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Opinion Article

Development and Detection of *Yersinia pestis* and Protection against Pneumonic Plague

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INTRODUCTION

The zoonotic illness known as plague is brought on by *Yersinia pestis*, a rod-shaped, gram-negative *Coccobacillus* that is typically found in rodents and can be spread to people by a flea bite. Bubonic (caused by a flea bite), pneumonic (caused by respiratory droplets), and septicemic plague are the three main clinical types of the illness. Due of its high mortality and simple person to person transmission, *Y. pestis* is categorised as a category "A" agent by the NIAID, USA. The traditional diagnostic techniques for *Y. pestis* are ineffective because of cross-reactivity with other enteropathogenic bacteria. To accurately detect *Y. pestis*, a molecular test that is sensitive and focused is required [1].

Although PCR is a good molecular biology method for a quick diagnosis of the plague, it takes more time to evaluate the amplified result using agarose gel electrophoresis once the heat cycling processes are finished. For the quick identification of *Y. pestis*, a lateral flow strip assay has been devised. Gel electrophoresis is an option, although lateral flow strips provide for simple and quick detection of PCR results. *Y. pestis* specific 5′ 6-FAM and biotin-tagged primers were used in the PCR to target the chromosomal *yihN* gene. Latitudinal flow strips were used to examine the PCR product, and the results were available in 2 minutes–3 minutes.

The PCR-Lateral Flow (PCR-LF) assay's analytical sensitivity was 1 g of *Y. pestis* genomic DNA and 500 copies of the target DNA sequence contained in a recombinant plasmid. The test was able to identify *Y. pestis* DNA isolated from human blood samples that had been spiked and contained 104 CFU per mL of bacteria [2]. The test was shown to be precise and to have no cross reactions with bacterial species that are closely related to it. The newly created test was very sensitive, extremely specific, and did not require post-amplification electrophoresis on agarose gels. Humans are an unintentional host for the plague's causative agent, *Yersinia pestis*, which mostly affects rodents. It is challenging to detect *Y. pestis* since the traditional diagnostic techniques are cross reactive with other enteropathogenic bacteria.

For prompt treatment to begin, *Y. pestis* must be quickly and accurately detected at the point of care. A pair of loop mediated isothermal amplification (LAMP) assays has been created in the current work for the quick identification of *Y. pestis*. In order to selectively target the *Caf1* and *3a* genes on the pFra plasmid and chromosome of *Y. pestis*, two sets of LAMP primers, each containing six primers, were created [3]. For the *caf1* target, isothermal amplification was completed at 65°C for 40 minutes, and for the *3a* choromosomal target, at 63°C for 50 minutes. The assay's analytical sensitivity for the *caf1* and *3a* targets was discovered to be 500 g and 100 g of *Y. pestis* genomic DNA, respectively.

DESCRIPTION

As few as 100 copies of the *Caf1* and *3a* gene targets, respectively, and 10 copies of the *3a* gene targets, were found by the *caf1* and *3a* LAMP tests. Using SYBR green 1 dye, the enhanced products could be seen under visible and UV light. Due to the test pair's lack of cross-reactivity with other bacterial species and closely related bacteria, it was discovered to be highly specific. If left untreated, the pneumonic plague's *Yersinia pestis* causes an extremely fatal illness. However, USAMRIID has created a recombinant fusion protein, F1-V, which has been demonstrated to generate protection against pneumonic plague. There is currently no FDA-approved vaccine against this infection [4].

There are few examples of quick induction of protective immunity (14 days post-vaccination (DPI)), and many F1-V based vaccine formulations need prime-boost immunisation to produce protective immunity. Cyclic Dinucleotides (CDNs), which are the stimulator of interferon genes agonists, have demonstrated promise as vaccine adjuvants. Due to their dual roles as adjuvants and delivery mechanisms, polyanhydride nanoparticle-based vaccinations (*i.e.*, nanovaccines) have also demonstrated the ability to improve immune responses. A combination nanovaccine was created that combines CDN, dithio-RP, RP-cyclic di-guanosine monophosphate, and F1-V-loaded nanoparticles to quickly and effectively develop protective immunity against pneumonic plague.

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At challenge doses of 7000 CFU *Y. pestis* CO92, all mice vaccinated with a single dose combination nanovaccine showed improved protection over F1-V adjuvanted with CDNs alone and were protected against *Y. pestis* fatal challenge within 14 DPI. Additionally, 78% of mice who received a single dose of a combination nanovaccine were resistant to being challenged at 182 DPI while maintaining high levels of antigen-specific blood IgG. In mice inoculated with CDN adjuvanted F1-V or the combination nanovaccine, ELISPOT examination of vaccinated animals at 218 DPI indicated long-lived plasma cells specific for F1-V.

CONCLUSION

These immunised mice's serum was examined using a microarray and found to contain serum antibodies that bound to a variety of F1 and V linear epitopes. These findings show that inducing fast and long-lasting resistance mechanisms against *Y. pestis* is possible when CDN adjuvanticity is combined with such a nanovaccine delivery system.

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