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Nano coronavirus recombinant vaccine taking graphene oxide as carrier

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ABSTRACT :

(EN)
The invention belongs to the field of nano materials and biological medicines, and relates to a vaccine, in particular to development of a 2019-nCoV coronavirus nuclear recombinant nano vaccine. The invention also comprises a preparation method of the vaccine and application of the vaccine in animal experiments. The novel coronavirus vaccine contains graphene oxide, carnosine, CpG and novel coronavirus RBD; The carnosine, the CpG and novel coronavirus RBD are **combined on a framework of the graphene oxide**; the coding sequence of the CpG is as shown in SEQ ID NO 1; and the novel coronavirus RBDrefers to that a novel coronavirus protein receptor binding region can generate a high-titer specific antibody aiming at the RBD in a mouse body, and strong support is provided for prevention and treatment of the novel coronavirus.

DESCRIPTION :
https://patentscope.wipo.int/search/en/detail.jsf?docId=CN317065497&tab=PCTDESCRIPTION

Soon after the SARS-CoV-2 outbreak, different laboratories in China have completed the isolation of the virus strains. This is a big step towards vaccine research and development. I believe we will soon have the ultimate goal of eliminating SARS-CoV-2. Weapons, however, until now, there has not been an approved vaccine or drug for the treatment of CoV infection. Therefore, it is very urgent to develop an effective drug treatment or prevent the infection and outbreak of the coronavirus.

According to research on coronavirus vaccines such as SARS and MERS, the main target of coronavirus vaccines at present is the S protein of coronavirus. Vaccines need not only induce humoral and cellular immune responses, but also induce mucosal immune responses, and use adjuvants to induce a balanced Th1 and Th2 pathway to produce a truly effective vaccine. At

present, more researches on SARS and MERS vaccines are mainly focused on viral vector vaccines and subunit vaccines. A large number of studies have shown that the difficulty of SARS and MERS is that they cannot stimulate the production of long-term memory B cells. In cured SARS and MERS patients, long-term memory cells are only Can last for 2-3 years, unable to produce immune memory, causing vaccine development failure

Failure, so far only 6 potential coronavirus vaccines have entered the clinical research stage, but no effective vaccine has been approved for marketing.

Summary of the invention

The purpose of the present invention is to provide a recombinant coronavirus vaccine.

Another object of the present invention is to provide a method for preparing the virus recombinant vaccine.

Another object of the present invention is to provide the application of the virus recombinant vaccine.

In view of the various problems existing in traditional vaccines, how to change the problems in existing vaccines and enhance the immune response is a problem we have been thinking about. In order to improve the immune activity of immunogens and strengthen the body's immune response capacity, the most basic method is The immunogen is mixed with an adjuvant. The immune adjuvant is a type of promoter that can strengthen the body's immune response to the immunogen. CpG oligodeoxynucleotide (ODN) is a very promising adjuvant discovered in recent years. CpG ODN has been proved to have good adjuvant activity in animals, in vitro and in clinical studies. The most well-researched are CpG7909 and CpG1018. On November 9, 2017, the U.S. FDA approved Dynavax disease cells with limited activating effects and easy It is rapidly hydrolyzed by exonuclease, making it insufficient in vivo stability and causing side effects; CpG oligodeoxynucleotides (ODN) synthesized in the sequence can also enhance the stimulating effect, coupling CpG with antigens and other proteins After combined use, it has a very significant immune activation effect.

Graphene is a two-dimensional carbon nanomaterial with a hexagonal honeycomb lattice composed of carbon atoms and sp^2 hybrid orbitals. Its basic structural unit is the most stable six-membered benzene ring among organic materials, which is currently the most ideal two-dimensional material. Graphene oxide (GO), as a derivative of graphene, is an exfoliated product of graphite oxide. Due to its unique SP2 hybridization, perfect two-dimensional structure, and high reactivity at the edge, it can be used as an ideal load and graft carrier in the design and development of a therapeutic platform based on it, in the nano drug delivery system, Biological testing, tumor treatment, and cell imaging play an important role.

The present invention is completed on the basis of the above research.

The present invention develops a new vaccine development method based on the graphene oxide material to load CpG molecules and recombinant proteins into the skeleton.

Based on this technology platform combined with the recombinant protein of the RBD region of the Spike protein of SAR-CoV-2, a new nano-coronal vaccine was prepared. The prepared nano-new crown vaccine has shown strong immunogenicity in mouse experiments and can produce high-titer antibodies.

In one aspect, the present invention provides a coronavirus vaccine containing graphene oxide, carnosine, CpG, and RBD. In a preferred embodiment of the present invention, it is called GO-Car-carnosine-CpG-RBD vaccine.

Graphene oxide (GO, graphene oxide) is the oxide of graphene. After oxidation, the oxygen-containing functional groups on it increase, making it more active than graphene. For example, hydroxyl groups and epoxy groups are randomly distributed on a graphene oxide monolith, while carboxyl and carbonyl groups are introduced at the edge of the monolith. Common graphene oxide commercially available products are in powder, flake and solution form, and the color is brown-yellow.

Carnosine, the scientific name β-alanyl-L-histidine, is a dipeptide composed of β-alanine and L-histidine, a crystalline solid. Carnosine has a strong antioxidant capacity, which can remove reactive oxygen free radicals (ROS) and α-β unsaturated aldehydes formed by excessive oxidation of fatty acids in cell membranes during oxidative stress.

The CpG motif has the effect of activating the body's immune system and can be used as an adjuvant. Preferably, the coding sequence of CpG is shown in SEQ ID NO 1.

RBD (spike receptor binding domain) is the receptor binding domain. The RBD in the present invention specifically refers to the coronavirus protein (S protein) receptor binding domain (RBD). For example, you can select an RBD protein with the following sequence: The coronavirus vaccine of the present invention is obtained by combining carnosine, CpG and new coronavirus RBD on activated graphene oxide.

In the coronavirus vaccine of the present invention, GO is used as the backbone, and the dosage is usually excessive, and the dosage of carnosine can be about twice that of GO. As biological macromolecules, CpG and new coronavirus RBD are used in a small amount, and the amount of the two is usually one ten thousandth of GO, the mass ratio. The dosage of RBD is more than twice that of CpG, for example, CpG:RBD=1:2-10, preferably, the dosage of RBD is 3-6 times of CpG.

On the other hand, the present invention provides a preparation method of the coronavirus vaccine. The preparation method includes the following steps:

Obtain CpG, RBD recombinant protein and carnosine;

Add the GO freeze-dried powder to the phosphate buffer solution, and sonicate;

Add EDC and NHS to activate the GO solution, remove excess EDC/sulfo-NHS in the reaction solution by ultrafiltration, and adjust the pH of the reaction solution to neutral; Add carnosine, CpG and RBD recombinant proteins to the reaction solution and incubate with activated GO;

Remove excess uncoupled protein from the reaction solution, sterilize, and set aside.

Preferably, the duration of ultrasound is 2-3 hours. The ultrasonic conditions are 200 W, 40 kHz.

In a preferred embodiment of the present invention, the GO-Car-carnosine-CpG-RBD vaccine is prepared by coupling GO to carnosine using an improved method of EDC-NHS reaction, and adding 26 mg of GO freeze-dried powder to 5. In 20 mL of phosphate buffered saline (PBS, pH = 7.4), sonicate (200 W, 40 kHz) at 25°C for 3 h. Add 6.82 mg EDC (N1-((ethylimino)methylene)-N3,N3-dimethylpropane-1,3-diamine at 25°C, Chinese: 1-(3-dimethylaminopropyl)-3-ethylcarbodiamine Imine) and 7.73 mg NHS (NN-hydroxysuccinimide) to activate the GO solution. The excess EDC/sulfo-NHS was removed from the reaction solution by ultrafiltration, and then the pH of the solution was adjusted to 7.4. Then, 40 mg of carnosine, 1.2ug of CpG, and RBD recombinant protein of different concentrations were added to the solution and reacted with activated GO at 25°C for 2 h. Subsequently, the excess uncoupled protein is removed from the reaction solution by ultrafiltration. The prepared product is labeled GO-Car-carnosine-CpG-RBD vaccine. Finally, use a sterile filter
0.22um) Contact GO-Car-carnosine-CpG-RBD vaccine solution and store it in a sterile container at 4°C for subsequent experiments.

The invention establishes a nano recombinant protein vaccine preparation technology platform that can quickly stimulate the human immune system, and can quickly produce a large number of preventive vaccines after the infectious virus is confirmed.

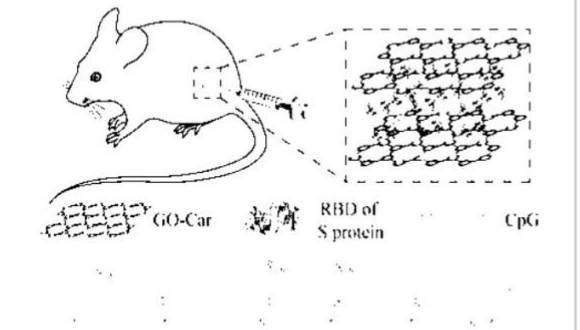
This technology platform makes full use of the characteristics of COOH, hydroxyl and other groups on the surface of graphene oxide, and uses the interaction between π-π bonds to assemble the selected recombinant protein of RBD with CpG molecules and carnosine to prepare A nano recombinant protein vaccine based on graphene oxide as the backbone. The vaccine can stimulate the body to produce high-titer RBD neutralizing antibodies against SAR-CoV-2, laying a solid technical foundation for the prevention and treatment of coronavirus infections and similar outbreaks in the future.

In another aspect, the present invention provides the application of the above-mentioned GO-Car-carnosine-CpG-RBD vaccine, that is, the application of the GO-Car-carnosine-CpG-RBD vaccine in the preparation of drugs for preventing the new coronavirus.

Preferably, the application can improve the body's immunity to the new coronavirus.

Even better, the GO-Car-carnosine-CpG-RBD vaccine can produce specific antibodies against RBD, and the specific antibody has a high titer. In the embodiment of the present invention, the nano-new crown vaccine has shown strong immunogenicity in mouse experiments…

DRAWING:


GO-Car
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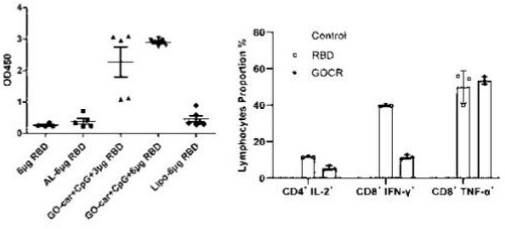
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GO-car+CpG+3μg RBD
GO-car+CpG+6μg RBD
Lipo-6μg RBD
Lymphocytes Proportion %
Control
RBD
GOCR
CD4+ IL-2+
CD8+ IFN-γ+
CD8+ TNF-α+