Towards a Cross-Protective Influenza A Vaccine

Armando van Bruggen, Universitair Medisch Centrum Groningen, Groningen, The Netherlands.

Abstract

The threat of a future influenza A pandemic necessitates the development of a vaccine eliciting a cross-protective immune response to influenza A. While several antibodies showing cross-reactive properties to different influenza A subtypes have been identified, the essential and at the same time the most difficult part remains to stimulate the immune system in such a way that its primary response will be the production of such cross-reactive antibodies. This process is especially hindered by the mechanisms in which influenza evolves and causes influenza to evade the immune system. It appears that most cross-reactive antibodies bind to the stem region of hemagglutinin, as it is also noticed in the elderly showing cross-protection to the 1918 H1N1 influenza subtype and the H1N1 pandemic virus which emerged in 2009. In this review article it is revealed that prime-boost immunization fulfills these criteria and could be a possible candidate for the next generation of vaccines.

Keywords: Influenza A, vaccine, antibody, hemagglutinin, cross-protection

1. Introduction:

1.1. The global impact of influenza A

Influenza A infections pose a major burden worldwide in terms of healthcare and control and its global impact is greatly underestimated. For example it is estimated that the damage done annually by influenza A infections in US healthcare alone costs around 87,1 billion dollars (1). The global expenses therefore should be considered to be far higher. Influenza is also accountable for significant death tolls; The World Health Organization estimates influenza associated deaths in between 250.000-500.000 annually worldwide (2). These numbers seem to be in stark contrast considering previous influenza pandemics and its annual impact is often well underestimated. However, it has been calculated that the cumulative death toll for yearly influenza related deaths greatly exceeds that of the last 3 major pandemics combined emphasizing the severity of the disease (3, 4). The high annual cost and death count clearly point out the severity of influenza infections hence methods controlling influenza infection and spread should greatly be improved.

Although most healthy individuals have the capability to effectively neutralize an influenza infection, certain individuals show more susceptibility to influenza than the average individual. Various reasons can cause higher susceptibility and individuals showing higher susceptibility are classified to risk groups as defined by the European Centre for Disease Prevention and Control. These are though not limited to the elderly above the age of 65, persons with chronic or medical conditions, pregnant woman and children below the age of 5 (5). It is recommended that these individuals should receive yearly vaccination. However, each year individuals in risk groups still show to be affected more severely to influenza and show a higher mortality rate than healthy individuals, especially the elderly aged 65+ (5, 6).

Currently influenza A subtypes H1N1 and H3N2 are circulating among the human population (2, 7), and other subtypes of influenza are currently circulating amongst birds, pigs and even bats (8). Because of this, influenza has proven to be a highly adaptable virus and it is this ability to adapt which makes influenza being the global problem in terms of high death counts and economical losses today.

1.2. A history of influenza A pandemics

In the last century three major pandemics have passed and can be distinguished. All three pandemics have claimed millions of lives and were caused by different subtypes (9, 10). The designation of subtypes to different influenza strains will be explained in chapter 2.1. The Spanish flu pandemic in 1918 alone was estimated to claim 50 million lives (9), and was caused by the influenza H1N1 subtype. Other pandemics such as the Asian flu pandemic which emerged in 1957 and eventually disappeared in 1958 was caused by subtype H2N2 and it is still thought that antigenic counterparts of the human Asian pandemic strain continue to circulate in avian reservoirs (11). The third major pandemic known as the Hong Kong flu emerged in 1968 and lasted to 1969 and was caused by an H3N2

virus. What these pandemics have in common is that they were all caused by major genetic alterations causing massive spread among the naïve population. These previous pandemics have caused damage on a global scale and it is estimated a future pandemic would cause great economic losses which are strongly correlated to the loss of lives (6). Because the recurring influenza pandemics it is only logical that a method providing protection to current and future emerging influenza subtypes must be developed.

1.3. Vaccination remains the best solution to control influenza, though it should be improved

Two methods of protection against influenza are available today, one being the antiviral drugs and the other being vaccination. Antiviral drugs can be divided in 2 groups; the M2 inhibitors such as amantadine and rimantadine, and the second group which are neuraminidase (NA) inhibitors such as zanamivir and oseltamivir. Although antiviral medication provides protection against influenza, immunity is not acquired, and antiviral resistant influenza strains have already been detected (12, 13). For the sake of the focus of this thesis, these antiviral medications will not further be discussed. The most efficient way to prevent mass infection by influenza remains the administration of vaccines (14). Vaccination induces a protective response in the individual and is more suitable in preventing infection spread as well as mediating herd immunity. Because of these reasons vaccination is a method which should be more invested upon. However, as it is known that influenza vaccines only elicit a very narrow response to the hemagglutinin of the virus, future pandemics could still pose a threat.

Indeed, the possibility of a future pandemic of the highly virulent avian influenza H5N1 is a serious cause for concern (15) and infections in humans by the lethal H5N1 have already been observed (16). The problem though, is the uncertainty of the origin of a future pandemic and when it will appear. Moreover even the severity of the disease and the influenza subtype which will cause the pandemic are far from predictable (17). Another problem with current influenza vaccines is the need for annual vaccination to sustain individual and herd immunity as recommended by Advisory Committee on Immunization Practices (18). In conclusion, the previous stated factors necessitate the need for vaccines which induce an immune response capable of neutralizing multiple lineages of influenza A. A future vaccine should thus induce cross-reactive antibodies with a high possibility to neutralize influenza subtypes which have not even emerged yet.

The mechanism to create such an antibody has been investigated with promising results. To understand how this is being done understanding the mechanics in which influenza A works is necessary. In the following chapters the function and structure of HA as well as its variation in genetic structure and preservation will be discussed. Other topics such as obstacles and issues in current vaccination strategies and significant developments in creating a broadly neutralizing antibody will also be addressed. In this paper the main scope remains to look into a method suitable and capable of creating a broadly neutralizing antibody response by means of vaccination will be discussed. It will also be determined if this method can overcome obstacles and shortcomings in current vaccination methods.

2. Hemagglutinin as prime protein

2.1. The subtypes and structure of hemagglutinin

Influenza A expresses three surface proteins; hemagglutinin (HA), neuraminidase (NA) and M2 (a hydrogen ion channel protein integrated in the influenza A membrane). Neuraminidase however is not required in entry, replication, assembly or budding. Its function is to release the virus from the surface of the host cell by destroying the sialic acid, and to counteract the effects of aggregation therefore promoting the spread of influenza virions in vivo (19, 20). Because the main focus of this thesis will be on the HA protein, no further details of mechanics and structure of NA and M2 will be discussed here. HA is the most immunogenic influenza protein and as it is required for binding to the host and entry into the host cell, it is the most important protein on the influenza virus surface membrane.

At least 17 types of HA are known today known as H1 to H17 of which H16 and H17 have been discovered recently in black headed gulls and fruit bats respectively (8, 21). Influenza A subtypes are named after two of the surface proteins expressed on the influenza virion namely HA and NA. For example; the subtype responsible for the 1918 pandemic was called H1N1 because it was the first influenza subtype to be characterized in humans. From that day HA subtypes were classified according to antibody specificity. Today influenza HA subtypes are classified according to molecular structure. Of those 17 HA types, three of them are incorporated in human influenza strains, namely H1, H2 and H3. Today, HA has been extensively researched and it function as well as structure have been well defined (22).

The structure of HA was first elucidated in 1981 by Wiley and Skehel (23). The HA influenza protein is a transmembranal homotrimer attached to the viral surface membrane. HA consists of 2 regions, the globular head and the stalk region. The stalk region consists of 3 homologous monomers of α-helices creating the stalk section of HA (24). Connected to the stalk are three globular heads, each head with the

ability to bind to sialic acids. More precisely, HA can be divided into two parts; HA1 and HA2. HA2 being part of the stalk region and containing the α-helix, while the HA1 subunit extends from the base to top and contains the globular receptor for sialic acids. The globular heads are formed by an antiparallel β-sheet (22, 23). In total, the hemagglutinin protein contains three subunits of HA1 and 3 subunits of HA2 assembled into a fully functional sialic acid receptor that is giving the influenza virion the capability to infect the host.

2.2. Hemagglutinin is essential for the pathway of infection

Hemagglutinin has distinct functions; Firstly HA is tightly packed on the influenza viral membrane and is assembled into rafts which make virus-cell fusion easier (25). When an influenza virion enters a hosts system, HA binds to sialic acids to attach the influenza virion to the host cell membrane. Subsequently, when HA binds to the host cell membrane, the host cell engulfs the virion through a process called endocytosis. H^+-pumps keep pumping H^+ ions into the endosome containing the influenza virion causing a drop in pH. When the pH reaches 5.8 the acidic environment causes HA to undergo conformational changes which allow the host membrane and that of the influenza virion to fuse and thus releasing the contents of the virus into the host cell (22, 26-28). These functions enable influenza to recognize and infect human cells. Thus by blocking the functions of HA, Influenza will be unable to replicate in vivo.

2.3. Antigenic shift and drift cause variation in HA

Certain processes cause changes in HA. Antigenic drift and antigenic shift are the two mechanisms contributing to variation in influenza HA and causes influenza to mutate into other variants and escape neutralization. Antigenic drift is a mechanism in which single mutations can gradually change certain proteins in the virus to escape neutralization by the immune system of the host. The proteins involved in this process are HA and NA, although NA evolves at a slower rate (29). These surface proteins are subject to frequent changes in the amino acid sequence caused by point mutations (30). These mutations can occur any time the DNA of the influenza virus is being replicated and are random (31). Most of the mutations occurring in influenza are silent, meaning they do not change the amino acid sequence. While some mutations do not cause any change in the structure of viral proteins, some mutations can cause a slight change in the structure of the HA protein. This does not mean that those mutations are beneficial to the virus. However, in some cases these point mutations can cause the immune system of the host to fail to recognize the viral protein and thus the virus escapes neutralization. Thus influenza A virions are subject to selection resulting from the immune response of the host and occasionally mutations can lead to immune escape variants.

The second mechanism responsible for changes in influenza proteins is antigenic shift and can occur in two different ways. Antigenic shift results in a new type of Hemagglutinin and is seen when an influenza subtype is transmitted directly from the animal reservoir to the human population. Major changes in the HA protein can cause influenza to be compatible to replicate in humans. Shift also occurs when the HA molecule of influenza A is being replaced by another subtype, and this process has only been detected in influenza A strains. This can occur when a host is infected by two types of influenza strains. The co-circulation of these strains in one host can cause genetic reassortment and is responsible for a new influenza subtype construct (32). Occasionally antigenic shift can cause avian influenza strains to infect humans directly or via indirect pathways and it is this mechanism which is responsible for influenza pandemics as it was seen in pandemics in the past. Both antigenic shift and drift thus contribute to the fact that influenza A is continuously changing its surface receptor causing the creation of immune escape variants. Concluding from this section and as will be explained in next chapters, developing a method which targets more conserved regions in HA instead of targeting the more variable regions of HA to sustain immunity will be necessary and more effective.

As stated previously, antigenic drift occurs because of the selective pressure on influenza virions of the infected host (33). Increased variability in antigenic sites of the globular head lead to the creation of immune escape variants of influenza A. Wiley et al. identified four regions in the globular domain of HA based on the fact that in each of these regions mutations tended to cluster (34). These clusters are called antigenic sites which contain multiple epitopes and are named site Sa, site Sb, Site Ca_1, site Ca_2 and site Cb (35), and it was noted that only 1 amino acid substitution in each immunogenic site was needed to create new epidemic strains between 1968 and 1975 (34). The antigenic sites are mapped to certain regions of the HA influenza protein. Antigenic sites Sa and Sb are located in the upper part of the HA1 subunit of HA (the globular domain), and are separated by a polypeptide loop (35). Because these antigenic sites are in close proximity to another, binding by antibodies could cause steric hindrance and thus competition of antibodies of these epitopes exist (35, 36). Although Wiley et al. defined 4 immunogenic clusters which were expanded to 5 by Caton et al. (34, 35). Some of these antigenic clusters have proven to show differences in immunogenicity, namely that one immunogenic site can show

immunodominance over another (37). This means that the host immune system as shown in the work of Popova et al. tends to select an immunogenic site probably because of the higher neutralizing effect it has (37). This immediately illustrates the current dilemma of immunity to influenza; while the globular domain of HA is immunodominant it is also the area of HA subject to frequent mutations and substitutions in amino acid sequence thus increasing the chance of immune escape variants of influenza.

3. Development of vaccines and obstacles in current vaccine development

3.1. The development of vaccines

It has been over sixty years since the discovery that influenza A virus particles could be mass produced in the allantoic cavity of chicken embryo's (38), and the mass production of influenza particles was an important step in the production of vaccines and development (39). When vaccines were in early development, they still contained impurities which provoked immunological reactions (40). While the latter was considered a lesser disadvantage compared to the advantages of an influenza A vaccine its discovery eventually led to the start of vaccination programmes and the development of better vaccines. Indeed, in subsequent years zonal centrifugation (41), the splitting of Influenza virions and the treatment of vaccines with deoxycholate led to more purified vaccines that provoked less side reactions to the recipient host (42, 43), thus beginning a new era in influenza vaccine development. Nonetheless, an influenza vaccine eliciting protection to multiple influenza subtypes has yet to be developed.

3.2. Current commercially used vaccines and their deficits

Methods of vaccination in the 1960s are still being used today and while they have been adapted and improved they are not capable of ensuring protection to multiple subtypes of influenza. However, today vaccines provoke far less immunogenic reactions to the recipient host compared with the early vaccines. An influenza vaccine today contains 2 strains of influenza A subtypes and 1 strain of the influenza B subtype. These vaccines are carefully composed by the World Health Organization, based on the predictions of future emerging influenza subtypes potentially causing outbreaks. There are several types of vaccines which are used today or are being researched. The first type of vaccine which is being used today contains non-replicating antigens such as split virus or subunit preparations. The split virus vaccine contains virion particles and the subunit vaccine is a vaccine purified for a specific influenza A protein generally containing NA and HA. The idea behind these vaccines is that while the virus is destroyed, the immune system will still be capable of eliminating this virus as a whole by recognizing its surface protein HA. Today split vaccines and subunit vaccines have proven to be efficient in 70-90% of the cases (18). Another type of vaccine, though less common in use, is called cold-adapted live attenuated influenza vaccines (CAIV) and contains the influenza as a whole still having the capability to replicate although it is weakened. CAIVs are often administered intranasally. The virus is cold-adapted meaning its replication is optimal at 25 degrees Celsius. The virus is thus attenuated at normal body temperature and should reduce the possibility of the virus regaining virulence (44). The idea behind CAIV is that the vaccine should resemble a natural infection more closely than that of split or subunit vaccines. CAIVs stimulate local immune response while split and subunit vaccines stimulate systemic immune reactions (45).

Split vaccines and subunit vaccines and CAIVs do have shortcomings. Due to the fact that influenza A is subject to genetic drift and shift, both vaccines have to be constantly updated. Split vaccines and subunit vaccines are only capable of inducing an immune response in the host to the antigens they contain, hence why yearly vaccination is necessary. Another problem which arises is that current vaccines are unable to induce antibodies capable of neutralizing multiple subtypes of influenza A in sufficient amounts. As well as split and subunit vaccines CAIVs has its deficiencies. For example; while CAIVs are attenuated to reduce the virulence, chances are that co-circulation with another influenza subtype may cause genetic reassortment and cause the attenuated virus to (partly) regain virulence or become even more virulent. The latter is illustrated by Scholtissek et al. in mice in which nonneurovirulent or weakly neurovirulent influenza viruses recombined into a lethal variant (46). While it must be noted that Scholtissek's research did not include attenuated influenza virions, the possibility of such a scenario must not be denied. Another problem with CAIVs is that they are not applicable to individuals with impaired immunity. These drawbacks in current vaccines imply the need for a vaccine which overcomes these deficiencies.

3.3. Influenza escapes neutralization via multiple pathways

Influenza escapes the immune system not only via antigenic shift and antigenic drift. And it is shown that while vaccination ensures protection against recurring infections of the homotypic strain, there exists no immunity against novel influenza variants as reviewed by Webster et al. (47). The shortcomings of current vaccines are thus not the only problems in developing the future vaccine.

Numerous obstacles on molecular level are present and will be explained next.

The highly conserved stem region of HA as well as the globular domain contain sites which are glycosylated. Glycosylation can mask HA from being recognized by the hosts immune system and thus forms a problem to neutralize influenza A itself (48-50). This means that the stem region is shielded from antibody interaction preventing neutralization. Based on the observations that many broadly reactive antibodies rely on binding to the stem of HA (51, 52), this could very well be a major obstacle for the human immune system in generating antibodies that have cross-reactive capabilities.

A second obstacle is that of original antigenic sin (OAS) in which antibodies produced in response to an infection closely related to a previously encountered infection are directed to the previous encountered infection. Because the immune system expresses antibodies to the original antigen of exposure, the neutralizing effect tends to be much less effective in consecutive infections related to previously encountered infections than it should be. To emphasize this, in a recent study Kim et al. revisited the effect of OAS. Although Kim et al. noted that in consecutive immunization with inactivated viruses lead to a minimal effect of OAS, they concluded that sequential infection with live mouse-adapted PR8 and FM1 (both a H1N1 subtype) virus in mice severely increased the response of OAS when subsequently infected with the FM1 virus (53). The latter illustrates the dilemma of original antigenic sin; if the influenza A virus is constantly changing while remaining closely related to it preceding strains, possibly future vaccines could be impaired in stimulating the immune system to elicit cross-reactive antibodies. Original antigenic sin appears to be another mechanism in which influenza A succeeds to escape the host immunity.

3.4. Immunodominance of HA1 hinders induction of cross-reactive antibodies

Another key problem which affects the effectiveness of vaccines and the induction of broadly reacting antibodies is that of immunodominance of the globular domain of HA; HA1. In section 2.3 it was noted that the globule of HA of the H1 subtype consists 5 immunogenic sites Sa, Sb, Ca_1, Ca_2 and site Cb (35). The immune system specifically targets HA possibly because of the immunodominance in one or more of these 5 antigenic sites of the viral HA. Indeed, in a recent experiment conducted by Popova et al. while investigating 2 antigenic sites on the HA1 subunit, it was found that the majority of the antibodies produced to the H3 Hemagluttinin were directed to one certain epitope which would be the immunodominant one (37). Because the HA1 is immunodominant, the attention of the immune system is pulled away from the stem region which is conserved (54). The importance of the conserved stem region of HA in respect to cross-reactive antibodies will be addressed in the following chapter.

4. Lessons learned from the 2009 pandemic

4.1. Pre-existing immunity in the elderly

Not too long ago a minor pandemic was triggered by a variant of H1N1 in 2009 called the swine flu or Mexican flu. It was thought as with every influenza infection to be more virulent in the elderly while this appeared not to be the case as the 2009 influenza expressed an unusual infection pattern affecting the younger individuals more (55). This observation was not expected and it implied that these elder individuals should somehow have had immunity to the 2009 H1N1 strain before contacting this virus. This pre-existing immunity exists in individuals exposed to the 1918 H1N1 and to the 2009 Influenza H1N1 strain, and appeared indeed to be age related (23, 56-58). The question is, do these antibodies show any cross-reactivity or is the 2009 H1N1 influenza strain similar to the 1918 H1N1 virus therefore reactivating memory B-cells in the elderly and neutralizing the virus while the naïve population is susceptible to infection by the 2009 H1N1 influenza strain?

In recent work by Hancock et al. it was described that individuals born before 1950 showed more pre-existing immunity to the 2009 H1N1 virus than individuals born after 1980 (59). Also conducted in their research, Hancock et al. concluded that the seasonal vaccine elicited little cross-reactive antibodies in children or adults (59). This raises the question how adults born before 1950 came to acquire cross reactive antibodies if they were not induced by the seasonal vaccine. The fact could be due to conserved patches in epitopes in the 2009 H1N1 virus to the H1N1 virus circulating among humans before 1950 (56). And some believe pre-existing immunity in the elderly to the 2009 H1N1 influenza was because it structurally hardly differed from the 1918 H1N1 virus (60). Although some similarities exist, the CDC has noted in a weekly report that the H1N1 2009 strain had characteristics never seen before in humans, and stated their concern of an outbreak because of possible dissimilarities between the 2009 H1N1 influenza virus and the H1N1 seasonal human influenza A (61). Garten et al. investigated the genetic past of the H1N1 2009 influenza and suggested that it might have been circulation amongst swine herds undetected (62). While the last statement implies a tighter surveillance for influenza in swine herds, it seems that opinions about the origin of the 2009 H1N1 influenza strain are divergent. However, it is

important to note that there were antibodies discovered in the elderly which bound to the stem region of HA.

4.2. Cross-reactivity is related to the stalk region of hemagglutinin

While most vaccines elicit immune responses to the globular domain of hemagglutinin, it has been noted that some individuals express neutralizing antibodies to the stalk region of HA capable of neutralizing heterosubtypic influenza strains (51, 58, 59, 63, 64). As shown in past research, the stalk of HA contains a region which is conserved (54). Using this property, one could try to trick the immune system into creating antibodies to the stalk region as to create a broadly reactive immune response. A prerequisite of this immune response is that it has to have a neutralizing response on influenza itself and preferably being the major component of the immune response itself. Indeed, recent research showed that mice injected with HA lacking the globular head (while still including element of HA1 supporting the stalk region) contributed to the induction of broadly reacting antibodies capable of neutralizing influenza virus (65). Although it must be noted that immunization with whole HA provided much better immunity probably because the globular domain is more immunogenic.

Continuing from the case of the elder showing more protection to the 2009 H1N1 virus than expected, it is important to know how these cross-reactive antibodies in the elderly came to exist. It has been noted that most cross-reactive antibodies predominantly bind to the stalk region of HA and inhibit pH induced conformational changes (51, 52). Knowing this Wrammert et al. proposed that pre-existing immunity in the elderly was due to the fact that memory B-cells became reactivated by the 2009 H1N1 influenza A strain (63). This mechanism is similar (or equal to) original antigenic sin and understanding its mechanism could provide beneficial in future vaccine development. On the contrary, based on the results of Xu et al. conserved patches in epitopes on the globular domain of HA are also a factor contributing to cross reactivity in the elderly (56). Possible both findings are correct. Even so it is important to focus the attention on conserved regions in the stalk domain since it is this region which is far less susceptible to changes in amino acid sequence than the globular domain.

4.3. A possible mechanism for the 2009 H1N1 protection in the elderly

In a recent study a possible explanation for pre-existing immunity and the cross reactivity among the elderly has been proposed in an experimental mice model (50). Mice immunized with the 1918 H1N1 strain appeared to be protected to a subsequent 2009 H1N1 influenza strain, while mice immunized with a 1999 H1N1 subtype failed to neutralize the 2009 H1N1 subtype. Considering the evolutionary gap between the 1999 and 1918 in respect to the 2009 subtype, this is an unexpected observation. A possible explanation which arises from these observations as proposed by the investigators is that a certain conserved epitope in the 1918 H1N1 subtype became glycosylated shielding it from recognition by the host immune system (50). In this way the new population would be unable to detect this epitope and produce antibodies against it, while the older population still would have this ability. These findings illustrate the importance of glycosylated HA, and could prove to be valuable in constructing more advanced vaccines eliciting cross-protection as suggested (66).

5. A promising approach to cross-protective vaccines

5.1. The solution to a cross-protective vaccine

Thus far the significance of the HA protein was described as well as existing problems which make creating a cross-protective immune response from a vaccine difficult. As previously described, antigenic shift and drift can cause influenza to infect species of different kinds and gradually change influenza respectively. Original antigenic sin can prevent the immune response to adapt to a new influenza subtype challenge, and glycosylation shields HA from being recognized. The question remains how these obstacles can be overcome or bypassed. Different methods can be used to elicit cross-protection to multiple influenza A strains (25, 67). Prime-boost vaccination appears to be the best method to elicit cross-protective antibodies directed to the stem region of HA. Also important is finding a method to break original antigenic sin as it hinders vaccine efficiency. The ultimate vaccine should elicit an immune response which complies with 3 criteria; first it has to produce an antibody that is cross-protective to many influenza A strains, secondly the antibody created has to bind to conserved regions of HA and lastly these cross-reactive antibodies have to be the major component of the immune response and thus neutralize the virus. Knowing these criteria, a possible candidate would be an antibody that binds to the stem region of HA. This is because this region is conserved and binding with an antibody prevents fusion of the virion with the host cell (51, 52). Numerous cross-reactive antibodies have already been identified such as C179, CR6261, CR6262 and F10, and have all been reported to bind to the conserved regions in the stem of HA (51, 52, 63, 68, 69). In this chapter prime-boosting is discussed which succeeds to elicit stem-reactive antibodies capable of neutralizing

lethal influenza infections. Also the challenging obstacle of OAS will be addressed.

5.2. Overcoming original antigenic sin

Although the breaking of OAS is not about creating an antibody that is cross-protective, it is a problem in modern vaccination programs and has been observed in humans (70). As described in section 3.3 OAS is the mechanism in which a second related influenza infection induces the production of antibodies to a related virus encountered in the past reducing the effectiveness of the immune response. The importance of breaking original antigenic sin is illustrated by the fact that the majority of the individuals today have been inoculated to influenza A, and that there is no effective way of telling whether future vaccines eliciting cross-protective antibodies will have an effect in already-immunized individuals. Fortunately a method has been proposed to counteract original antigenic sin.

In a recent study Kim et al. demonstrated that immunization with dendritic cell-activating adjuvants could effectively diminish the effects of OAS (71). A possible explanation for this finding is that antigen presentation is focused on activated dendritic cells instead of memory B cells using squalene-based adjuvants. By doing so the recruiting of naïve B cells is more likely to take place instead of reactivating the memory B cells as stated by the investigators (71). A second method to counter OAS is to repeat the exposure with the same antigen to the patient. The data shown also by Kim et al. proved to expand memory cells and effectively decrease the effects of OAS (71). These methods could prove useful for individuals in which a vaccine appears to be less effective than expected and where original antigenic sin appears to be the underlying cause.

5.3. Prime-boost strategies for cross-protection

New strategies involving priming the immune system with DNA vaccines and subsequently vaccinating with an appropriate heterologous boost elicit cross-protection to a variety of influenza strains and have already proven to provide protection to lethal challenge in an experimental mice model through eliciting antibodies which are stem-specific (67). The thought behind prime-boosting is that the more conserved regions of HA will be the target of the immune response instead of the globular domain of HA. The DNA vaccine itself uses solely the DNA encoded for certain influenza A protein in plasmids and has proven to be beneficial in priming the immune response for subsequent boosting by conventional vaccination (72). Prime-boost strategies thus take advantage of the this ability of DNA vaccines by priming the immune system by the administration of HA DNA containing plasmids. This technique has been proven to be effective (if not more effective) in mice (73). Research shows that two injections are needed separated by a certain time span, and although single dose injections do neutralize influenza A subtypes and even show some cross-protection, they do not produce broadly reactive antibodies in detectable amounts (74). In contrast, DNA prime boost vaccination shows superiority in neutralizing influenza over single dose injections (73) and antibodies elicited in the immune response by DNA prime-boosting bind to conserved regions of the stem-area of HA (67). Because of the fact that prime-boost vaccination elicits more broadly neutralizing antibodies, this method could prove useful to be effective to future emerging influenza strains. In recent work it was indeed shown that mice immunized with H1 HA DNA/vaccine neutralized homologous as well as heterologous strains and even showed cross-protection to H2N2 and H5N1 influenza subtypes (67). Also concluded in this research was that the cross-protective antibodies elicited by the DNA prime/boost vaccination were specific to the stalk region of HA emphasizing the implications of prime-boosting vaccination (67).

A second method to boost memory B-cells to produce cross-protective antibodies directed to the stem region of HA which does not use the priming ability of DNA vaccines was conducted in an experiment involving individuals pre-vaccinated with a H5N3 influenza vaccine containing the adjuvant MF59. It was found that when subsequently boosting these individuals with an MF59 adjuvated H5N1 vaccine these individuals expressed cross-protection to multiple clades of the H5N1 subtype while also showing a better immunological response than individuals which received the boost lacking the adjuvant MF59 (75). It was also discovered that the antibodies which were produced bound to the stem region of HA and did not have the preference of binding to the original antigen of exposure (75). Because of this the investigators conclude that there should be no concerns about original antigenic sin when using their method of immunization. A possible explanation for this could be due to the fact that squalene based adjuvants such an MF59 were used to support boosting the immune response. As showed by Kim et al. the use of these adjuvants can significantly reduce the effects of original antigenic sin (71), hence why the presence of OAS was not observed.

While prime-boost immunization has been experimentally tested in humans while succeeding in generating stem-reactive antibodies (76), it has yet to be accepted to be used commercially. Concluding from this section, these prime boost strategies show promising results to fight a future influenza pandemic and could well be the next generation vaccines.

6. Conclusion:

Prime-boost strategies can induce antibodies directed to the stem region of hemagglutinin of influenza A. And antibodies produced via prime-boost vaccination possess cross-reactive capabilities. Influenza cannot induce replication without its most important protein hemagglutinin and will be effectively neutralized when HA is bound to antibodies. Thus it is this protein which must be the target of the immune system, especially the stem region as it appeared to provoke a higher degree of cross-protection (51, 52, 63). Prime-boost vaccination already proves to be a good candidate in fulfilling this requirement and the enormous annual cost as well as the annual death toll of influenza A associated deaths and the threat of a possible H5N1 pandemic in near future definitely justifies investments in extensively researching these methods.

Concluding from previous sections we are still some time away from creating a commercially approved cross-protective influenza A vaccine. While many problems still have to be overcome the solutions lie within reach and current research already succeeded in generating cross-protection by prime-boost vaccination (67, 73, 76). DNA prime-boost vaccination thus seems to be excellent methods to induce cross-reactive antibodies thus potentially solving the threat of future pandemics. In addition, vaccine efficiency can be assured with the addition of adjuvants to diminish effects of OAS and improve the immune response as previously discussed. Also contributing to vaccine efficiency is the timespan in which an individual expresses the cross-protective antibodies. If a vaccine eliciting a cross-protective immune response were to be efficient, immunity acquired has to be sustained for a long period of time, preferably a lifetime. A common opinion is that influenza vaccination does create a protective immune response but that this immunity diminishes within a year. This however appears not to be the case. In elder individuals neutralizing antibodies have been found against the 1918 H1N1 influenza subtypes aged over 90 years (64). While this has yet to be determined for prime-boost vaccination, this thus clearly states that antibodies do have the capability to circulate for many decades even after the influenza infection has been neutralized. Hence it is possible for a future vaccine to not only induce cross-protection to multiple influenza subtypes but also to sustain this immunity for decades.

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