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An oral mucosal DNA vaccine for SARS coronavirus infections

Key Messages

1. When different forms of SARS coronavirus (SARS-CoV) spike protein-based vaccines for generation of a neutralising antibody response to SARS-CoV were injected into a mouse model, all the mice immunised with intramuscular tPA-optimise800 DNA vaccine boosted with intraperitoneal recombinant spike polypeptide generated by *Escherichia coli* and intramuscular CTLA4HingeSARS800 DNA vaccine boosted with intraperitoneal S-peptide had neutralising antibody titres of $\geq 1:1280$.
2. This observation may have major practical value for field studies, such as the immunisation of civet cats, as the cost of recombinant proteins produced by *E coli* is much lower than those produced by eukaryotic systems.
3. This study indicates that the type of vaccine used for priming is crucial for determining the type of immune response developed. Subsequent doses will boost the immune response generated by the first dose of vaccine.

Introduction

The 2003 severe acute respiratory syndrome (SARS) outbreak was the first epidemic caused by a coronavirus and resulted in a fatality rate of approximately 10%.

Coronavirus spike proteins have been shown to be highly immunogenic, and able to produce neutralising antibodies effective for prevention of infections caused by the corresponding coronaviruses when introduced into animals. There are no data on less expensive modalities of immunisation, such as DNA vaccination followed by booster doses of recombinant vaccine produced by *Escherichia coli* or oral mucosal DNA vaccines.

Methods

This study was conducted from September 2004 to August 2006.

Study design

To compare the neutralising antibody response to SARS-CoV generated by different forms of SARS-CoV spike protein-based vaccines comparison groups of mice were immunised with the following vaccines: recombinant spike polypeptide vaccine produced by *E coli*, two different types of intramuscular spike polypeptide DNA vaccine with and without boosters of recombinant spike polypeptide vaccine produced by *E coli* and two different types of oral mucosal spike polypeptide DNA vaccine with and without boosters of recombinant spike polypeptide vaccine produced by *E coli*.

Animals and immunisation schedule, ELISA, and neutralising antibody assay

Details of the experimental protocol have been reported.¹

Results

Among all groups of mice, sera of all the mice immunised with i.m. tPA-S-DNA boosted with i.p. S-peptide and i.m. CTLA4-S-DNA boosted with i.p. S-peptide showed the highest neutralising antibody titres of $\geq 1:1280$. Details of the results have been reported.¹

Discussion

Of all groups, the mice primed with SARS-CoV human-codon-usage-optimised spike polypeptide DNA vaccines and boosted with S-peptide produced by *E coli* generated the highest neutralising antibody titres against SARS-CoV. It has been observed, and was confirmed by the present study, that S-peptide produced by *E coli* does not induce neutralising antibodies to SARS-CoV infection. This is probably because when S-peptide produced by *E coli* was used, the three dimensional folding and/or the glycosylation of the S-peptide was not optimal for the generation of neutralising antibodies. In this study, we documented that, although recombinant S-peptide produced by *E coli* itself was not able to generate neutralising antibodies against SARS-CoV infection, mice primed with spike polypeptide DNA vaccine and boosted with S-peptide from *E coli* were able to generate high titres of neutralising antibody against SARS-CoV. This indicates that the type of vaccine used for priming is crucial for determining the

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type of immune response that develops. Subsequent doses will boost the immune response generated by the first dose of vaccine.

This observation may have major practical value in areas such as the immunisation of civet cats. Production of recombinant proteins from *E coli* is far less expensive than production of recombinant proteins using eukaryotic systems, such as transfection of cell lines, or DNA vaccines. Although it has been shown that DNA vaccines are able to successfully generate both humoral and cellular immunity to various pathogens in mice, one of the major limitations for their clinical use is their ineffectiveness when used in humans, unless a large amount of DNA is used for immunisation. It is difficult to scale up the levels of production of eukaryote-generated recombinant proteins to industrial levels. Therefore, the large amount of S-peptide that is produced by *E coli* in a relatively inexpensive way could be used as booster doses instead of being injected alone as vaccine. This principle can also be examined in vaccination for other pathogens, where 'more effective' modalities of vaccination, such as DNA vaccines, can be used for priming, and the 'less

expensive' recombinant protein produced by *E coli*, instead of eukaryotic systems, can be used as booster doses.

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Reference

1. Woo PC, Lau SK, Tsoi HW, et al. SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from *Escherichia coli* as booster induces high titer of neutralizing antibody against SARS coronavirus. *Vaccine* 2005;23:4959-68.