The prospects of cross-reactive T cell peptide vaccines as an effective universal influenza A combatant

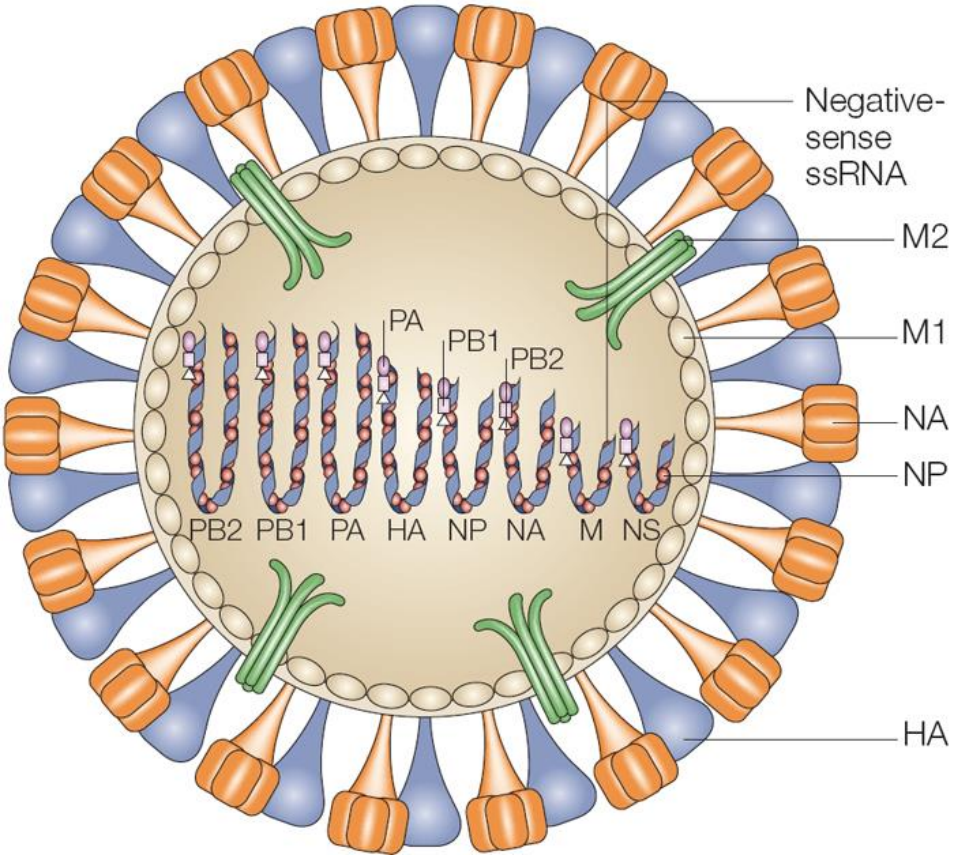

Fig. 1: The influenza A virus (Taisuke Horimoto & Kawaoka, 2005).

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Abstract

Vaccines are the most cost-effective way of preventing influenza A infections. However, most of the current vaccines are based on eliciting an antibody response against the mutation-sensitive HA and NA surface proteins. These proteins differ between strains of influenza and therefore current vaccines cannot elicit a cross-reactive immune response. Peptide vaccines provide a promising way to combat this issue. These vaccines use peptides from conserved regions of the influenza virus and elicit a cross-reactive T cell response over multiple strains of influenza A. Furthermore, this helps in inducing pre-existing immunity to prevent a new emerging influenza strain from causing widespread infections. Also, peptide vaccines can be produced more easily than current vaccines.

However, there are multiple challenges in the development of peptide vaccines. Foremost they need to contain conserved peptides. but these peptides can differ in their HLA restriction. Conserved peptides can be found in proteins with a low frequency of mutations like PB1, M1, M2 and NP. For the HLA restriction, peptides can be used that target the globally most frequent HLA types. There are already some peptide vaccines in development that use this approach called M-001, FLU-v and Fp-01.1. These vaccines show to be safe, well tolerated and can induce a cellular immune response. However, populations across the globe differ in which HLA type is most abundant. A new approach that could improve the binding of peptides to these specific HLA types is chemically altering peptides at anchor points. This can help the peptide vaccines in eliciting a stronger immune response at different areas around the globe. Influenza A peptide vaccines should also include peptides for both CD4+ and CD8+ T cells to induce an effective cellular response. Moreover, vaccines should contain peptides of multiple proteins as the virus is less likely to escape the immune response and the virus can be targeted at different stages of infection.

When these different conditions are met, peptide vaccines can prove to be a very effective combatant of influenza A virus infections. The chemical altering of peptides could provide an effective tool in the further refinement and improvement of the vaccines.

Contents

Introduction

The influenza virus causes millions of infections worldwide, leading to high treatment costs and deaths in susceptible groups like the elderly and children (WHO, 2015). Vaccines are the most (cost-)effective tool for the prevention of infections and are thus the main method used to prevent influenza disease (Frieden et al., 2011). Two types of influenza, influenza A and B, circulate among humans. This thesis will focus on influenza A, which can cause seasonal infections of influenza but more importantly is the major cause of pandemics of influenza. Influenza A is subdivided into different strains by their distinct hemagglutinin (HA) and neuraminidase (NA) surface proteins (Dolin, 2011; WHO, 2015). These surface proteins are known to change due to antigenic drift and shift (T. Horimoto & Kawaoka, 2001). The main problem with current influenza A vaccines is that they are in general not able to elicit a cross-reactive immune response between different influenza strains. The vaccines are aimed at eliciting an antibody response to the two variable surface proteins. The first consequence of this is that every year a new seasonal vaccine needs to be formulated and produced, leading to higher production costs. Furthermore, it means that for a new emerging strain there is little to no pre-existing immunity which in turn results in high risks of infection and even pandemic spread (Sridhar, 2016).

One kind of vaccine that can be used to solve these problems are peptide vaccines. Peptide vaccines, in contrast to conventional vaccines, contain only parts of proteins from the influenza A virus. These so-called peptides contain epitopes of conserved regions of the virus, regions that are not susceptible to mutations and therefore are the same among the different strains of influenza A. Peptide vaccines can thus elicit an immune response to multiple strains. This induced cross reactivity of the vaccines is the major benefit over current vaccines because only one vaccine should be needed for multiple strains (De Groot et al., 2014). Moreover, peptide vaccines can be synthetically produced rather easily, in contrast to current seasonal vaccines (Huber at al., 2014).

Peptides can target both the humoral immune system using B cell epitopes, or target the cellular immune response by using T cell epitopes. The T cell peptide vaccines are taken up in a dendritic cell (DC) that processes the peptides and presents the epitopes they contain on their major histocompatibility complex-I (MHC-I) and MHC-II molecules (Abbas et al., 2014). These epitopes are the part of the peptide that get recognized by the immune system. The epitopes can then activate T cells. Some of the activated T cells will remain as memory T cells which stay in the body for years and are already primed for the specific epitope. This leads to long term immunity against an actual infection with influenza as the virus gets recognized immediately and cleared effectively. B cell peptide vaccines can be directly recognized by the B cells, meaning no processing is needed by DCs (Abbas et al., 2014).

The function of the B cell response in the humoral immunity is producing antibodies against a specific protein of a pathogen, which helps in the neutralization of the pathogen and its clearance from the body. The cellular immune response can be divided into two types of T cells; CD4+ and CD8+ T cells. The function of CD4+ cells (also known as T helper cells) is both assisting in the maturation of B cells into antibody producing plasma cells and memory B cells, and the activation of the CD8+ cells and macrophages. CD8+ cells (also known as cytotoxic T cells) are vital for the specific killing of virus infected cells (Abbas et al., 2014). This review will limit itself to the cellular immunity, therefore focussing on peptides directed at a T cell response.

When the T cell epitopes are presented on the MHC molecules they can activate T cells, which elicits a specific cellular response against these epitopes. This is crucial for effective clearance of the virus (Huber et al., 2014). The MHC molecules I and II differ in their function and structure. MHC-I molecules present their epitopes to CD8+ cells and MHC-II molecules present their epitopes to CD4+ cells. The molecules also differ in the way antigens are processed. For MHC-I, virus proteins and peptides from the cytosol are degraded to epitopes by proteasomes and then presented on the MHC-I molecule. For MHC-II, antigens taken from outside the cell are presented on the molecule. The structure of the MHC molecules differs too as MHC-I molecules can present antigens of 8 to 11 amino acids in length. MHC-II can present antigens of 10 up to 30 amino acids, with an optimum of 16 (Abbas et al., 2014). First off, the differences between the two MHC types have implications for the way the peptide vaccines are administered to the DC cell. For binding MHC-I, peptides should enter the cytosol. Whereas for MHC-II, peptides can be taken from outside the cell. More importantly however, the differences in the structure of MHC-I and MHC-II means certain peptides might only be able to bind to MHC-I or MHC-II and therefore only elicit a CD4+ or CD8+ T cell response.

Wilkinson et al. (2012) studied the importance of pre-existing influenza CD4+ T cells for the protection against influenza infection. The study showed that a higher amount of pre-existing CD4+ T cells was associated with lower virus shedding and less severe illness. Sridhar et al. (2013) showed the importance of the specific CD8+ T cell response during an influenza infection. It showed that people with higher amounts of pre-existing T cells against conserved CD8 epitopes also developed less severe illness after infection with the H1N1 influenza virus. As peptide vaccines are aimed at eliciting this specific T cell response, in contrast to current influenza vaccines, peptide vaccines could prove to be a more efficient combatant of influenza infections.

Peptide vaccines do however have their challenges as well. The main issue encountered in developing peptide vaccines is the choice of peptide to use. First of all, peptides to be used must be conserved over different influenza A strains. However, peptides can differ in the strength of immune response they elicit, for example based on their abundancy in the virus. (Grant et al., 2013). Therefore, a consensus of which peptides to use in a vaccine is hard to make. Moreover MHC molecules, known in humans as human leukocyte antigen (HLA) molecules, can differ between individuals. There are a lot of different HLA alleles, with different affinity to epitopes (Abbas et al., 2014). The consequence is that epitopes of peptide vaccines can sometimes not effectively bind to the HLA molecules of certain individuals, the epitopes are HLA-restricted. This is a major obstacle in making a universal vaccine that works for all individuals.

As illustrated, there are a lot of advantages but also challenges to be found with peptide vaccines. This thesis first aims to examine the peptides that can be used in peptide vaccines of influenza A. It will then discuss how the characteristics of these peptides can influence the effectiveness of peptide vaccines and how some characteristics can be changed to improve the vaccines.

Proteins to target for an influenza A peptide vaccine

The first issue with peptide vaccines is the subject of peptide selection. For this, a crucial element is which regions of the influenza A virus are well conserved. The influenza A virus consists of many different proteins. The following section will discuss some parts of the influenza A virus that could be targets for use in peptide vaccines.

HA and NA

The envelope of the virus consists of both HA and NA. HA cleaves into subunits HA1 and HA2, which helps the virus to fuse with the host cell (Cross et al., 2009; Worch, 2014). The function of NA is mediating the release of new virus particles from infected cells. Epitopes of these two proteins are known to mutate easily and are different between influenza strains (T. Horimoto & Kawaoka, 2001; Tewawong et al., 2015). These proteins are therefore in general not suitable targets for peptide vaccines.

PB1 and M1

Possible good candidates for a peptide vaccine were studied by Assarsson et al. (2008). This study focussed on the proteins polymerase basic 1 (PB1) and matrix protein 1 (M1). PB1 is a polymerase of influenza. Its function is the replication and transcription of the viral RNA (Fodor, 2013). The protein M1 plays a pivotal role in stabilizing the HA and NA on the surface (Sharma et al., 2001). Both PB1 and M1 proteins have previously been shown to be well conserved and therefore associated with cross-reactivity between different influenza strains (Lee et al., 2008). The study by Assarsson et al. (2008) studied both the CD4+ and CD8+ T cell response by using six types of HLA class I molecules that are common across the globe, and an HLA class II (HLA-DR) type that encompasses many common HLA-DR alleles. This study again showed that both PB1 and M1 peptides contain well-conserved

epitopes and that the peptides are major targets for an effective CD4+ and CD8+ T cell response.

M2

The other matrix protein, M2, was studied by Fiers et al. (2004). M2 is a protein that forms an ion channel, helping in the release of virions from infected cells. The protein M2 is scarcely present on the surface of the virus itself but is expressed on cells infected with influenza. M2 has an internal domain for anchorage in the membrane of the infected cells and it has an external domain, called M2e (Fiers et al., 2004; Lamb et al., 1985). Fiers et al. (2004) first studied how well conserved this M2e-sequence was by compiling reported human influenza M2e-sequences. M2e showed to be very well conserved, only displaying alternative amino acids at two positions.

The next step in the study was testing whether or not a vaccine based on this M2e could induce immunity. The results of the study showed that their vaccine provided long lasting, protective immunity against all tested influenza A strains, indicating a good cross-reactive response. The peptide mainly induced an antibody mediated immune response with a B cell epitope, but a significant cellular response can also be induced especially by activation of CD4+ T cells (Pejoski et al., 2010). The study by Fiers et al. (2004) ends with a possible outlook for the M2e protein to be used for CD8+ T cell epitopes, indicating the possibilities for this protein to be used in other peptide vaccines.

NP

The last major protein to target is the nucleoprotein (NP). Viruses that contain single-stranded RNA, like influenza, encode a single-strand RNA-binding protein called NP. Its function is key in effective RNA transcription and replication for the virus (Portela & Digard, 2002; Turrell et al., 2013). Grant et al. (2013) studied which proteins of the influenza A virus elicit the highest CD8+ T cell immune response, e.g. which proteins are the most immunodominant. The study focussed on individuals expressing different HLA types then HLA-A2 to avoid a previously identified immunodominant response to the M1 mentioned earlier. The research showed that NP induced the largest response of CD8+ T cells compared to other proteins (like M2). This might be due to the high abundance of the NP protein found in the virions of influenza A. The NP was however found to be less conserved than the earlier mentioned M1.

These studies show that multiple proteins of influenza A are well conserved and elicit effective T cell responses. Therefore, peptides of these proteins could potentially be used in the development of a universally working influenza A peptide vaccine.

HLA diversity between individuals

The next thing that is important for developing a universally working vaccine is a clear understanding of the differences in HLA molecules between individuals. There are a lot of distinct alleles that code for HLA molecules. Each individual has three types of HLA class I genes: HLA-A, HLA-B and HLA-C. Next to that, everyone has three HLA class II genes: HLA-DP, HLA-DQ and HLA-DR. There are over 5000 different HLA alleles of these genes

all with their different affinity to antigens, in this case the epitopes of the peptide vaccines. (Abbas et al., 2014). The diversity in HLA types leads to problems with making peptide vaccines universally effective as they cannot effectively bind to every HLA type, they are HLA-restricted.

HLA alleles can be clustered together based on their overlapping peptide repertoires. A study by Sidney et al. (2008) showed that the most commonly found subtypes worldwide for HLA class I, the HLA for CD8+ T cells, are HLA-A1, -A2, -A3, -A24, -B7 and -B44. Peptides and epitopes HLA-restricted to these dominant HLA subtypes have been discussed in a study by Alexander et al. (2010). This research studied protein sequences from influenza A and evaluated the most conserved sequences that bind to the globally most frequent HLA-A and HLA-B types. The research found 25 potential inducers of specific CD8+ T cell response.

However, looking at different populations around the globe, abundancy of alleles still differs between populations. For example, in African populations HLA-A3 and -A6 are more dominant than in European individuals and in the European population HLA-A2 is the most frequently found allele. Within a population, frequencies are however mostly consistent (Gonzalez-Galarza et al., 2011; Gourraud et al., 2014).

This shows that different populations around the world differ in which HLA molecules are most frequent. Therefore, it is vital to know which HLA molecules are most common in an area to know whether or not the peptide can effectively bind to them and thus work effectively. Peptide vaccines can then be focussed specifically on the most frequent HLA molecule to target the biggest group of people in an area. This does mean that for different areas around the globe it could be needed to develop other peptide vaccines that are HLA-restricted to the specific HLA molecules in that area. This also does not solve the problem of people with rare HLA alleles as vaccines are then not specifically made for them and therefore might not effectively bind and be presented to T cells.

Chemically altering peptides for improved HLA binding

A promising approach for tackling this problem is chemically altering peptides that are suitable for a peptide vaccine. The affinity of a peptide for an HLA molecule is determined by its shape, size and electrostatic interactions (Silver et al., 1992). The specific binding of a processed peptide depends on the positively charged N terminus and the negatively charged C terminus of the peptide (Abbas et al., 2014). For example, HLA-A3 has a preference for long positively charged residues on the C-terminus of peptides whereas HLA-A2 prefers long hydrophobic residues (McMahon et al., 2011). To chemically change a peptide to increase its affinity, modifications could therefore be performed at positions near this N and C-terminus.

A study by Schumacher et al. (2014) focussed on the commonly found HLA class I (MHC-I) subtype HLA-A2 and how several influenza A peptides restricted to this HLA type could be further improved in terms of their binding levels. Peptides with already high binding affinity and peptides with moderate binding affinity to the HLA-A2 molecule were used. The study tried to substitute amino acids at anchoring points close to the N and C-terminus, making chemically enhanced altered peptide ligands (CPLs). Proteogenic amino acids (amino acids that are translationally incorporated into proteins) and nonproteogenic amino acids (amino

acids that are not encoded for by humans) were used (Ambrogelly et al., 2007). Both conditions lead to enhanced affinity of the HLA molecule to the peptides used, but introduction of nonproteogenic amino acids was the most effective. The increase in affinity led to a stronger and longer induction of T cell activation.

The results of this study are comparable with a more recent study by Huber et al. (2016). This study first focussed on peptides for HLA-A2, by introducing the more effective nonproteogenic type amino acids as discussed by Schumacher et al. (2014). The binding groove of the HLA-A2 molecule prefers long hydrophobic residues and therefore amino acids with hydrophobic side chains, e.g. norleucine and norvaline, were introduced. The CPLs were again able to improve the T cell responses. Peptides that normally have a low affinity for the HLA molecule and therefore induce a lower T cell response were improved significantly. Besides the HLA-A2 allele the HLA-A3 allele was also studied to find out if these modifications could be extended to other alleles, which was the case.

These two studies show that it is possible to take known peptides for specific alleles and increase their binding affinity to the HLA molecule. This provides an important tool for increasing the T cell response induced by a peptide vaccine and thereby the effectiveness of T cell peptide vaccines. Moreover, this could also help improving peptide vaccines for people with less frequent HLA types as the binding affinity of the peptides can be improved by studying the characteristics of the HLA molecule binding groove.

However, Huber et al. (2016) mentioned the fact that modifications need to be performed carefully. Too many substitutions might also affect the recognition by the T cell receptor to which the peptide is presented as the amino acid chain is changed. In order to limit the consequences these modifications have on the recognition by T cells, a maximum of two changed amino acids was used. Another thing to keep in mind is that changes in the amino acids at the anchor position might still influence the structure of the peptide as a whole. This could also affect the recognition of the peptide by T cells (Sharma et al., 2001). Therefore, when developing new, better binding peptide vaccines by chemically modifying anchor points care must be taken to analyse if the T cell response is still effective.

Current development of influenza A peptide vaccines

In the following section, several vaccines that are currently in the process of being tested will be discussed to show how current insights about conserved proteins and peptide HLA restriction can be applied to the actual development of vaccines.

M-001

The vaccine that is currently furthest in the process of development is Multimeric-001 (M-001). M-001 is a peptide-based vaccine in the form of a synthetic recombinant protein. This synthetic protein is made up of multiple conserved epitopes from peptides of influenza A, but also of the influenza B virus. It therefore targets both seasonal infections caused by influenza A and B and the pandemic infections caused by influenza A. The vaccine uses in total 9 B- and T-cell epitopes, coming from HA, NP and M1 which have been mentioned earlier (Atsmon et al., 2014; van Doorn et al., 2017).

The vaccine has already gone through a phase IIa trial where the safety and tolerance was tested in an elderly population (ages 65 years and older). The vaccine was found to be safe and well tolerated. It also showed to elicit influenza specific T cell responses and did so across multiple strains of influenza. The vaccine was also tested as a primer to an HA-based influenza vaccine, this showed increased elevations in HA antibodies (Atsmon et al., 2014). However, the sample size used in the trial was in many cases too small to draw actual significant statistical conclusions, mainly in the case of using M-001 as a primer to an HA-based vaccine. Therefore, in order to understand the actual effectiveness of the vaccine, a larger scale research is needed. The vaccine is currently being tested in a phase IIb trial to again study its safety and focus more on its immunogenicity, this time in a large group of healthy individuals of 18 to 60 years of age. Both the effects as a standalone vaccine and as a primer will be studied (van Doorn et al., 2017)

The cell mediated immunity and the elevations of HA antibody levels as seen in the phase IIa trial show the potential of M-001 to be used as a universal working influenza vaccine. However, the phase IIb still has to show whether these effects are indeed consistent and significant.

FLU-v

A second peptide vaccine that has already gone through some testing is FLU-v. This vaccine contains several peptides of conserved regions of NP, M1 and M2 proteins. The phase 1a trial used both FLU-v with and without an adjuvant. The trial showed that FLU-v is safe and well tolerated and showed to induce cellular immunity with and without the adjuvant. No significant antibody response was elicited as the vaccine specifically targets the cellular immune response (Pleguezuelos et al., 2012).

A phase 1b trial again tested the capacity of the vaccine to induce T cell responses to the influenza virus in humans. Volunteers received either a placebo containing only the adjuvant or the vaccine plus adjuvant. The groups were then challenged with live influenza A virus (Pleguezuelos et al., 2015). Questions can be raised at the ethics of such a treatment, especially for the group that was administered only the placebo. The people did however apply voluntarily. The results of the study were said to be consistent with the phase 1a trial results; the vaccine was found to be safe and well tolerated and increased cellular immunity, correlating with decreased virus shedding and less severe symptoms (Pleguezuelos et al., 2015). However, the results of the study actually showed that in the group of volunteers who were administered the FLU-v vaccine most volunteers only produced a weak immune response. In some cases, the placebo group even showed lower virus shedding and symptom scores than the FLU-v group. Only the few cases of strong immune responses were associated with decreased virus shedding and less severe symptoms. The dose used in this trial (500 μg) was not enough to induce these strong immune responses consistently. Therefore, a general statement about the capability of the vaccine to induce a strong immune response cannot yet be made. Further research is needed to elucidate whether or not higher doses of FLU-v are able to result in more consistent and effective immune responses.

Fp-01.1

Another vaccine that is currently in testing is called Fp-01.1. This vaccine specifically targets influenza A and consists of a total of 6 well-conserved peptides containing multiple CD4+ and CD8+ T cell epitopes. The used peptides come from NP, M1, M2 and PB. Sequences of these proteins were selected on their HLA restriction, sequences that were chosen contained

binding motifs that target the globally most present HLA class I and II molecules (Francis et al., 2015). The study by Francis et al. (2015) tested the vaccine for its safety and immunogenicity in a phase 1 trial in a group of healthy adults. Fp-01.1 was found to be a safe and well-tolerated vaccine. All of the six peptides induced an immune response of both CD4+ and CD8+ T cells. The vaccine also showed that the T cells activated after vaccine administration were cross-reactive among the multiple types of H1N1 and H3N2. This is important for Fp-01.1 to induce a broad protection and it means the vaccine doesn't need to be changed for new emerging strains of influenza. The phase 1 trial shows the potential of Fp-01.1 as an effective vaccine for a broad range of different influenza strains.

The vaccines mentioned here show that the use of peptide vaccines against influenza A is currently being tested and in general shows promising results, but more extensive research is needed. The vaccines use multiple peptides of various proteins and target both the CD4+ and CD8+ T cell responses. The vaccines mainly use the method of targeting the most common HLA types over the globe by choosing sequences that are restricted to these HLA types. This is in order to provide a broad effectiveness and it aims to provide a universally applicable influenza peptide vaccine.

Discussion

T cell peptide vaccines are a promising new combatant of the influenza A virus. The peptide vaccines are taken from conserved regions of specific proteins; mainly PB1, M1, M2 and NP. Because the sequences are conserved, the peptides can provide a cross-reactive T cell response over multiple strains giving the possibility of creating a universally working vaccine for influenza A. Moreover, the vaccine-induced immune response provides pre-existing immunity to protect against newly emerging influenza A strains. This counters the need for new vaccines to be produced every time a new strain emerges, leading to a higher cost-effectiveness of the vaccines. The production of peptide vaccines is also a simple and scalable process, in contrast to current vaccines. For the conventional influenza A vaccines, development of a new vaccine can take over 30 weeks (Jin & Chen, 2014). The peptide-based vaccine M-001 however, is produced in *Escherichia coli* and takes only 6 to 8 weeks in total. It can be manufactured year round which means that producing the vaccine can also be planned to coincide with differences in market demands at different points in time (van Doorn et al., 2017).

Three major vaccines are currently in development; M-001, FLU-v and Fp-01.1. These vaccines show to be safe to use and mostly show a promising induction of T cell response in testing. However, further research has to show if the induction of this immune response is indeed effective and consistent. The vaccines use a combination of peptides that are HLA class I restricted and peptides that are HLA class II restricted to induce both a CD4+ and CD8+ T cell response. Huber et al. (2014) show that a combination of CD4+ and CD8+ T cells in response to a viral infection is vital to effectively clear a virus. The CD8+ T cells are required for killing of the infected cells and therefore removing the virus from the body, but to do this effectively the CD4+ T cells play an important role. The CD4+ T cells help in the

activation and growth of the CD8+ T cells. Moreover, CD4+ T cells can also induce the humoral response through CD4+ T cell induced maturation of B cells (Abbas et al., 2014). To induce simultaneous activation of the T cells, vaccines therefore need to include peptides for both CD4+ and CD8+ T cells. A different interesting approach is looking for peptides that are HLA class I and HLA class II double restricted. Pedersen et al. (2016) studied this method by testing eight influenza A derived peptides that should bind to both HLA-A2 (HLA class I) and HLA-DRB1 (HLA class II). Results showed that 4 of the 8 peptides did indeed bind both classes of HLA and were therefore double restricted. These peptides elicited both CD4+ and CD8+ T cell responses against the same peptide epitope. This study by Pedersen et al. (2016) shows a potential area of interest for peptide vaccines that simultaneously induce CD4+ and CD8+ T cell responses by using only one peptide. This may help in the effectiveness of development of peptide vaccines and may increase their immunogenicity.

The next vital component in the development of an effective vaccine is using peptides derived from multiple proteins. The vaccines that are currently in development all use a combination of peptides from different proteins. First, this is important for a higher chance of the vaccines to be effective. Immune responses targeted against multiple parts of the virus will be induced, providing a broader immune response. This makes it more difficult for the virus to escape the response. Besides this, the virus has different levels of protein expression at certain stages of the viral infection. For example, for the initial infection of the cells by the virus HA, NA and M1 are important. After the actual infection proteins like M2, PB1 and NP begin to play a role. Therefore, for an effective immune response against the influenza virus, multiple protein peptides are key. This will induce an immune response against multiple stages of the virus infection and therefore provide a higher level of immunity (Assarsson et al., 2008).

The chemical altering of peptides could provide a useful tool in increasing the effectiveness of vaccines that are currently being developed or that will be developed in the future. It can improve the binding of the peptides to HLA molecules and therefore help in inducing a more effective immune response. Next to that, it could also help in increasing the effectiveness of the vaccines in individuals or populations with less frequent HLA molecules by altering the peptides and changing their HLA restriction to the less frequent HLA types.

An important topic for peptide vaccines that has not yet been discussed in this thesis is that of the actual delivery of the vaccine and its peptides to the DC cell. This delivery is of course also pivotal in the development of an effectively working vaccine as it is vital in order to activate DCs to present the peptides to T cells. There are multiple ways of delivery to the DC, for example with peptide loaded virosomes or in liposomes. The different methods of delivery have their specific advantages and disadvantages which have been discussed thoroughly in a thesis by P.C. Soema (2015).

In summary, influenza A peptide vaccines could prove to be a useful combatant of influenza A virus infections. Vaccines that are currently in development show promising first results eliciting both CD4+ and CD8+ T cell responses simultaneously. Peptide vaccines use conserved peptides derived from multiple proteins, helping them elicit an effective cellular immune response against multiple stages of the infection. Because the regions are conserved, these peptide vaccines can elicit immune responses against multiple strains of influenza and even new emerging strains, which makes them more (cost)-effective than vaccines that are currently being used. Also, peptide vaccines are more easily produced than current vaccines.

The chemical altering of peptides could provide an effective tool in improving the immune response they elicit. This could likewise help in altering peptides in vaccines that are currently being developed to be able to bind to less frequent HLA types across the globe.

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References

Abbas, A. K., Lichtman, H. A., & Pilai, S. (2014). *Cellular and Molecular Immunology*. *Elsevier* (Vol. 8ª ed.).

Alexander, J., Bilsel, P., del Guercio, M. F., Marinkovic-Petrovic, A., Southwood, S., Stewart, S., … Sette, A. (2010). Identification of broad binding class I HLA supertype epitopes to provide universal coverage of influenza A virus. *Human Immunology*, *71*(5), 468–474. http://doi.org/10.1016/j.humimm.2010.02.014

Ambrogelly, A., Palioura, S., & Söll, D. (2007). Natural expansion of the genetic code. *Nature Chemical Biology*, *3*(1), 29–35. http://doi.org/10.1038/nchembio847

Assarsson, E., Bui, H.-H., Sidney, J., Zhang, Q., Glenn, J., Oseroff, C., … Sette, A. (2008). Immunomic analysis of the repertoire of T-cell specificities for influenza A virus in humans. *Journal of Virology*, *82*(24), 12241–51. http://doi.org/10.1128/JVI.01563-08

Atsmon, J., Caraco, Y., Ziv-Sefer, S., Shaikevich, D., Abramov, E., Volokhov, I., … Ben-Yedidia, T. (2014). Priming by a novel universal influenza vaccine (Multimeric-001)-A gateway for improving immune response in the elderly population. *Vaccine*, *32*(44), 5816–5823. http://doi.org/10.1016/j.vaccine.2014.08.031

Cross, K. J., Langley, W. A., Russell, R. J., Skehel, J. J., & Steinhauer, D. A. (2009). Composition and functions of the influenza fusion peptide. *Protein and Peptide Letters*, *16*(7), 766–78. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19601906

De Groot, A. S., Moise, L., Liu, R., Gutierrez, A. H., Terry, F., Koita, O. A., … Martin, W. (2014). Cross-conservation of T-cell epitopes: now even more relevant to (H7N9) influenza vaccine design. *Human Vaccines & Immunotherapeutics*, *10*(2), 256–62. http://doi.org/10.4161/hv.28135

Dolin, R. (2011). Chapter 187. Influenza | Harrison's Principles of Internal Medicine, 18e | AccessMedicine | McGraw-Hill Medical. Retrieved February 22, 2017, from http://accessmedicine.mhmedical.com/content.aspx?bookid=331&sectionid=40726944

Fiers, W., De Filette, M., Birkett, A., Neirynck, S., & Min Jou, W. (2004). A "universal" human influenza A vaccine. In *Virus Research* (Vol. 103, pp. 173–176). http://doi.org/10.1016/j.virusres.2004.02.030

Fodor, E. (2013). The RNA polymerase of influenza A virus: Mechanisms of viral transcription and replication. *Acta Virologica*, *57*(2), 113–122. http://doi.org/10.4149/av_2013_02_113

Francis, J. N., Bunce, C. J., Horlock, C., Watson, J. M., Warrington, S. J., Georges, B., & Brown, C. B. (2015). A novel peptide-based pan-influenza A vaccine: A double blind, randomised clinical trial of immunogenicity and safety. *Vaccine*, *33*(2), 396–402. http://doi.org/10.1016/j.vaccine.2014.06.006

Frieden, T. R., Khabbaz, R. F., Redd, S. C., Bell, B. P., Fenton, K., Schuchat, A., & Cock, K. M. De. (2011). A CDC framework for preventing infectious diseases: Sustaining the essentials and innovating for the future. *CDC ID Framework*, 1.

Gonzalez-Galarza, F. F., Christmas, S., Middleton, D., & Jones, A. R. (2011). Allele frequency net: A database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Research*, *39*(SUPPL. 1), D913-9. http://doi.org/10.1093/nar/gkq1128

Gourraud, P.-A., Khankhanian, P., Cereb, N., Yang, S. Y., Feolo, M., Maiers, M., … Oksenberg, J. (2014). HLA diversity in the 1000 genomes dataset. *PloS One*, *9*(7), e97282. http://doi.org/10.1371/journal.pone.0097282

Grant, E., Wu, C., Chan, K.-F., Eckle, S., Bharadwaj, M., Zou, Q. M., … Chen, W. (2013). Nucleoprotein of influenza A virus is a major target of immunodominant CD8+ T-cell responses. *Immunology and Cell Biology*, *91*(2), 184–194. http://doi.org/10.1038/icb.2012.78

Horimoto, T., & Kawaoka, Y. (2001). Pandemic Threat Posed by Avian Influenza A Viruses. *Clinical Microbiology Reviews*, *14*(1), 129–149. http://doi.org/10.1128/CMR.14.1.129-149.2001

Horimoto, T., & Kawaoka, Y. (2005). Influenza: lessons from past pandemics, warnings from current incidents. *Nature Reviews Microbiology*, *3*(8), 591–600. http://doi.org/10.1038/nrmicro1208

Huber, S. K. R., Luimstra, J. J., Van Beek, J., Hoppes, R., Jacobi, R. H. J., Hendriks, M., … De Jonge, J. (2016). Chemical modification of influenza CD8+ TCell epitopes enhances their immunogenicity regardless of immunodominance. *PLoS ONE*, *11*(6), e0156462. http://doi.org/10.1371/journal.pone.0156462

Huber, S. R., van Beek, J., de Jonge, J., Luytjes, W., & van Baarle, D. (2014). T cell responses to viral infections - opportunities for peptide vaccination. *Frontiers in Immunology*. Frontiers Media SA. http://doi.org/10.3389/fimmu.2014.00171

Jin, H., & Chen, Z. (2014). Production of live attenuated influenza vaccines against seasonal and potential pandemic influenza viruses. *Current Opinion in Virology*, *6*, 34–39. http://doi.org/10.1016/j.coviro.2014.02.008

Lamb, R. A., Zebedee, S. L., & Richardson, C. D. (1985). Influenza virus M2 protein is an integral membrane protein expressed on the infected-cell surface. *Cell*, *40*(3), 627–633. http://doi.org/10.1016/0092-8674(85)90211-9

Lee, L. Y.-H., Ha, D. L. A., Simmons, C., de Jong, M. D., Chau, N. V. V., Schumacher, R., … Dong, T. (2008). Memory T cells established by seasonal human influenza A infection cross-react with avian influenza A (H5N1) in healthy individuals. *The Journal of Clinical Investigation*, *118*(10), 3478–90. http://doi.org/10.1172/JCI32460

McMahon, R. M., Friis, L., Siebold, C., Friese, M. A., Fugger, L., & Jones, E. Y. (2011). Structure of HLA-A*0301 in complex with a peptide of proteolipid protein: insights into the role of HLA-A alleles in susceptibility to multiple sclerosis. *Acta Crystallographica. Section D, Biological Crystallography*, *67*(Pt 5), 447–54. http://doi.org/10.1107/S0907444911007888

Pedersen, S. R., Christensen, J. P., Buus, S., Rasmussen, M., Korsholm, K. S., Nielsen, M., & Claesson, M. H. (2016). Immunogenicity of HLA Class I and II Double Restricted Influenza A-Derived Peptides. *PLOS ONE*, *11*(1), e0145629. http://doi.org/10.1371/journal.pone.0145629

Pejoski, D., Zeng, W., Rockman, S., Brown, L. E., & Jackson, D. C. (2010). A lipopeptide based on the M2 and HA proteins of influenza A viruses induces protective antibody. *Immunology and Cell Biology*, *88*(5), 605–611. http://doi.org/10.1038/icb.2010.15

Pleguezuelos, O., Robinson, S., Fernández, A., Stoloff, G. A., Mann, A., Gilbert, A., … Caparrós-Wanderley, W. (2015). A Synthetic Influenza Virus Vaccine Induces a Cellular Immune Response That Correlates with Reduction in Symptomatology and Virus Shedding in a Randomized Phase Ib Live-Virus Challenge in Humans. *Clinical and Vaccine Immunology : CVI*, *22*(7), 828–35. http://doi.org/10.1128/CVI.00098-15

Pleguezuelos, O., Robinson, S., Stoloff, G. A., & Caparrós-Wanderley, W. (2012). Synthetic Influenza vaccine (FLU-v) stimulates cell mediated immunity in a double-blind, randomised, placebo-controlled Phase I trial. *Vaccine*, *30*(31), 4655–4660. http://doi.org/10.1016/j.vaccine.2012.04.089

Portela, A., & Digard, P. (2002). The influenza virus nucleoprotein: a multifunctional RNA-binding protein pivotal to virus replication. *Journal of General Virology*, *83*(4), 723–734. http://doi.org/10.1099/0022-1317-83-4-723

Schumacher, M., Mutis, T., Rodenko, B., Ovaa, H., Emmelot, E., Spaapen, R. M., … Schumacher, T. N. M. (2014). Altered Peptide Ligands Revisited: Vaccine Design through Chemically Modified HLA-A2–Restricted T Cell Epitopes. *The Journal of Immunology*, *193*(9), 4803–4813. http://doi.org/10.4049/jimmunol.1400800

Sharma, A. K., Kuhns, J. J., Yan, S., Friedline, R. H., Long, B., Tisch, R., & Collins, E. J. (2001). Class I major histocompatibility complex anchor substitutions alter the conformation of T cell receptor contacts. *The Journal of Biological Chemistry*, *276*(24), 21443–9. http://doi.org/10.1074/jbc.M010791200

Sidney, J., Peters, B., Frahm, N., Brander, C., & Sette, A. (2008). HLA class I supertypes: a revised and updated classification. *BMC Immunology*, *9*, 1. http://doi.org/10.1186/1471-2172-9-1

Silver, M. L., Guo, H.-C., Strominger, J. L., & Wiley, D. C. (1992). Atomic structure of a human MHC molecule presenting an influenza virus peptide. *Nature*, *360*(6402), 367–369. http://doi.org/10.1038/360367a0

Soema, P. C. (2015). *Formulation of influenza T cell peptides : in search of a universal influenza vaccine*. Leiden. Retrieved from https://openaccess.leidenuniv.nl/handle/1887/35908

Sridhar, S. (2016). Heterosubtypic T-cell immunity to influenza in humans: Challenges for universal T-cell influenza vaccines. *Frontiers in Immunology*. Frontiers Media SA. http://doi.org/10.3389/fimmu.2016.00195

Sridhar, S., Begom, S., Bermingham, A., Hoschler, K., Adamson, W., Carman, W., … Lalvani, A. (2013). Cellular immune correlates of protection against symptomatic pandemic influenza. *Nature Medicine*, *19*(10), 1305–1312. http://doi.org/10.1038/nm.3350

Tewawong, N., Prachayangprecha, S., Vichiwattana, P., Korkong, S., Klinfueng, S., Vongpunsawad, S., … Poovorawan, Y. (2015). Assessing Antigenic Drift of Seasonal Influenza A(H3N2) and A(H1N1)pdm09 Viruses. *PLOS ONE*, *10*(10), e0139958. http://doi.org/10.1371/journal.pone.0139958

Turrell, L., Lyall, J. W., Tiley, L. S., Fodor, E., & Vreede, F. T. (2013). The role and assembly mechanism of nucleoprotein in influenza A virus ribonucleoprotein complexes. *Nature Communications*, *4*, 1591. http://doi.org/10.1038/ncomms2589

van Doorn, E., Liu, H., Ben-Yedidia, T., Hassin, S., Visontai, I., Norley, S., … Hak, E. (2017). Evaluating

the immunogenicity and safety of a BiondVax-developed universal influenza vaccine (Multimeric-001) either as a standalone vaccine or as a primer to H5N1 influenza vaccine. *Medicine*, *96*(11), e6339. http://doi.org/10.1097/MD.0000000000006339

WHO. (2015). WHO | Influenza vaccines. Retrieved from http://www.who.int/immunization/research/development/influenza/en/

Wilkinson, T. M., Li, C. K. F., Chui, C. S. C., Huang, A. K. Y., Perkins, M., Liebner, J. C., … Xu, X.-N. (2012). Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nature Medicine*, *18*(2), 274–280. http://doi.org/10.1038/nm.2612

Worch, R. (2014). Structural biology of the influenza virus fusion peptide. *Acta Biochim Pol*, *61*(3), 421–426. Retrieved from http://www.actabp.pl/pdf/3_2014/421.pdf