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# Study of immunogenicity of recombinant proteins based on hemagglutinin and neuraminidase conservative epitopes of Influenza A virus

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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**Background:** Recombinant hemagglutinin (rHA) and neurominidase (rNA) developed in our investigation are amino acid sequence consensus variants of H1N1 2009 subtype influenza virus strain, also including immunogenic epitopes typical for other influenza virus subtypes (H3N1 and H5N1). Substitutions were made: typical for Russian virus isolates (in HA – S220T, NA – D248N) and in active centers of molecules – R118L, R293L, R368L; C92S, C417S to increase recombinant proteins stability in *E. coli*. The aim of the present work was to study immunogenicity of the obtained rHA and rNA.

**Material/Methods:** Fragments aa 83–469 of NA and aa 61–287 of HA were chosen because they include the main B-cell epitopes and are the minimal structures for correct folding of target proteins. The designed nucleotide sequences were synthesized and purified and the expression of rNA and rNA were analyzed. For immunization and virus challenge we used influenza viruses A/California/04/2009 (H1N1), A/PR/8/34 (H1N1), A/Perth/16/2009 (H3N2), A/Chicken/Kurgan/05/2005 R.G. (H5N1), and B/Florida/04/2006. Specific IgG levels were determined by ELISA in 96-well ELISA plates. Significant differences of survival in mouse groups were analyzed by Mantel-Cox (log-rank) and Gehan-Breslow-Wilcoxon tests.

**Results:** The obtained results demonstrate the high immunogenicity and ability of indicated proteins mixture to provide similar cross-protection against influenza viruses of the H1N1 subtype.

**Conclusions:** The data obtained suggest efficient pluripotent vaccine creation based on HA and NA conservative regions.

**Key words:** conservative epitopes • immunogenicity • influenza virus • recombinant haemagglutinin • recombinant neuraminidase

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## Background

According to WHO data, 250 000 to 500 000 people (most of them older than 65 years) die each year from of all variants of the virus during seasonal epidemics in the world; in some years the number of deaths could reach a million [1,2]. Many researchers around the world are working on a universal flu vaccine [3,4]. However, existing vaccines only have effectiveness against certain strains of influenza virus.

A common focus for creating a universal vaccine is the development of substances that affect the virus structure, which is conservative and highly homologous among the different types of viruses. At the moment, the flu vaccine should be created every year because the virus is constantly mutating, forming new antigenic determinants, and every time the disease is caused by a new type of virus.

Influenza viruses belong to the Ortomyxoviridae family, which includes the genera influenza A, B, and C, which are defined depending on presence of 2 internal virion proteins (M1 and NP). Further division is carried out in accordance with subtypes (serotypes) of the surface proteins hemagglutinin (HA) and neuraminidase (NA) [5,6]. Currently, 16 HA and 9 NA subtypes are known.

It is of epidemiological importance that human have viruses containing 3 subtypes of hemagglutinin (H1, H2, and H3) and 2 subtypes of neuraminidase (N1, N2). The major virion antigenic components of influenza A and B is the NA and HA; the C virus does not contain neuraminidase. Antibodies produced in response to the hemagglutinin constitute the basis of immunity against a specific subtype of influenza virus [7–9].

The immune system usually does not respond to the "stem" part of hemagglutinin, which is difficult to reach, but instead it rapidly produces antibodies to surface determinants, which are more accessible. However, their high variability allows the virus to remain unrecognized. Even in case of administration of specially isolated conserved regions of hemagglutinin, the immune system will respond with the formation of a wide spectrum of antibodies, most of which will not be able to recognize a live virus because this area is deeply hidden [10].

Our study was conducted in an attempt to create 2 recombinant short conserved regions of viral surface proteins – hemagglutinin and neuraminidase integrated epitopes – against which both T- and B-cells are active.

NA- and HA-coding genes were obtained and expressed in *E. coli* during the decade of the 1980s to study their immunological properties [11–13].

The first hemagglutinin subunit (HA1) amino acids (aa) 193–199 forming QNPTTYI epitope remained conservative among influenza H5N1 virus strains recently in circulation and could be recognized by monoclonal antibodies to HA1 of influenza virus A/Vietnam/1203/04 (H5N1). HA1 region aa 42–75 (HA1 N-terminus) in H5N1 viruses, aa 262–295 in H1N1 viruses, aa 57–90 in H3N2 viruses, and aa 28–61 in H7N7 viruses were shown to be highly conserved [14,15]. HA conservative epitope sequences of influenza H3N2 virus (aa 173–181, 227–239) and antibodies to these epitopes neutralizing strains of this subtype were demonstrated [16].

Highly homologious HA areas among H1, H3, and H5 subtypes of influenza A virus were detected. Nine HA1 sites have similar secondary structure (aa: 31-37, 89-96, 118-130, 200-208, 238-246, 272-277, 304-312, 317-334, 341-348) among H1, H2, H3, and H5 subtypes [7,17]. Similar structure regions were identified among influenza H1N1 and H5N2 virus subtypes, probably being the main epitope inducing formation of neutralizing antibodies to a vast variety of influenza virus strains [16]. These regions located behind the receptor-binding HA sites are conservative among H3N2 subtype of influenza virus strains and highly conservative in H1N1 and H5N1.

Nucleotide sequences coding rHA and rNA of influenza A and B virus and antigenic determinants with codon optimization for expression in *E. coli* are unknown.

The aim of the present work was to study immunogenicity of obtained rHA and rNA.

Fragments aa 83–469 of NA and aa 61–287 of HA were chosen because they include the main B-cell epitopes and are the minimal structures for correct folding of target proteins.

## Material and Methods

### Gene synthesis

Designed nucleotide sequences were synthesized as described by Young [18].

Vector pUC57 was used for cloning. Fragments were cut from the vector and amplified using the polymerase chain reaction (PCR) method. Nucleotide sequences coding HA and NA were synthesized by overlapping of elongation of oligonucleotides [19] and cloned into plasmid pET151/D-TOPO. For gene engineering work, DH10B/R *E. coli* cells (Gibko BRL, USA) were used.

### rHA and rNA expression and purificaion.

BL21(DE3) *E. coli* cells were transformed by electroporation. Induction of rHA and rNA expression was performed by 0.2%





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lactose in induction medium (1% peptone, 0.5% yeast extract, 50 mM $Na_2HPO_4$, 50 mM $K_2HPO_4$, 25 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.5% glycerol, 0.05% glucose, 0.2% lactose).

Fermentation was performed in induction medium containing ampicillin (100 µg/ml) in thermostatic rotor shaker (37°C, 250 rpm, 16–20 hours).

Disc-electrophoresis in PAAG of cell lysate of BL21(DE3)pPVNA and BL21(DE3)pPVHA *E. coli* strains was performed for densitometric measurement of rHA and rNA nucleotide sequences expression level in *E. coli*.

Proteins were purified according to the Ni-NTA Purification System protocol (Invitrogen, Catalog Number K950-01).

### Immunization and virus challenge

Balb/c mice (16 per each group, 16–18 g) were immunized by 20 µg rHA and rNA in the ratio 1: 1 with 0.5 mg of Alhydrogel. Two intraperitoneal immunizations were conducted 2 weeks apart. Control mice received PBS (priming) and vaccine filling agent (boosting). Blood samples (from 5–6 mice) were collected from the ventral vein 2 weeks after the second immunization.

Influenza viruses A/California/04/2009 (H1N1), A/PR/8/34 (H1N1), A/Perth/16/2009 (H3N2), A/Chicken/Kurgan/05/2005 R.G. (H5N1), and B/Florida/04/2006 were used. Immunized mice were challenged intranasally with $1LD/_{50}$ of A/California/04/2009 (H1N1) and A/PR/8/34 (H1N1) or $5LD/_{50}$ of A/California/04/2009 strain (50 µl per mouse)s. Animals were monitored daily for 2 weeks for survival and weight loss.

### Proteins denaturation

Tween-20 was added to the sample – the final concentration 1% (w/v), followed by water bath incubation (1 h, 37°C) with further centrifugation (1 h, 20°C, 2000 g). Supernatant including viral envelope proteins of influenza viruses A/PR/8/34 and A/Chicken/Kurgan/05/2005 R.G. was taken. Detergent was removed using the Detergent-OUT™Micro Kit (Millipore) and the sample was concentrated on SpeedVac by achieving initial volume. The preparation of additional elaboration was held with 8M carbamide in presence of DDT (0.02 M) with further night dialysis *versus* carbonate buffer (pH 8.5).

### Serum antibody determination

Specific IgG levels were determined by ELISA in 96-well ELISA plates (“Greiner”, Germany) coated overnight at 4°C with 100 µl/well of rHA, rNA (3 µg/ml) and influenza strains A/California/04/2009, A/PR/8/34, A/Perth/16/2006, A/Chicken/Kurgan/05/2005 R.G., B/Florida/04/2006 (2 µg/ml) resuspended in carbonate coating buffer. Plates were blocked with blocking buffer (PBS with 5% FCS) – 300 µl/well, 1 h at room temperature. Sera were serially diluted. The diluted sample (100 µl) was added to duplicate wells (incubated 1 h at room temperature). HRP-labeled rabbit anti-mouse IgG antibodies (Abcam, UK) and goat anti-rabbit IgG antibodies (Sigma-Aldrich) diluted in blocking buffer were added (100 µl/well) (incubation at room temperature for 1 h). Plates were developed with TMB substrate (BD Bioscience) according to the manufacturer’s instructions. ELISA endpoint titers were defined as inverse value of the highest dilution that yielded an OD 450 nm value above 2 times the mean of negative control wells.

### Statistical analysis

The life length evaluation was doned with instantaneous sampling (Kaplan-Meyer). Significant differences in survival in mouse groups were analyzed by Mantel-Cox (log-rank) and Gehan-Breslow-Wilcoxon tests.

## Results

### rHA and rNA design and expression

Alignment of 837 aa rNA and rHA sequences of influenza A and B virus strains (NCBI, 2009) resulted in aa sequence creation representing NA and HA consensus aa variants. Fragments including the main A (H1N1, H3N2, H5N1) and A influenza virus B-cell epitopes were chosen. Substitutions in an active center of the NA molecule and those typical for Russian virus isolates were made. Corresponding nucleotide gene sequences were codon-optimised for *E. coli*.

The obtained expression of pPVNA and pPVHA plasmids provide rNA and rHA synthesis in *E. coli* under specific promoter induction (Figures 1 and 2). Sequenation has shown the absence of mistakes in the gene structure.

### rHA and rNA separation and purification

NA accumulation assessed with densitometry was 23% of total cell protein amounts in BL21(DE3)pPVNA *E. coli* cells and 43% in BL21(DE3)pPVHA *E. coli* cells. Expression levels did not change during 6 passages, which confirms the expression stability. After purification, rHA was obtained with 96% purity and rNA with 95% purity.

### rHA+rNA immunogenicity in mice

The specific serum IgG antibodies to rHA, rHA+rNA, A/California/04/2009, A/PR/8/34, A/Perth/1620/09, and A/Chicken/





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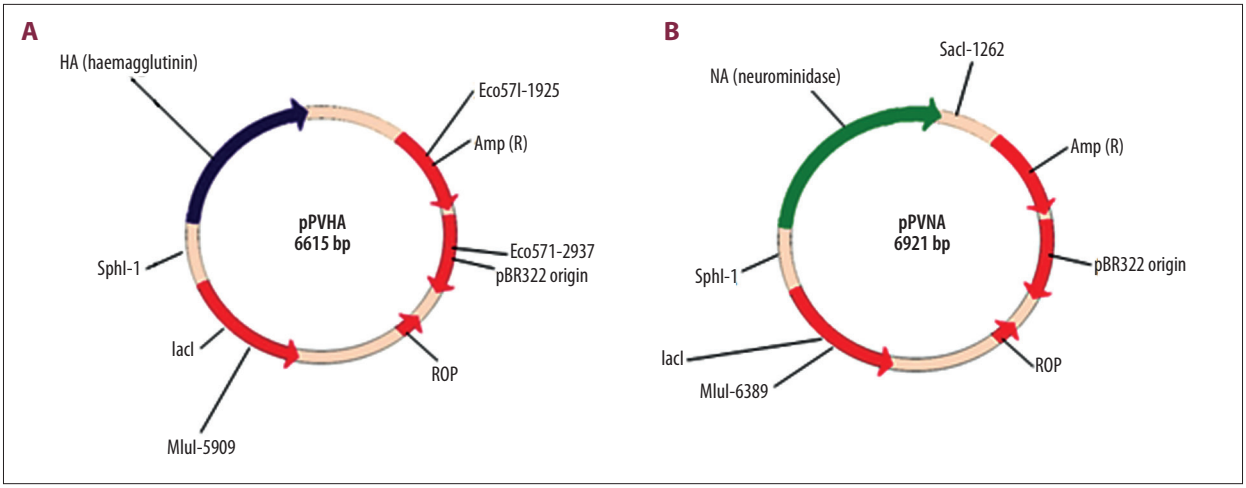


**Figure 1.** Plasmid schemes (**A**) pPVHA, coding and expressing rHA in *E.coli* cells; (**B**) pPVNA coding and expressing rNA in *E.coli* cells.

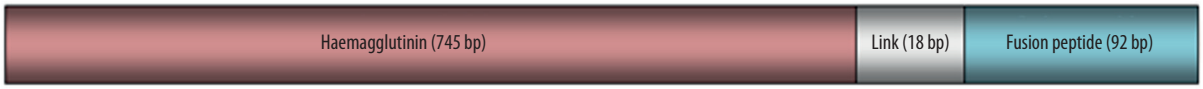


**Figure 2.** rHA-coding influenza virus nucleotide sequence.

**Table 1.** Titers of serum IgG to rHA, rNA and influenza viruses after immunization with rHA+rNA.

| Mice groups | Fixed antigens | | | | | |
|---|---|---|---|---|---|---|
| | rHA | rHA+ rNA | HA A/California/ 04/2009 | A/California/ 04/2009 | A/PR/8/34 | A/Perth/ 16/2009 |
| Mice immunized with rHA+rNA | 409 600 | 819 200 | 6 400 | 51200 | 12 800 | 3200 |
| Control mice | 200 | 200 | 200 | 200 | 200 | 200 |

**Table 2.** Titers of serum IgG to A/PR/8/34 and A/Kurgan/05/2005 R.G. before and after influenza viruses surface glycoproteins denaturation.

| Mice groups | Fixed antigens | | | |
|---|---|---|---|---|
| | A/PR/8/34 | A/PR/8/34 Tw | A/Kurgan/ 05/2005 | A/Kurgan/ 05/2005 Tw |
| Mice immunized with rHA+rNA | 12 800 | 51 200 | 200 | 1 600 |
| Control mice | 200 | 200 | 200 | 200 |

Kurgan/05/2005 R.G were determined by ELISA. Immunization induced high titers of antibodies to rHA and rNA and titers were 2 times lower to influenza viruses surface glycoproteins (Table 1).

After denaturation of influenza virus surface glycoproteins, the antibody titers increased by 4 times to A/PR/8/34 and by 8 times to A/Chicken/Kurgan/05/2005 R.G. (Table 2).





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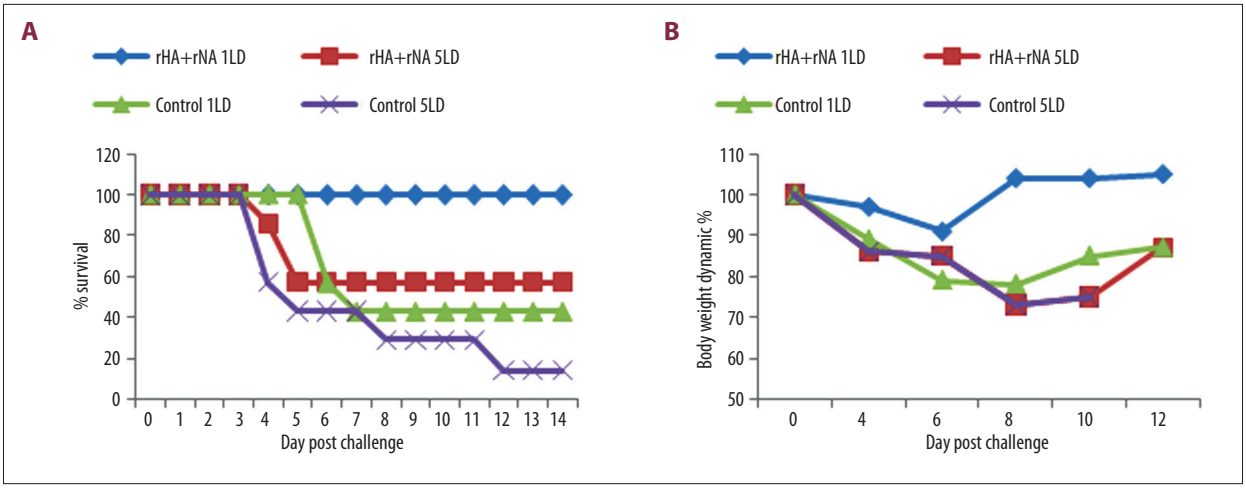


**Figure 3.** Survival (**A**) and body weight dynamics (**B**) after challenge with $1LD/_{50}$ and $5LD/_{50}$ A/California/04/09 (H1N1).

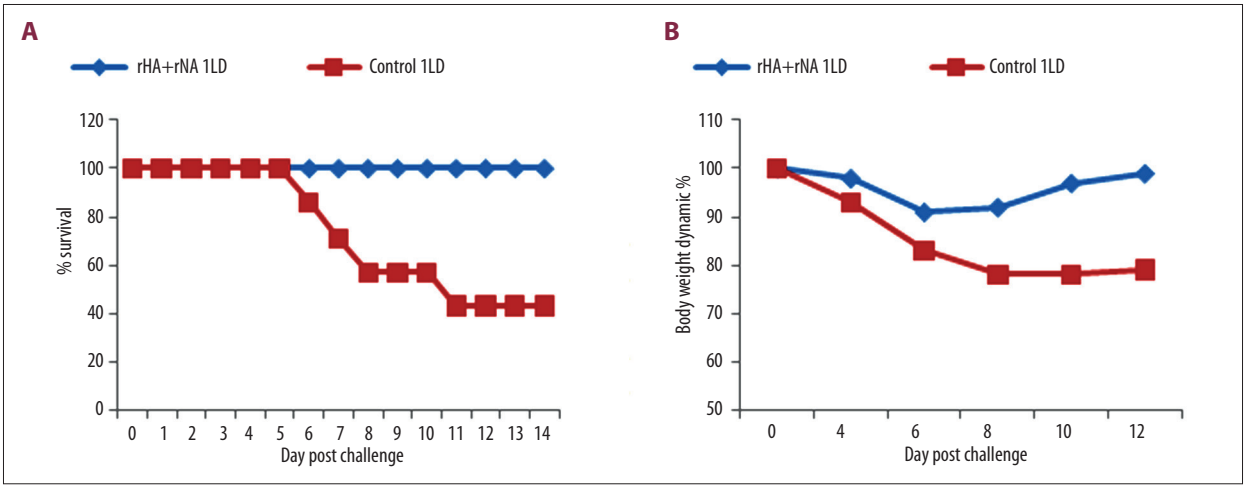


**Figure 4.** Survival (A) and body weight dynamics (**B**) after challenge with $1LD/_{50}$ A/PR/8/34 (H1N1).

Anti-HA and anti-NA serum IgG induced by immunization with inactivated A/California/04/2009 were shown to be capable of binding to rHA and rNA of this virus. Two months following immunization of mice, IgG titers to influenza virus A/California/04/2009 were 102 400, titers to rHA were 12 800, and titers to rNA were 3200. Serum of rabbits immunized with influenza B virus was shown to be capable of binding to both rHA and influenza virus B/Florida/04/2006. The serum IgG titers were 409 600 and 204 800, respectively.

### Mice protection from influenza challenge

We evaluated the ability of mice to resist a lethal dose after immunization with rHA+rNA containing virus. Immunized mice challenged with $1LD/_{50}$ of influenza virus A/California/04/2009 showed 100% survival compared to 43% ($p<0.05$) survival in the control group (Figure 3). Immunized mice lost less body weight and started rapidly gaining weight compared to the control group ($p<0.05$). These findings indicate a recovery process starting 6 days after viral challenge. Administration of $5LD/_{50}$ of influenza virus A/California/04/2009 resulted in 57% survival level among immunized mice compared to 14% among control animals (Figure 3) and also resulted in less body weight loss than in unvaccinated controls. Inoculation with $1LD/P_{50}$ of A/PR/8/34 showed 100% survival in the group of immunized mice (Figure 4) and 53% in the control group ($p<0.05$). Animals of the control group had more pronounced body weight loss compared with immunized mice ($p<0.05$).

## Discussion

Nowadays recombinant proteins based on the conservative epitopes of influenza virus HA, M2, and NP genes obtained in *E. coli* are used for the development of influenza vaccines, including the so-called "universal" vaccines requiring little seasonal modifications and designed with gene engineering methods representing minimal outlay [4,20–22].





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Antibodies to conservative HA regions can induce a broad cross-reactive immune response to new influenza A virus strains [16], hence HA1-conservative regions could become potential targets for universal influenza vaccines. Strain-specific antibodies to HA are considered to have a weak cross-activity with other HA types. Antibodies to definite epitopes having cross-reactive neutralizing activity also exist. Furthermore, H1N1-induced infection stimulates the production of protective cross-reacting antibodies to H5N1 [6], which are supposed to serve as the basis for development of universal vaccines against flu and theurapeutic immunbiological medications [23,24]. Descriptive studies have been conducted of antibodies interacting with different influenza virus subtypes, as well as corresponding antigen determinants [25–28]. Synthetic immunogens based on such epitopes inducing the formation of antibodies displaying high cross-reactivity with H1N1, H3N2, H2N2, H5N1, H7N2, H7N3, H7N7, and H9N2 virus antigens were described and their protective immune response were shown [29]. Recombinant protein consisting of 6 HA epitopes highly protective against influenza virus A strains H1N1, H2N2, and H3N2 was characterized [3]. According to the obtained data, the synthesis of neutralizing antibodies to influenza H1N1 virus strains that emerged during the last 73 years was induced by H1 HA-based immunogens [30].

The originality of the suggested approach is that HA and NA (inducing immunogenic reactivity spectrum in vaccine composition) antigen determinants of 2 influenza virus types (A and B) were used for the first time and sequences presenting combination of B- and T-cell conservative epitopes (HA – 61–287 aa, NA –83–469 aa) are codon-optimized for increase of rHA and rNA nucleotide sequences expression level. The obtained rHA and rNA are influenza A (H1N1) virus aa sequences consensus variants also containing immunogenic epitopes typical for other influenza virus strains (H3N1 and H5N1).

High immunogenicity of rHA and rNA was shown. Intraperitoneal immunization efficiently stimulated specific serum IgG production in equal concentrations to both rHA and rNA. Antibodies to influenza virus A/California/04/2009 A/PR/8/34 and A/Perth/16/2006 surface glycoproteins were revealed in lesser titers. Nearly 12% of total antibodies to rHA+rNA bind with influenza virus A/California/04/2009, likely because synthesized recombinant proteins are not structurally identical to influenza virus HA and cannot bind with antibodies specific to HA tetrameric molecule conformational epitopes. To make some linear HA and NA epitopes accessible for post-immunization antibodies, surface proteins denaturation was performed; IgG titers to them increased by 4–8 times. Immunization with rHA+rNA was shown to protect mice, fully or partially, from lethal challenge with A/California/04/2009 and A/PR/8/34 viruses (depending on injected virus dose) and to significantly reduce weight loss.

## Conclusions

Most current efforts to create universal vaccines hinge on the idea of generating antibodies against a portion of the virus that is relatively unchanged year-to-year. A vaccine, based on using antibodies simultaneously to two conservative and highly homologous virus structures serves to increase the degree of defence from different types of viruses.

The results obtained for rHA and rNA immunogenicity and protective efficacy demonstrate the possibility and necessity of this approach for development of a universal pandemic influenza vaccine.

## References:

1. Webster RG, Bean WJ, Gorman OT et al: Evolution and ecology of influenza A viruses, Microbiol Rev, 1992; 56: 152–79
2. Influenza WHO Fact sheet No. 211 revised March 2003. Retrieved 22 October 2006. *http://www.who.int/mediacentre/factsheets/fs211/en/*
3. Adar Y, Singer Y, Levi R, Tzehoval E et al: A universal epitope-based influenza vaccine and its efficacy against H5N1. Vaccine, 2009; 27(15): 2099–107
4. Jeon SH, Ben-Yedida T, Arnon R: Intranasal immunization with synthetic recombinant vaccine containing multiple epitopes of influenza virus. Vaccine, 2002; 20: 2772–80
5. Fouchier RA, Munster V, Wallenstein A et al: Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls J Virol, 2005; 79: 2814–22
6. Van Reeth K, Braeckmans D, Cox E et al: Prior infection with an H1N1 swine influenza virus partially protects pigs against a low pathogenic H5N1 avian influenza virus. Vaccine, 2009; 27(45): 6330–39
7. Clements ML, Betts RL, Tierney RL, Murphy BR: Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. J Clin Microbiol, 1986; 22: 157–60
8. Sahini L, Tempczyk-Russell A, Agarwal R: Large-Scale Sequence Analysis of Hemagglutinin of Influenza A Virus Identifies Conserved Regions Suitable for Targeting an Anti-Viral Response. PLoS One, 2010; 5(2): e9268
9. Skehel JJ, Wiley DC: Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem, 2000; 69: 531–69
10. Ekiert DC, Bhabha G, Elsliger M-A et al: Antibody recognition of a highly conserved influenza virus epitope: implications for universal prevention and therapy. Science, 2009 (April 10); 324(5924): 246–51
11. Davis AR, Nayak DP, Ueda M et al: Expression of antigenic determinants of the hemagglutinin gene of a human influenza virus in Escherichia coli. Proc Natl Acad Sci USA, 1981; 78(9): 5376–80
12. Emtage JS, Carey NH.(: The production of vaccines by recombinant DNA techniques. Prog Clin Biol Res, 1981; 47: 367–75
13. Jones LV, Compans RW, Davis AR et al: Surface expression of influenza virus neuraminidase, an amino-terminally anchored viral membrane glycoprotein, in polarized epithelial cells. Mol Cell Biol, 1985; 5(9): 2181–89
14. Veljkovic V, Veljkovic N, Muller CP et.al: Characterization of conserved properties of hemagglutinin of H5N1 and human influenza viruses: possible consequences for therapy and infection control. BMC Structural Biology, 2009; 9: 21
15. Wang K, Holtz KM, Anderson K et al: Expression and purification of an influenza haemagglutinin – one step closer to a recombinant protein-based influenza vaccine Vaccine, 2006; 24: 2176–85





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16. Yamashita A, Kawashita N, Kubota-Koketsu R et al: Highly conserved sequences for human neutralization epitope on hemagglutinin of influenza A viruses H3N2, H1N1 and H5N1: Implication for human monoclonal antibody recognition. Biochem Biophys Res Commun, 2010; 393: 614–18
17. Arias CF, Escalera-Zamudio M, Soto-Del Rio MD et al: Molecular anatomy of 2009 Influenza virus A (H1N1). Arch Med Res, 2009; 40: 643–54
18. Young L, Dong Q: Two-step total gene synthesis method. Nucleic Acids Res, 2004; 32(7): e59
19. Majumder K: Ligation-free gene synthesis by PCR: Synthesis and mutagenesis at multiple *loci* of a chimeric gene encoding OmpA signal peptide and hirudin. Gene, 1992; 110(1): 89–94. Erratum in: Gene, 1992; 122(2): 389, Gene, 1992; 116(1): 115–16
20. Biesova Z, Miller MA, Schneerson R et al: Preparation, characterization, and immunogenicity in mice of a recombinant influenza H5 hemagglutinin vaccine against the avian H5N1 A/Vietnam/1203/2004 influenza virus. Vaccine, 2009; 27(44): 6234–38
21. Faulkner L, Buchan G, Slobbe L et al: Influenza haemagglutinin peptides fused to interferon gamma and encapsulated in liposomes protects mice against influenza infection. Vaccine, 2003; 21: 932–39
22. Livingston BD, Higgins D, Van Nest G: Evolving strategies for the prevention of influenza infection: potential for multistrain targeting. BioDrugs, 2006; 20(6): 335–40
23. Sui J, Hwang WC, Perez S: Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nat Struct Mol Biol, 2009; 16: 265–73
24. Yoshida R, Igarashi M, Ozaki H et al: Cross-Protective Potential of a Novel Monoclonal Antibody Directed against Antigenic Site B of the Hemagglutinin of Influenza A Viruses. PLoS Pathog, 2009; 5(3): e1000350
25. Brown JD, Stallknecht DE, Berghaus RD et al: Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. Clin Vaccine Immunol, 2009; 16(6): 824–29
26. Heckler R, Baillot A, Engelman H et al: Cross-protection against homologous drift variants of influenza A and B after vaccination with split vaccine. Intervirology, 2007; 50(1): 58–62
27. Lee VJ, Tay JK, Chen MI et al: Inactivated trivalent seasonal influenza vaccine induces limited cross-reactive neutralizing antibody responses against 2009 pandemic and 1934 PR8 H1N1 strains. Vaccine, 2010; 28(42): 6852–57
28. Zanvit P, Havlíčkováb M, Táčnerb J et al: Protective and cross-protective mucosal immunization of mice by influenza virus type A with bacterial adjuvant. Immunol Lett, 2008; 115(2): 144–52
29. Alexander J, Bilsel P, del Guercio MF et al: Identification of broad binding class I HLA supertype epitopes to provide universal coverage of influenza A virus. Hum Immunol, 2010; 71(5): 468–74
30. Chih-Jen W, Boyington JC, McTamney PM et al: Induction of Broadly Neutralizing H1N1 Influenza Antibodies by Vaccination. Science, 2010 (August 27); 1060–64





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