Research Article

Short Peptides Vaccines For Early Detection And Treatments Of Coronavirus

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ABSTRACT

Background and Purpose: Coronavirus is a RNA virus with an envelope protein, a protease enzyme which is required for its infection into the host cells. This study can determine if the polyclonal antibodies produced from short peptides in rabbits or human can be used for early detection and treatment of the coronaviral infection.

Experimental Approach: Rabbit polyclonal antibodies will be produced from two short peptides at N-Terminal and C-terminal of the coronavirus membrane protein. The human vaccine will be produced from a short peptide of C-terminal by transcutaneous active and passive immunization.

Results and Conclusions: The rabbit polyclonal antibodies produced from short peptides will be used for early detection of coronavirus infection by using ELISA against saliva or serum taken from infected subjects. The infected human subject will be treated with passive immunization. For prevention of viral infection, volunteer human subjects will participate in active immunization by transcutaneous route.
INTRODUCTION:
Coronaviruses also known as CoVs, or COVID-19 primarily infect birds and animals, but recently have been shown to infect human with symptoms similar to severe acute respiratory syndrome (SARS). Coronavirus is a RNA virus with a protein envelope which is required for its infection into the host cells (Schoeman and Fielding 2019). The coronavirus protein is a short chain membrane protein of 76-109 amino acids with 8.4to 12 kDa in size. There are three sections in the membrane protein, N-Terminal, Transmembrane, and C-Terminal. The N-terminal is consisting of hydrophilic 7-12 amino acid residues, followed by a hydrophobic transmembrane domain of about 25 amino acids, and ending with a hydrophilic carboxyl of about 44-72 amino acids residues. An antibody (Ab), also known as an immunoglobulin (Ig) is a large, Y-shaped glycoprotein produced mainly by blood cells to neutralize or inhibit the infection of pathogenic bacteria or viruses. An enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies and color change to identify an antigenic substance. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries.

An amino acid is an organic acid having one or more than one alkaline radicals such as amino, guanidino, imino, or hydrazine radical attached at any carbon atom other than carbon one. There are 20 common amino acids which are represented by chemical names, such as “glycine”, or abbreviated symbols such as three letters, “Gly” or one letter “G. In this report, the one letter will be used. Except glycine, all other common amino acids have stereoisomers, i.e., enantiomer, D or L form. The amino acids in most natural peptides and proteins are all in L-form. The one letter and three letter symbols used for the 20 common amino acids are as follows: alanine (A, Ala), arginine (R, Arg), aspartic acid (D, Asp), asparagine (N, Asn), cysteine (C, Cys), glycine (G, Gly), glutamic acid (E, Glu), glutamine (Q, Gln), histidine (H, His), isoleucine (I, Ile), leucine (L, Leu), lysine (K, Lys), methionine (M, Met), phenylalanine (F, Phe), proline (P, Pro), serine (S, Ser), threonine (T, Thr), tryptophan (W, Trp), tyrosine (Y, Tyr) and valine (V, Val). The letter “O” or “Non” represents there is no amino acid. A peptide is formed between at least two amino acid molecules when the carboxyl group on one amino acid reacts with the amino group of the other amino acid in a dehydration synthesis reaction. A short peptide contains between 2 and 50 amino acid residues. The preferred short peptide for antibody production is between 10-20 amino acid residues. Because very few pharmaceutical agents are therapeutically effective against viral infection, prevention of viral infection through vaccine immunization has been the best approach. There are two types of immunizations. The active immunity uses live but attenuated virus or killed virus in the vaccine preparation. The human vaccine produced from live but attenuated virus has various safety issues; including causing hypersensitivity and activating virus infections in the volunteer human subject. On the other hand, the human vaccine produced from killed virus does not have safety issues but the vaccine thus produced is less sensitive and much less active or effective because the protein is denatured during the killing process.

One solution is to use unadenatured short peptide of 12 -18 amino acid residues. The rabbit polyclonal antibody produced from a short peptide has been used for early detection of breast cancer. The short peptide is EEASPEAVAGVFESK, a 16 amino acid residue identified as an epitope or binding site of G-Protein Coupled Receptor-Associated Sorting Protein-1 (GASP-1) with serum albumin (Chang FN and Tuszynski GP. 2012. Zheng X, Chang F, Zhang X, Rothman V, and Tuszynski GP). Over the past years, more than 300 rabbit polyclonal antibodies have been successfully produced from short peptides with 10-30 amino acid residues for biopeptide research related to breast cancer, pancreatic cancer, and Alzheimer’s disease (Yu RJ. 2019)

METHODS
For rabbit polyclonal antibody production, each biopeptide is emulsified in Complete Freund’s Adjuvant (CFA) which contained keyhole limpet hemocyanin (KLH) for initial subcutaneous injections (S.C.). Incomplete Freund’s Adjuvant (IFA) was used
for subsequent boost injections. Two to four rabbits are used for each biopeptide at the amount of 3.0 mg per rabbit. The following was the procedure:

**Rabbit Polyclonal Antibody Production Procedures:**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Day</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary immunization</td>
<td>1</td>
<td>0.5 mg per rabbit peptide-KLH conjugate, subcutaneous injection</td>
</tr>
<tr>
<td>1st boost</td>
<td>14</td>
<td>0.5 mg per rabbit; peptide-KLH conjugate, S.C.</td>
</tr>
<tr>
<td>Test bleed and ELISA</td>
<td>24</td>
<td>100 µl serum per rabbit. ELISA and immune response evaluation.</td>
</tr>
<tr>
<td>2nd boost</td>
<td>35</td>
<td>0.5 mg per rabbit; peptide-KLH conjugate, S.C.</td>
</tr>
<tr>
<td>Production bleed</td>
<td>45</td>
<td>ELISA test</td>
</tr>
<tr>
<td>3rd boost</td>
<td>49</td>
<td>0.5 mg per rabbit; peptide-KLH conjugate, S.C.</td>
</tr>
<tr>
<td>Production bleed</td>
<td>59</td>
<td>ELISA test</td>
</tr>
<tr>
<td>Production bleed 4th boost</td>
<td></td>
<td>Day 63</td>
</tr>
<tr>
<td>5th boost</td>
<td>77</td>
<td>0.5 mg per rabbit; peptide-KLH conjugate, S.C.</td>
</tr>
<tr>
<td>Production bleed</td>
<td>87</td>
<td>40 ml serum per rabbit</td>
</tr>
</tbody>
</table>

Note: keyhole limpet hemocyanin (KLH)

For a short peptide, it is necessary to add a cysteine amino acid at the beginning or at the end of the peptide chain for binding with KLH to stimulate vaccine production. The short peptide used for rabbit polyclonal antibodies production has 17 amino acid residues including cysteine: CRVKNLNSSEGVPDILLV (17C) based on C-Terminal motif or epitope. As a backup measure, another short peptide including cysteine can be used for rabbit polyclonal antibody production; CMYSFVSEETGTLIVNS (17N) based on N-Terminal motif.

**RESULTS**

After the rabbit polyclonal antibodies are produced and purified, the production company will send a report on each antibody produced, including HPLC and mass chromatography. Each rabbit will produce about 3ml of purified serum containing polyclonal antibodies, usually having 50% titers higher than 64,000.

For early detection of viral infection, saliva or serum from a suspected human subject will be taken, diluted to 1:20 ratio and be tested against rabbit polyclonal antibodies by ELISA method. A healthy human volunteer will be used as control. If the infection is confirmed, the subject will be treated with vaccine produced from the epitope short peptide by human volunteer. The suspected human subject can also be treated with a pharmaceutical agent by intramuscular injection of RVKLNLSSEGVPDILLV-NH(C=NH)NH₂. If the patient has not responded to the above treatments, it can be assumed that the infection might be due to different genus or strain of virus. In such case, different antibodies produced from the group consisting up to 16 short peptides of different strains can be used initially as detection and identification of viral infections. Once identified, a specific or combination of several antibodies can be used for treatment, using active and passive immunization.

The following up to 16 peptides can be developed for antibodies production:

1. CCoV; CIKAYNPDEALLV(13)
2. PRCV; CIKAYNPDGALLV(13)
3. TGEV, mutant; CIKAYNPDGDLLV(13)
4. FeCoV; CIKAYNPDEFLV(13)
5. HCoV-299E; CHIDPFPKRVIDF(13)
6. PEDV; CRIDPLPVSTVIDF(13)
7. HCoV-NL63; CQIAPVPAEVNLV(13)
8. SARS-CoV; CLNSSEGVPDILLV(13)
9. MERS-CoV; CDSKPPLPDEWV(13)
10. HCoV-4408; CDVKPPVLVDVDDV(13)
11. HEV; CEVKVPLVDVDDV(13)
12. HCoV-OC43; CDVKPPVLDVDDV(13)
13. MHV; CEMRLPLLEEVDI(13)
14. HCoV-HKU1; CEHVIPSTLDLDDI(13)
15. BatCoV; CLNSSEGVPDILLV(13)
16. IBV; CNFQDVQRDKLYS(13)
17. TCoV; NEFPKNGWKNGC(12)
It is interesting to note that SARS-CoV and BatCoV have the same amino acid sequences as coronavirus C-Terminal motif

**DISCUSSION:**
At present, there is no effective treatment for coronavirus infection, and the same as common cold virus infection because constant mutation of the viral infections. After a specific vaccine is developed for specific viral strain, the virus appears to mutate to different strains, and the vaccine becomes ineffective. It appears the best approach is to use a combination of vaccines with up to 16 short peptides.

**Prevention and Treatment of Viral Infection (Common cold)**
The author, male age 88, would like to share his personal experience about Common cold.

**PREVENTION:**
1. Always wear dry cloth: change immediately from wet cloth due to sweat, rain or others.
2. After washing hair, immediately dry it with hair dryer using hot air.
3. Take hot shower or hot bath immediately after getting wet from rain.
4. When drinking beer or wine, keep a distance from coughing person, because alcohol, corticosteroid medication is quite immunosuppressive or weakening the immune system in the body.

**TREATMENT:**
1. Take hot bath to sweat it out when you feel a tingle throat or slight fever. Dry body completely, take two tablets of aspirin, go to bed and keep warm.
2. Always keep body warm and wear dry cloth; change to dry cloth from sweating wet cloth as many times as necessary
3. Do not go outside after catching bad cold, and the body recover or heal much faster with warm body.
4. Eat a lot of hot soup such as home-made chicken soup with garlic, ginger, carrot, celery etc. Avoid any beer or wine drinking when still running a fever.
5. Unless kissing your spouse intensively usually a person cannot catch viral infection from your coughing spouse.
6. The viral infection usually disappeared within a week without any complications.

Because everybody has different body functions, the author disclaims any validity or liability following the above suggestions.

**REFERENCES**
(7) Yu RJ. (2019) Unpublished Results; Entitled “Cancer Peptides For Early Detection and Treatment”

**ABOUT AUTHOR**
Dr. Yu was born on March 23, 1932 in Taiwan. After receiving BS, MS in Chemistry from National Taiwan University, and PhD in Chemistry from University of Ottawa, Canada, Dr. Yu was hired initially as a research scientist, later promoted as associate professor at Temple University Skin Cancer Hospital. In association with Dr. Eugene Van Scott, Dr. Yu discovered the use of alpha-hydroxyacids for topical treatment of aging skin. In 1980, Dr. Yu continued his postgraduate studies at several universities, and received a Doctorate of Oriental Medicine, and was licensed to practice acupuncture and Oriental medicine. After about 20 years at Temple University, Dr. Yu and Dr. Van Scott left Temple and formed a skin care company, NeoStrata Company which was
later sold to J&J in June, 2016. About ten years ago, Dr. Yu became interested in peptide biochemistry and pharmacology. His major research interests are (a) find biomarker peptides for Alzheimer’s Disease (b) find biomarker peptides for breast cancer and pancreatic cancer (c) find physiologic peptides as analgesic substances. The University of Ottawa has published a book about Dr. Yu, entitled “Journey of a Thousand Miles, An Extraordinary Life of Dr. Yu”. The book is available on Amazon.

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