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THE UNIVERSITY of EDINBURGH

RAPID RESEARCH IN COVID-19 PROGRAMME

A novel platform technology for industrial-scale production of an immune-targeted Covid-19 vaccine

AIMS

The aim of this project was to develop a new platform to mass produce a high-efficacy vaccine against SARS-CoV-2 by adding immune cell-specific signals to part of the Spike protein of SARS-CoV-2 to enhance the immune response generated against the virus. The platform is compatible with existing pharmaceutical production methods and can be adapted to produce vaccines against new variants of SARS-CoV-2 and other emerging public health threats.

KEY FINDINGS

- A novel vaccine against SARS-CoV-2 was designed by adding a ‘signal’ for uptake by human immune cells to the part of the SARS-CoV-2 Spike protein that binds to cells in the lungs. To detect and purify the vaccine in the lab, green fluorescent protein and 6XHis tags, respectively, were also added that could be removed later.
- DNA sequences coding for the whole vaccine, as above, and the individual parts were synthesised in the lab. A version of the vaccine in which the signal for uptake by immune cells was inactive was also synthesised as a control.
- These synthetic ‘genes’ were maintained in a lab workhorse strain of the bacterium *Escherichia coli* but were then transferred to another bacterial species, *Streptomyces lividans*, which can both make the vaccine and also produce the ‘signal’ for uptake by human immune cells.
- Production of the vaccine in *Streptomyces lividans* was found to have toxic effects on the bacteria. We have partially solved this problem by changing the medium in which the cells are grown but efforts are ongoing to alleviate this metabolic burden on *Streptomyces lividans* and improve vaccine production.

WHAT DID THE STUDY INVOLVE?

In this project, our aim was to develop a novel vaccine platform technology using the inherent properties of a bacterium called *Streptomyces* which can potentially produce a 'signal', by addition of specific sugars, to improve uptake of vaccines by human immune cells. These sugars are recognised by immune cells, which makes uptake of vaccines more efficient, thus generating a better immune response. To make a high-efficacy vaccine against SARS-CoV-2, we selected a part of the SARS-CoV-2 Spike protein that binds to human lung cells to act as the antigen or immune target. To this protein, we added a specific sequence which would allow addition of immune cell-specific sugars. We synthesised unique genes encoding this novel vaccine and then introduced them into *Streptomyces*, a bacterium routinely used as microbial factories for industrial-scale production of important antibiotics. Developing a vaccine platform that would work in *Streptomyces* would therefore allow large-scale vaccine production using existing infrastructure and with reduced costs compared to other methods. Our vaccine is a unique fusion protein consisting of a number of distinct parts: (1) a *Streptomyces*-specific secretion signal, to release the vaccine from the bacterial cells; (2) immune cell-specific signal, to enhance vaccine uptake by immune cells; (3) the part of the SARS-CoV-2 Spike protein that binds to human cells; (4) green fluorescent protein (GFP), to allow visualisation of vaccine uptake by immune cells; and (5) a 6xHis tag, to allow vaccine purification. A form of the vaccine was also designed to make the 'signal' inactive and serve as a control which would allow us to measure the effectiveness of the 'signal'.

The unique DNA sequences to produce vaccines with active and inactive signals were optimised for production in *Streptomyces*. For risk management, DNA sequences to produce individual parts of the vaccine were also generated. These synthetic genes were introduced into plasmids, which are small circular segments of DNA that can be transferred between bacterial species. The plasmids were maintained in *E. coli*. Once their fidelity was confirmed by DNA sequencing they were transferred into *Streptomyces lividans* for vaccine production. This species of *Streptomyces* was chosen because it has been shown to be a good system for protein production at industrial scale.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

We first introduced the plasmids with the synthetic genes to produce vaccines with active and inactive signals (Figure 1), into *Escherichia coli*. PCR screening was then performed to confirm that the plasmids had been successfully transferred. This was followed by DNA sequencing to re-confirm the fidelity of the plasmids and ensure no changes had occurred. We then tried to introduce the plasmids into *Streptomyces* using a method which uses electricity to punch holes in the cells to allow the DNA to enter. Despite repeated attempts using numerous different protocols, the frequency of DNA entry remained low. To overcome the potential problem of *Streptomyces* destroying foreign plasmids, we attempted to introduce the plasmids by interspecies mating in which DNA can be transferred by direct contact between cells. The plasmids were introduced into a specific strain of *E. coli* that was then mated with *Streptomyces.* Control experiments using only the plasmids without the synthetic genes demonstrated high frequencies of plasmid transfer but mating frequencies for plasmids with the synthetic genes were low suggesting there was a biological penalty when introducing the synthetic genes into *Streptomyces*. The small number of *Streptomyces* that did maintain the plasmids with the synthetic genes were cultured and vaccine production was induced using an agent called thiostrepton. Cultures of these strains grew slowly compared to control cultures and examination by microscopy showed the presence of considerable amounts of cell debris, indicating that cells were dying. Fluorescence microscopy revealed low levels of fluorescence in both the bacteria and debris, suggesting that the vaccine was being produced by *Streptomyces*, albeit at low levels and at a significant cost to fitness. At this stage, we do not know which part(s) of the vaccine could be responsible for this fitness cost, but subsequent modifications to the medium in which the cells are grown have improved the viability of the *Streptomyces* cells and will allow us to improve vaccine production.

Figure 1. Design of the novel vaccine against SARS-CoV-2. This fusion protein consists of a number of distinct parts: (1) a *Streptomyces*-specific secretion signal, to release the vaccine from the bacterial cells; (2) immune cell-specific signal, to enhance vaccine uptake by human immune cells; (3) the part of the SARS-CoV-2 Spike protein that binds to human cells; (4) green fluorescent protein (GFP), to allow visualisation of vaccine uptake by immune cells; and (5) a 6xHis tag, to allow vaccine purification. The control vaccine with an inactive signal was made by modifying the signal so that the sites for sugar addition were removed.

WHAT IMPACT COULD THE FINDINGS HAVE?

This ‘proof of concept’ study demonstrates the successful construction of synthetic genes which express a part of the SARS-CoV-2 Spike protein that binds to human cells and which is fused to an immune cell-targeting signal that could enhance vaccine efficacy. In addition to being a useful tool to produce vaccines against COVID-19, this vaccine platform could be used as a ‘plug and play’ system to produce high-efficacy immune-targeted vaccines against other pathogens by replacing the Spike protein with any target from a pathogen of interest. As this vaccine platform is designed for *Streptomyces* that are routinely used as microbial factories for industrial-scale production of important antibiotics, existing infrastructure can be re-purposed for rapid vaccine production on national and global levels. Producing vaccines cheaply in systems that require minimal modification or purification steps, and with reduced requirements for a significant cold-chain is particularly relevant for countries with limited finances and infrastructure, especially when millions of doses are required.

HOW WILL THE OUTCOMES BE DISSEMINATED?

The outcomes of this research will be presented via seminars and posters at the local level and disseminated to the scientific community at scientific meetings. Public engagement will be achieved using outreach activities such as the Edinburgh Science Festival, the Midlothian Science Festival, and the University of Edinburgh Open Doors events. In the immediate future, funding will be sought to address the issues raised by this research, especially understanding which parts of the vaccine could be affecting the viability of *Streptomyces*. This will be done by using the DNA sequences designed to produce individual parts of the vaccine that were generated as part of the risk management strategy. Our ongoing work will therefore focus on improving the growth of the *Streptomyces* vaccine strain and enhancing vaccine production.

CONCLUSION

This project represents a step towards a second generation of SARS-CoV-2 vaccines with enhanced efficacy that are cheaper, easier to produce and do not require extremely low temperatures for storage. National and global efforts to immunise populations against SARS-CoV-2 require large-scale production at a cost sufficient to sustain repeated vaccination since immune responses potentially reduce over time. In addition, the mutations that are arising in the Spike protein of the SARS-CoV-2 coronavirus demonstrate that new vaccines will need to be produced rapidly on a regular basis, and at a reasonable cost, to enable continuous protection of communities from infection. The societal disruption cause by COVID-19 has emphasised the importance of vaccines for public health and has highlighted the need for a Scottish centre for vaccinology which has the expert scientific knowledge required to respond to imminent threats caused by new infectious diseases. The vaccine platform developed during this work has the potential to be expanded to develop vaccines against other important and emerging pathogens but will require significant financial support from government, funding bodies and relevant industrial partners.

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ADDITIONAL INFORMATION

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