Entwicklung eines synthetischen Peptid-Vaccins gegen H5N1 basierend auf MHC-I-Epitopen
Development of a synthetic peptid vaccine against H5N1 based on MHC-I epitopes

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The avian influenza virus: H5N1

Avian influenza is caused by the influenza-A-Avirus H5N1 which is found in birds.
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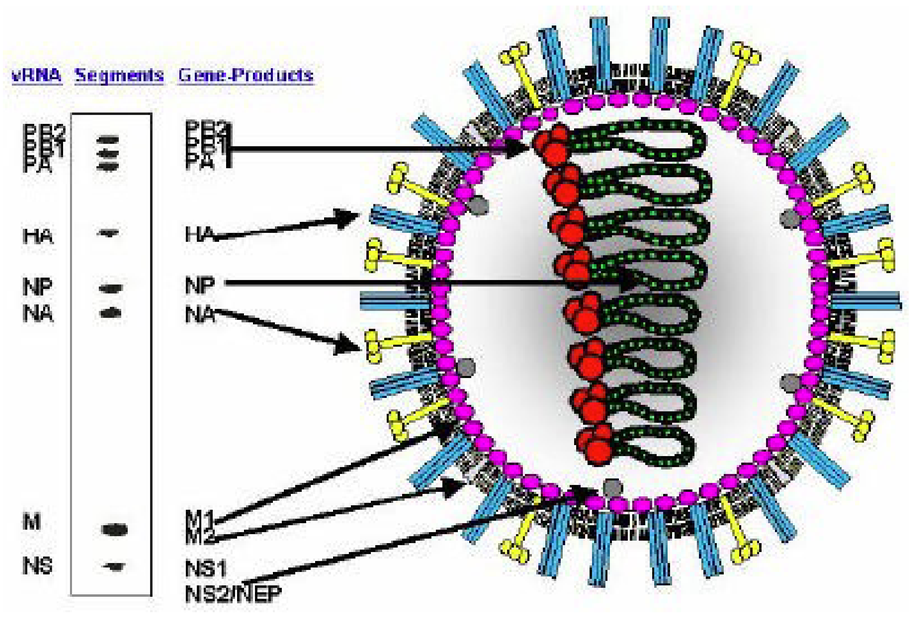

Figure 1: Influenza-A-virion.
The influenza-A-type virus is characterized by the HA (hemagluttinin) and NA (neuraminidase) proteins. H5N1 has one HA type 5 protein (H5) as well as one NA type 1 protein (N1)..

Prerequisites for a potential avian influenza pandemic among humans

There is growing concern that the H5N1 virus might cause a severe pandemic in the near future. Two out of three prerequisites for such a pandemic have already been fulfilled [1]:
● The majority of humans does not possess any kind of immunity against the H5N1 virus and second,
● the virus is able to replicate efficiently inside the human body.

The third prerequesite which has not been fulfilled yet is:
● a fast and effective spreading of H5N1 in the human population through the means of human to human transmission of the virus [2][3].
However, a mutation in the H5N1 virus, leading to these human pathogenic characteristics, could at any time fulfill this third prerequesite [3][4][5].

One of the dreaded potential mutations
Only two amino acid exchanges at the HA receptor binding site of H5N1 are required in order to optimize binding of the virus to N-acetyl-neuraminic acid, which is found on epithelial cells of the human lung [6]. These mutations would enable direct human to human transmission of the H5N1 virus.

Figure 2. Depiction of the trimeric HA protein
Interaction of HA with NA (in green) is shown [7].

Goal

Development of a vaccine for H5N1
Identification of peptides of the potential H5N1 mutants which might elicit an immune response in humans. Humans could be immunized with these peptides before the outbreak of a pandemic.

Approach

● Computer-based analysis of candidate peptides of the mutated H5N1 which bind to MHC-I with high affinity and hence are immunogenic.
● Verification of these candidates through ELISA and IFN-γ ELISPOT analysis in a laboratory setting.
● Animal testing of the candidate peptides which have found to be promising in the above mentioned tests.

References

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Methods

Computer-based MHC-I prediction

● Prediction of immunogenic MHC-I epitopes from the modified H5N1 protein by NetCTL 1.2.
● The same method of prediction was also used for the H5N1 nucleoprotein (NP), the polymerase protein (PB1) and the matrix protein (M1), since these proteins also harbor MHC-I epitopes in their amino acid sequence.
● The NetCTL 1.2 prediction has been employed for all pre-defined 12 HLA (human leukocyte antigen) types; these encompass 99% of MHC-I alleles of the entire human population.

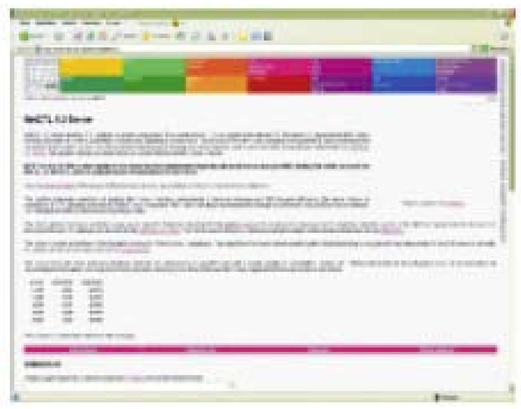

Figure 2. The NetCTL 2.1 Server

ELISA Experiment

The actual binding affinity between the synthetical peptides and the MHC-I alleles from the respective HLA types for which peptides have been predicted by NetCTL 1.2, need to be investigated by using quantitative ELISA (see Sylvester-Hvid et al., 2002 [9]).
In order to do this, all candidates (9mer peptides) need to be synthesized with a purity of 95%.

Animal studies and IFN-γ ELISPOT analysis

The immunogenic potency of the peptides needs to be verified using IFN-γ ELISPOT analysis (see Wang et al., 2007). For this purpose we are planning to utilize murine PBMC cells (*peripheral blood mononuclear cells)* infected with the H5N1 virus (see Gao et al., 2006).
To assess the effectiveness of the peptide vaccine, mice need to be treated with the MHC-I peptides which had been verified to be immunogenic. Subsequently these mice will be infected with the H5N1 virus. As a control, mice which have not received the vaccine, will be infected with H5N1.

Clinical Studies

Clinical studies are crucial, since results from animal studies cannot always be directly extrapolated to humans. For example, the majority of substances (about 90%) which had proven safe in animal studies failed in successive human trials. The typical duration of clinical studies is: Phase I – several weeks; Phase II – weeks to months, and Phase III – months up to several years.

Preliminary Results and Discussion

Identification of candidate epitopes using the computer-based MHC-I epitope prediction method

The number of epitopes for the 12 HLA types, which are hypothesized to being presented as CTL ligands in humans, range from n=193 for the modified H5 protein, n=189 for the NP protein, n=272 for the PB1 protein and n=85 for the M1 protein, with a total of n= 739 epitopes.
Of these, n=12 had also been identified as true CTL ligands in a study published by Wang et al., 2007) [7]. These 12 immunogenic epitopes are highly conserved and found in a variety of Influenza A subtypes, so also in the H5N1 virus.
20 epitopes which have been predicted for the modified H5 protein are also found in the sequence stretch of H5 which has been shown to be immunogenic by Gao et al., 2006 [8]. It is therefore highly likely that these are true CTL ligands, able to trigger an immune reaction in humans, and consequently potent to confer protective immunity for H5N1.
Two of these epitopes harbor the mutation found in the modified H5 protein. However, these epitopes only bind to two HLA types, HLA-B27 und -39.
It can be concluded that the method used in this study (using mutated H5 which is adapted to humans in its receptor binding site, and subsquently designing a vaccine from these H5 epitopes for protection from the human-adapted version of H5N1) might not prove successful.

Summary and Discussion

32 peptides wich are true CTL ligands and hence valid candidates for a MHC-I based vaccine, have been identified in this study. Theses peptides bind to 11 out of 12 HLA types, leaving only HLA type B44 with no true immunogenic CTL ligand identified. Nevertheless, the 11 HLA types A1, A2, A3, A24, A26, B7, B8, B27, B39, B58 and B62 cover 99% of HLA alleles of humans of all ethnicities. This means that a peptide vaccine based on these 32 epitopes could most likely confer sufficient immunity against H5N1 in humans.
Due to the highly conserved epitopes found in the NP, PB1 and M1 proteins of H5N1, such a vaccine might also protect from a human-adapted version of H5N1.

Verification of these data by ELISA experiments, animal studies and clinical studies are currently being planned.