaccine does not provide a protective immune response after one vaccination due to the fact that these vaccines are not as immunogenic as whole inactivated vaccines [24].
3. Split vaccines: the contents of these vaccines are small pieces of the virus that have been disrupted by a chemical agent. They contain not only surface proteins but also proteins from the viral core [23]. Split vaccines, just as subunit vaccines, are not as immunogenic as whole inactivated vaccines and thus a double dose is sometimes required for a satisfactory immune response that would protect the host against reinfection [24].
4. Life attenuated virus vaccines: Life attenuated influenza vaccines (LAIV) contain viruses with genes for the surface proteins and the other gene segments from a less virulent donor. Because the viruses are alive the infection follows a more natural course and can induce a mucosal antibody response and a broader T-cell response than inactivated vaccines [25]. Because these viruses are alive they are able to infect cells and even though they were made less virulent there still is a small chance the virus could become dangerous again. The virus could mutate spontaneously or it could reassert with other virus strains [18][26].

Vaccines still in their trial fase:

5. DNA/RNA vaccines: These vaccines contain synthetic DNA/RNA that encodes for the antigens to elicit an immune response [27]. Like life attenuated virus vaccines, DNA vaccines can induce an antibody response but also a T-cell response but they are not infectious and thus do not give the same problems life attenuated vaccines might give [28].
6. Synthetic protein vaccines: These vaccines only contain single proteins that have been harvested from a vector virus. They don't give as many side effects as whole inactivated or attenuated virus vaccines because they are not infectious but only elicit a humoral immune response[1].

4.2 A*djuvants*

Adjuvants are substances that are not immunogenic themselves but can be used to increase the immune response against the antigen of interest after vaccination. Adjuvants can be used to reduce the amount of virus, or viral particles necessary for inducing a sufficient immune response [6]. Also, they are beneficial in improving the immune response in newborns, elderly and immune-compromised [6][29]. They do this by increasing the presentation of antigens to T-cells and the recruitment of innate immune cells [1]. For a long time, only aluminum salts have been used as an adjuvant but are not very potent as adjuvants [30]. Most newly discovered adjuvants showed some level of toxicity in their trial fase and were rejected for human use and current adjuvant research is focused on minimizing the level of toxicity, while maximizing adjuvant effect [29]. There are several different types of adjuvants (see table 1).

Table 1. Adjuvants used and others under investigation in the context of influenza vaccines

Adjuvant category	Types
Mineral salts	• Aluminum salts*
Oil-in-water emulsions	• MF59* • AS03* • AF03** • CoVaccine HT**
Saponins and glycolipids	• QS-21*** • ISCOMATRIX** • Alpha-GalCer (alpha-galactosylceramide**
Liposomes	• Virosomes* • CCS (ceramide carbamoyl-spermine)** • CAF01 (cationic liposomes and synthetic mycobacterial cord factor)** • Vaxfectin**
Bacterial toxins/components	• CT (cholera toxin)** • LT (*Escherichia Coli* labile enterotoxin)*** • Chitosan** • Salmonella and *Escherichia Coli* flagellins**
Cytokines	• IL-12, IL-23, IL-28B** • GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor)** • Tyoe 1 IFN (IFNalpha)**
TLR agoninsts/ immunomodulators	• Synthetic lipid A adjuvant (TLR-4)** • Bacterial flagellines (TLR-5)** • CpG (oligodeoxynucleotide) (TLR-9)*** • Polyl:polyC12U [(synthetic double-stranded RNA (dsRNA)] (TLR-3)** • IC31 (oligodeoxynucleotide) (TLR-9)** • sLAG-3 (IMP321) (ligand for MHC class II)***
Biomedical polymers	• PCPP (polyphosphazenes)**

*(in clinical use); **(investigated in animal model); ***(in clinical development)

(source: [2] [29])

4. Cross-immune reactivity and the ideal influenza vaccine

As mentioned previously cross-immune reactivity, is immunity against a subtype of influenza A caused by infection with a different influenza A subtype [18]. The CD8+ T-cell appears to be important in inducing cross-immune reactivity but also CD4+ T-cells and antibodies play a role [31]. Here, the influence of humoral immunity and cellular immunity on cross-immune reactivity will be discussed.

5.1 Cellular immunity in cross-immune reactivity

As said before, cellular immunity is directed against the surface and internal proteins of an influenza A virus. Because these proteins are highly conserved, T-cell immunity against influenza is thought to be important in inducing cross-immune reactivity [32]. Several laboratories found that CTLs directed against a specific influenza A strain can kill cells infected with a different strain of influenza A [33][34]. The NP of influenza appeared to be a target of T-cell immunity in mice [32]. Cold-adapted viruses, which are viruses that are unable to replicate above 39°C, are currently used in life attenuated virus vaccines and have shown to induce cross-immune reactivity through CD8+ T-cells [35]. DNA vaccines have also shown to provide protection through cross-immune reactivity by inducing a CD8+ T-cell response [32]. CD4+ T-cells help to generate cross-protective antibodies and CD8+ T-cells by producing different cytokines[31].

5.2 Humoral immunity in cross-immune reactivity

As mentioned previously, an antibody mediated immune response is directed towards the HA and NA surface proteins of influenza A which leads to homosubtypic protection but hardly plays a part in cross-immunity because the surface proteins are to variable [32]. Antibodies directed toward more conserved epitopes might induce cross-immune reactivity. Two possible cross-immunity inducing proteins are the M2 protein and the NP. Antibodies against M2 might prevent the release of new virus particles and several studies have showed that this protein in a prime candidate for vaccines to induce cross-immune reactivity [36]. The NP is a protein in the virus core but can elicit an antibody response when they are freed from a host cell and in mice, immunization with NP has showed promising results [36]. The antibodies directed against NP cooperate with CD8+ T-cells to eliminate the virus. After natural infection with an influenza A subtype these antibodies are also produced but might not be sufficient enough to provide an immune response because these antibodies diminish after a while. Vaccination to boost these antibodies might be a possibility to induce successful cross-immune reactivity [36]. Another recent point of focus lays on the antibodies directed against the HA stem. This part gets exposed in the endosome and leads to fusion with the endosomal membrane. Antibodies against this more conserved part of the protein could block release of viral proteins into the host cell and this could be another possibility for vaccines [13].

5.3 What does an ideal influenza vaccine look like?

An ideal influenza vaccine has to meet several criteria; it has to be safe, elicit an immune response that follows a natural course of infection, result in long-lasting protection, elicit cross-immune reactivity and production time has to be short in case of an epidemic or pandemic outbreak [1]. Current vaccines against influenza mainly use the highly immunogenic HA surface protein but as this is the most variable part of the virus it will not lead to a sufficient cross-immune protection [1]. Natural infection with influenza is more powerful in inducing cross-immune reactivity than the current vaccines are and a reason for this could be the site of infection. The virus enters the body in the upper respiratory tract and induces the mucosal immunity. The most commonly used type of vaccination, an intramuscular injection, does not elicit a mucosal immune response [37]. The ideal vaccine should follow a natural course of infection, should induce cellular and humoral immunity, both a mucosal and serum antibody response and has to be cross-protective.

As said previously, to provide cross-immune protection, vaccination has to be directed against the more conserved epitopes of an influenza virus such as the NP, the matrix proteins or the more conserved parts of HA and NA proteins [1].

T-cells, in contrast to antibodies, are not capable of preventing an infection but can cause faster clearance of the virus and reduce pathology [32]. Antibodies are directed against surface proteins and thus can recognize a virus before it has entered a host cell. An ideal influenza vaccine would induce an antibody response against one of the more conserved proteins of influenza. The M2 protein seems to be a promising target for vaccination and has been studied thoroughly but antibodies directed against the M2 protein appear to not prevent infection but promote clearance [26]. Vaccines against the NP also seem promising but are also not capable of preventing an infection because the internal proteins are targeted by T-cells [26]. Although these vaccines might not be able to prevent infection, reducing morbidity and mortality would be beneficial in case of a pandemic outbreak. Another possible target for inducing a heterosubtypic reaction, one that seems to be able to induce a protective antibody response, is the highly conserved HA stem [26]. A vaccine developed against this part of the virus would not only provide protection against drifted strains but could protect against shifted strains that could cause a pandemic.

The role of adjuvants is still unclear because currently a lot of recently discovered adjuvants are still in their trial stage. Several of those adjuvants have shown promising results in inducing cross-immune reactivity and an adjuvant that could aid the mucosal immune response might be able to help with the induction of cross-immune reactivity [38].

5. Conclusion

The occurrence of the next influenza pandemic is unavoidable and to prevent worldwide morbidity and mortality the development of a universal vaccine is crucial. There are several possible candidate antigens for this universal vaccine but the NP, the M2 protein and the HA stem protein seem the most promising. A DNA vaccine or a life attenuated virus vaccine can induce the desired cross-protective response and are able to follow a more natural course of infection because they induce a broad immune response. However, DNA vaccines come with less risks and thus are a better candidate for an ideal influenza vaccine. Thus, development of a DNA vaccine that could cause a long-lasting production of protective antibodies against the HA stem, possibly with the use of an adjuvant, might be the ideal influenza vaccine.

6. References

[1] Linda C. Lambert and Anthony S. Fauci. Influenza Vaccines for the Future N Engl J Med 2010; 363:2036-2044

[2] Paolo Durando, Rocco Iudici, Cristiano Alicino, Marisa Alberti, Daniela de Florentis, Filippo Ansaldi and Giancarlo Icardi. Adjuvants and alternative routes of administration towards the development of the ideal influenza vaccine. Human Vaccines 7: Supplement, 29-40; January/February 2011; c 2011 Landes Bioscience

[3] Jiezhong Chen and Yi-Mo Deng. Influenza virus antigenic variation, host antibody production and new approach to control epidemics. Virol J. 2009; 6: 30.

[4] Ainai A, Tashiro M, Hasegawa H. Cross-protective immunity against influenza virus infections induced by intranasal vaccination together with a TLR3-mucosal adjuvant. Hum Vaccin. 2011 Jan-Feb;7 Suppl:174-82.

[5] Thomas Han and Wayne A. Marasco. Structural basis of influenza virus neutralization. Ann N Y Acad Sci. 2011 January; 1217: 178–190.

[6] Kanta Subbarao and Tomy Joseph. Scientific barriers to developing vaccines against avian influenza viruses. Nature Reviews Immunology 7, 267-278 (April 2007).

[7] Robert G. Webster, William J. Bean, Owen T. Gorman, Thomas M. Chambers and Yoshihiro Kawaoka. Evolution and Ecology of Influenza A Viruses. Microbiological reviews, mar 1992, p. 152-197

[8] Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, Zambon MC, Katz JM. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. J Infect Dis. 2005 Apr 15;191(8):1210-5.

[9] Maciej F. Boni. Vaccination and antigenic drift in influenza. Vaccine. 2008 July 18; 26(Suppl 3): C8–14.

[10] Raymond M. Welsh, Jenny Che, Michael A. Brehm and Liisa K. Selin. Heterologous immunity between viruses. Immunol Rev. 2010 May; 235(1): 244–266.

[11] Nicole M. Bouvrier and Peter Palese. The biology of influenza viruses. Vaccine. 2008 September 12; 26(suppl 4): D49-D53

[12] Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle AS, Moran D, Rogers S, Shi M, Tao Y, Weil MR, Tang K, Rowe LA, Sammons S, Xu X, Frace M, Lindblade KA, Cox NJ, Anderson LJ, Rupprecht CE, Donis RO. A distinct lineage of influenza A virus from bats. Proc Natl Acad Sci U S A. 2012 Mar 13;109(11):4269-74.

[13] C. A. Thomson et. Al. Pandemic H1N1 Influenza Infection and Vaccination in Humans Induces Cross-Protective Antibodies that Target the Hemagglutinin Stem. Front Immunol. 2012; 3: 87.

[14] Izuhara K, Ohta S, Shiraishi H, Suzuki S, Taniguchi K, Toda S, Tanabe T, Yasuo M, Kubo K, Hoshino T and Aizawa H. The mechanism of mucus production in bronchial asthma. Curr Med Chem. 2009;16 (22):2867-75.

[15] Shuhua Wu, Jordan P. Metcalf and Wenxin Wu. Innate immune response to influenza virus. Current Opinion in Infectious Diseases 2011, 24:235–240.

[16] Gill Diamond, Nicholas Beckloff, Aaron Weinberg and Kevin O. Kisich. The Roles of Antimicrobial Peptides in Innate Host Defense. Curr Pharm Des. 2009; 15(21): 2377–2392.

[17] Mary E. Klotman and Theresa L. Chang. Defensins in innate antiviral immunity. Nature Reviews Immunology 6, 447-456 (June 2006) | doi:10.1038/nri1860.

[18] R. J. Cox, K. A. Brokstady and P. Ograz. Influenza Virus: Immunity and Vaccination Strategies. Comparison of the Immune Response to Inactivated and Live, Attenuated Influenza Vaccines. 2004 Blackwell Publishing Ltd. Scandinavian Journal of Immunology 59, 1–15.

[19] Paul G. Thomas, Rachael Keating, Diane J. Hulse-Post and Peter C. Doherty. Cell-mediated protection in Influenza Infection. Emerging Infectious Diseases, Vol. 12, No. 1, January 2006.

[20] Veljkovic Vujaklija D, Sucic S, Gulic T, Dominovic M, Rukavina D. Cell death mechanisms at the maternal-fetal interface: insights into the role of granulysin. Clin Dev Immunol. 2012;2012: 180272.

[21] Furesz J, Scheifele DW and Palkonyay L. Safety and effectiveness of the new inactivated hepatitis A virus vaccine. CMAJ. 1995 Feb 1;152(3):343-8.

[22] Cohen JI. Strategies for herpes zoster vaccination of immunocompromised patients.J Infect Dis. 2008 Mar 1;197 Suppl 2:S237-41.

[23] T. Jefferson, E. Bianco and V. Demicheli. Influenza vaccines in adults. Occup. Med. Vol. 52 No. 5, p. 255-258, 2002.

[24] A. H. Ellebedy and R. J. Webby. Influenza Vaccines. Vaccine. 2009 November 5; 27(Suppl 4): D65–D68.

[25] Boris Ferko, Christian Kittel, Julia Romanova, Sabine Sereinig, Hermann Katinger, and Andrej Egorov. Live Attenuated Influenza Virus Expressing Human Interleukin-2 Reveals Increased Immunogenic Potential in Young and Aged Hosts. J Virol. 2006 December; 80(23): 11621–11627.

[26] Kaiser J. A One-Size-Fits-All Flu Vaccine? Science. 2006 Apr 21;312(5772):380-2.

[27] John J. Donnelly, Britta Wahren and Margaret A. Liu. DNA Vaccines: Progress and Challenges. The Journal of Immunology July 15, 2005 vol. 175 no. 2 633-639

[28] Sanjay Gurunathan, Dennis M. Klinman and Robert A. Seder. DNA VACCINES: Immunology, Application and Optimization. Annu. Rev. Immunol. 2000. 18:927–974.

[29] Atmar RL, Keitel WA. Adjuvants for pandemic influenza vaccines. Curr Top Microbiol Immunol. 2009;333:323-44.

[30] Lorena E. Brown. The role of adjuvants in vaccines for seasonal and pandemic influenza. Vaccine 28 (2010) 8043-8045

[31] K. K. McKinstry, T. M. Strutt and S. L. Swain. Hallmarks of CD4 T cell immunity against influenza. J Intern Med. 2011 May;269(5):507-18

[32] Kristie M. Grebe, Jonathan W. Yewdell, and Jack R. Bennink. Heterosubtypic Immunity To Influenza A Virus: Where Do We Stand? *Microbes Infect*. 2008 July ; 10(9): 1024–1029.

[33] Zweerink HJ, et al. Cytotoxic T cells kill influenza virus infected cells but do not distinguish between serologically distinct type A viruses. Nature 1977;267(5609):354–6.

[34] Braciale TJ. Immunologic recognition of influenza virus-infected cells. I. Generation of a virus-strain specific and a cross-reactive subpopulation of cytotoxic T cells in the response to type A influenza viruses of different subtypes. Cell Immunol 1977;33(2):423–36.

[35] Powell TJ, et al. Priming with cold-adapted influenza A does not prevent infection but elicits longlived protection against supralethal challenge with heterosubtypic virus. J Immunol 2007;178(2): 1030–8.

[36] Denise A. Kaminski and F. Eun-Hyung Lee. Antibodies Against Conserved Antigens Provide Opportunities for Reform in Influenza Vaccine Design. Front Immunol. 2011; 2: 76.

[37] Graeme E. Pricea, Mark R. Soboleskia, Chia-Yun Loa, Julia A. Misplona, Claudia Pappasb, Katherine V. Houserb, Terrence M. Tumpeyb, Suzanne L. Epsteina. Vaccination focusing immunity on conserved antigens protects mice and ferrets against virulent H1N1 and H5N1 influenza A viruses. Vaccine 27 (2009) 6512–6521.

[38] Cargnelutti DE, Sanchez MV, Alvarez P, Boado L, Glikmann G, Mattion N, Scodeller EA. Improved immune response to recombinant influenza nucleoprotein formulated with ISCOMATRIX. J Microbiol Biotechnol. 2012 Mar;22(3):416-21.