Exosomes decorated with a recombinant SARS-CoV-2 receptor-binding domain as an inhalable COVID-19 vaccine

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一、 簡述論文概要與重大發現

目前市面上的嚴重急性呼吸道症候群冠狀病毒 2 型(SARS-CoV-2)接種之疫苗，其製造、運輸和施打過程皆須具嚴謹的溫度控管，透過肌肉注射以引發全身免疫反應。此等過程仍耗人力及其他資源甚巨。因此、作者企望藉由 DSPE-PEG-NHS 將 SARS-CoV-2 的受體結合域(RBD) 與 lung spheroid cells 的 Exosome(LSC-Exo)綴合，製成吸入性疫苗，且由疫苗引起的特異性 IgG、IgA 抗體反應可使宿主免受過敏反應侵害。

由吸入性疫苗誘導的 $CD4^+$和 $CD8^+$免疫反應趨向 Th1 免疫路徑，在體內攻擊並清除 SARS-CoV-2 replicon 後，減輕了 SARS-CoV-2 感染所引發 的炎症，可保護宿主器官免受免疫反應引發的發炎損傷。而 SARS-CoV-2 作為呼吸道疾病，利用吸入性疫苗可大大增加黏膜免疫反應，誘導的 $CD4^+$、$CD8^+$等 T 細胞可更快的引發自體免疫反應，且黏膜疫苗接種可能對呼吸道產生殺菌效果，阻止病毒感染。

當前注射為主的接種疫苗需要儲存於-20ºC 或-70ºC 的低溫設備，確保疫苗的安全及穩定性，在運輸過程中需昂貴的運輸費用。此吸入性疫苗可於室溫存放，且已證明疫苗的穩定性，對於疫苗的運輸及存放具有優勢。

二、 對論文內容的提問

疫苗在運送過程中可能經過較高溫的環境，非常溫可比，而在此之中疫苗是否會因為高溫或途中其他變因造成品質的改變?

且作者提到 DSPE-PEG-NHS 與 RBD 之間存在空間位阻效應，其共軛效率是否因外在因素而受影響?

三、 論文的缺點與評論

當吸入性疫苗進入體內且清除體內病毒後，沒有研究證明進入體內的疫苗是否被降解，或是對宿主造成的影響，在接種後的安全性有待考量。

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ARTICLES

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The first two mRNA vaccines against infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that wer approved by regulators require a cold chain and were designed to elicit systemic immunity via intramuscular injection. Here w report the design and preclinical testing of an inhalable virus-like-particle as a COVID-19 vaccine that, after lyophilisation, stable at room temperature for over three months. The vaccine consists of a recombinant SARS-CoV-2 receptor-binding doma (RBD) conjugated to lung-derived exosomes which, with respect to liposomes, enhance the retention of the RBD in both th mucus-lined respiratory airway and in lung parenchyma. In mice, the vaccine elicited RBD-specific IgG antibodies, mucosal Ig responses and CD4⁺ and CD8⁺ T cells with a Th1-like cytokine expression profile in the animals' lungs, and cleared them SARS-CoV-2 pseudovirus after a challenge. In hamsters, two doses of the vaccine attenuated severe pneumonia and reduce inflammatory infiltrates after a challenge with live SARS-CoV-2. Inhalable and room-temperature-stable virus-like particle may become promising vaccine candidates.

The Coronavirus disease 2019 (COVID-19) pandemic has severely impacted financial and social systems[1,2]. Globally, there are at least 36 vaccines against COVID-19 that have been approved by at least one country[3]. Most of them require intramuscular injection, producing antibodies that circulate in the blood but do not necessarily elicit potent mucosal immune responses[4–6]. Because the transmission of acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily occurs via respiratory droplets and the respiratory mucosa is the primary route of viral entry, suboptimal mucosal immunity may limit the utility of intramuscularly administered COVID-19 vaccines. Additionally, some vaccines require deep-freezing for transportation and long-term storage (this is the case for the messenger RNA vaccines manufactured by Pfizer/BioNTech and Moderna). To circumvent such limitations, we sought to develop a vaccine candidate that provides efficient stimulation of mucosal immunity, allows for a non-invasive and needle-free delivery route, and is lyophilisable and stable at room temperature (r.t.) for months.

SARS-CoV-2 belongs to the coronavirus family of viruses. They are enveloped, positive-stranded RNA viruses with spike-protein complexes that recognize and bind to host-cell receptors[7,8]. Specifically, the receptor-binding domain (RBD) in the SARS-CoV and SARS-CoV-2 spike protein S1 subunit binds to the host airway epithelium angiotensin-converting enzyme 2 (ACE2) receptor and then fuses the viral and host membranes through the S2 subunit, making the RBD a specific target for neutralizing antibodies and vaccines[9–12]. Previous studies have demonstrated the efficacy of SARS-CoV RBD as the target of potently neutralizi antibodies[13,14]. In vitro studies of SARS-CoV-2 show host-antibo engagement with the RBD, binding to it and exerting a neutralizi effect[15]. It also blocked the entry of SARS-CoV-2 and SARS-Co into ACE2-expressing host cells, suggesting its potential as a vir attachment inhibitor. However, the administration of the RBD alo does not allow for specific targeted delivery and does not evade de radation or rapid clearance. The RBD must be protected through drug-delivery carrier that optimizes dosage to the antigen presen ing cells (APCs).

Virus-like particles (VLPs) and nanoparticles (NPs) are powe ful drug-delivery carriers[16]. In particular, exosomes are a type naturally occurring extracellular vesicle found in the body, whi makes them a native and ideal delivery vesicle for targeted dr delivery[17,18]. Because they carry and express their parent cell's RNA proteins and lipids, and because they express surface proteins ar receptors from the parent cell, they are superior at targeting san tissue-recipient cells[19,20]. They contain a cocktail of molecular con ponents composed of proteins, lipids and nucleic acids with ther peutic properties[21]. Furthermore, exosomes can be engineered b creating surface modifications to express proteins or peptides enhance targeting[19,22].

We have derived lung spheroid cells (LSCs) from human lu donor samples[23]. Their regenerative abilities have been demo strated in rodent models[22,24] and are being tested in a human clinic trial (HALT-IPF, Human Autologous Lung stem cell Transplant f Idiopathic Pulmonary Fibrosis) (www.clinicaltrials.gov)[25]. We ha

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