Influence of Different Antigen Administrations on the Production of Antibodies against Clostridium perfringens Beta Toxin

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Abstract: Clostridium perfringens beta toxin (CPB) is a toxin produced by C.perfringens that is responsible for hypertension and necrotic enteritis in mammals. Currently, DNA vaccines have been implemented to avoid such toxins. Previous studies have shown that heterologous prime boosting helps with the identification of CPB by producing more antibodies. However, there was no suggestion that differences in the administration of antibodies can further influence the production of antibodies. The study focuses on the optimal injection administration of DNA vaccine and the heterologous protein boost targeting CPB. The study provides important information to maximize the efficiency of immune response against CPB from the vaccine for mammals.

1 INTRODUCTION

Th1 and Th2 cells are two types of T helper cells which leads to an increase in cell-mediated response against virus, bacteria, and other possible pathogens (Kidd 2003). Specifically, Th1 cells produce interferon-gamma, interleukin -2, and tumor necrosis factor (TNF). Th2 cells produce IL-4, IL-5, IL-10, IL-13 (Romagnani 1999). These cytokines are responsible for a strong antibody production and the activation of eosinophil. These two immune cells are essential to the adaptive immune system to carry out its goal in fighting off infections.

Clostridium perfringens beta toxin (CPB) is responsible for necrotic enteritis, a disease that can be seen on humans after consuming unclean meat, in mammals and causes hypertension. It is one of the four major toxins produced by Clostridium perfringens. Recent studies have shown that by using a heterologous prime-boosting strategy in the DNA vaccine, the amount of anti-rCPB antibody response was stronger in comparison to a homologous boosting strategy. Heterologous strategy triggers both Th1 and Th2 response and the balance in response was necessary for the success of the vaccine. A possible link to the success is the increase in both levels of IgG1 and IgG2b antibodies in comparison to the IgG2b only reaction in homologous boosting response. However, there was no evidence suggesting that the administration of antigens influences the immune response by resulting in a better identification and neutralization of the toxin (Solanki, A. K., Bhatia, B., Kaushik, H., Deshmukh, S. K., Dixit, A., & Garg, L. C. 2017). Previous study has shown that Th1 and Th2 immune responses are not strictly synchronized because Th2 immune responses suppress the activities of phagocytosis. Under the infection of large eukaryotic pathogens, Th1 cell response can be seen as protective and Th2 cell response would resolve the inflammation (Romagnani 1999).

There are seven discovered routes which antigen administration has in the humoral immune response: intravenous, intraperitoneal, intermuscular, intranodal, intrasplenic, intradermal, and subcutaneous. This study will focus on intraperitoneal, intermuscular, and intradermal routes. Previous study suggests that different antigen administrations have no significant influence on antibody production. I hypothesize that the introduction of vaccine into the host of clostridium perfringens beta toxin will result in most improvement in the production of antibodies measured by ELISA and a better identification and neutralization of the toxin after immunization through the intradermal, following intramuscular, and lastly intraperitoneal.

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2 MATERIALS

2.1 Cells Used in the Study

E. coli DH5α and BL21 pLysS cells were used in the study along with DNA information and modifying enzymes.

2.2 Genetic Information of the CPB

Genetic information of the beta toxin in this study was used to optimize reactions. Genetic information will also be later used to introduce the booster shot in all mices. In order to fit the large requirements in this study. This piece of genetic information will be replicated through the process of PCR.

2.3 ELISA (Enzyme-Linked Immunosorbent Assay)

The antigen-specific antibodies of each group will be read by ELISA. In the study the antibody will be specific to Anti-rCPB antibodies.

2.4 Mice Immunization

In this study, each experimental group will contain 3 mice. Each mice will be immunized with 100ug of plasmid DNA following a booster shot of rCPB on day 15 after immunization. There will be a total of 29 groups of mice in this study to examine specific differences. They will be the 27 possible results presented by the different combinations of antigen administrations along with positive control group and negative control group. The positive control group will receive the inactivated bacteria. In addition, the negative control group will receive saline at all routes.

2.5 Data Analysis

Significance of all levels of antibody production measured by ELISA will be analyzed on TI-84 Plus CE. ($p<0.05$)

3 RESULTS

Possible results on the production of antibodies for different antigen administrations. All 27 possible results can be seen at Table 1 Different Antibody Production Measured by ELISA.

3.1 Possible Result #1

DNA vaccine administered through the intramuscular route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.2 Possible Result #2

DNA vaccine administered through the intradermal route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.3 Possible Result #3

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.4 Possible Result #4

DNA vaccine administered through the intramuscular route, receiving booster shot through the intradermal route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.5 Possible Result #5

DNA vaccine administered through the intramuscular route, receiving the booster shot through the intraperitoneal route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.6 Possible Result #6

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the intramuscular route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.7 Possible Result #7

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the intradermal route. The production of antibodies in these specific combinations will not

produce any antibodies against CPB.

3.8 Possible Result #8

DNA vaccine administered through the intradermal route, receiving the booster shot through the intramuscular route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.9 Possible Result #9

DNA vaccine administered through the intradermal route, receiving the booster shot through the intraperitoneal route. The production of antibodies in these specific combinations will not produce any antibodies against CPB.

3.10 Possible Result #10

DNA vaccine administered through the intramuscular route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.11 Possible Result #11

DNA vaccine administered through the intradermal route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.12 Possible Result #12

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.13 Possible Result #13

DNA vaccine administered through the intramuscular route, receiving booster shot through the intradermal route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.14 Possible Result #14

DNA vaccine administered through the intramuscular route, receiving the booster shot through the intraperitoneal route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.15 Possible Result #15

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the intramuscular route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.16 Possible Result #16

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the intradermal route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.17 Possible Result #17

DNA vaccine administered through the intradermal route, receiving the booster shot through the intramuscular route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.18 Possible Result #18

DNA vaccine administered through the intradermal route, receiving the booster shot through the intraperitoneal route. The production of antibodies in these specific combinations will produce high enough antibodies against CPB to efficiently eliminate the bacteria.

3.19 Possible Result #19

DNA vaccine administered through the intramuscular route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.20 Possible Result #20

DNA vaccine administered through the intradermal route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.21 Possible Result #21

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the same route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.22 Possible Result #22

DNA vaccine administered through the intramuscular route, receiving booster shot through the intradermal route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.23 Possible Result #23

DNA vaccine administered through the intramuscular route, receiving the booster shot through the intraperitoneal route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.24 Possible Result #24

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the intramuscular route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.25 Possible Result #25

DNA vaccine administered through the intraperitoneal route, receiving the booster shot through the intradermal route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.26 Possible Result #26

DNA vaccine administered through the intradermal route, receiving the booster shot through the intramuscular route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

3.27 Possible Result #27

DNA vaccine administered through the intradermal route, receiving the booster shot through the intraperitoneal route. The production of antibodies in these specific combinations will produce a low amount of antibodies against CPB that doesn't support an efficient response to eliminate the bacteria.

Table 1 Different Antibody Production Measured by ELISA

Results	Antibody Production	
Result 1	-	
Result 2	-	
Result 3	-	
Result 4	-	
Result 5	-	
Result 6	-	
Result 7	-	
Result 8	-	
Result 9	-	
Result 10	++	
Result 11	++	
Result 12	++	
Result 13	++	
Result 14	++	
Result 15	++	
Result 16	++	
Result 17	++	
Result 18	++	
Result 19	+	
Result 20	+	
Result 21	+	
Result 22	+	
Result 23	+	
Result 24	+	
Result 25	+	
Result 26	+	
Result 27	+	
Negative Control	-	
Positive Control	+	
Note: + represent low antibody production and ++represent high antibody production	Note: - represents no antibody production	

4 DISCUSSION

Previous studies have shown that there would be an increase in immune response when an extra booster shot is injected in addition to the shot of DNA vaccine targeting CPB. This study applied the ELISA method to examine how the production of antibodies can be influenced by different methods of administration. The hypothesis made for this experiment is based on a logical assumption. Intradermal injection is not common for vaccination because it requires a difficult technique which slows down the efficiency to vaccinate the population. However, areas of intradermal injection generally contain large amounts of dendritic cells, which are immune cells that present antigens to the adaptive immune system, where antibodies are produced. However, with livestock vaccination, it would be more ideal than intramuscular injection since some mammals have a thick skin layer. Therefore, I hypothesize that intradermal would be the most effective out of the three routes. Intermuscular, or the injection of vaccine in the muscle, is predicted to have less antibody production when compared to intradermal injection because muscles have more blood supplies which indicates that as cytokines are released, it will be easier for neutrophils to reach the site of injection and process the materials injected into the muscles and absorb the information. The least effective method of three would be intraperitoneal for various reasons. This injection method is largely used in mice rather than humans or larger cattles since larger mammals would require a larger needle to be able to

reach the abdomen. In addition, this injection route is usually for a large concentration of fluid which is not a typical feature of vaccines. Booster injections may also play a role in the production of antibodies. Since the immune system would already have memories of CPB after the injection of the vaccine, it would be the same logic as the initial exposure to antigen, placing the injection site at a location with easier absorption and presentation of antigens would result in the most production of antibodies.

The possible results that are provided by Table 1. It presents three different possible results in the production of antibodies for a specific injection combination. When there are no antibodies produced, the possible explanation is that the specific injection site doesn't trigger the immune system at all. Previous studies proved that the DNA Vaccine would trigger an immune response and produce specific antibodies against the toxin (Solanki, A. K., Bhatia, B., Kaushik, H., Deshmukh, S. K., Dixit, A., & Garg, L. C. 2017). However, if the method did not produce any antibodies for all mice in a group with the same route for both the injection of booster protein and vaccine, it signifies that the route somehow does not trigger an immune response inside the mice, meaning that the immune cells involved in the innate immune response does not recognize any pathogen and thereby not presenting any antigen for the production of antibodies. I believe that such a situation is not likely. However, there is no evidence to prove the nonexistence of such possibility nor evidence of the principle behind the observation.

When looking at the result, the results of mice groups of the two routes that are identical to each of the pathways should be compared. For instance, the results of possible result #14 presents two different routes: intramuscular and intraperitoneal. Hence the production of antibodies of result #1 and 3 should be compared with result #14. If both of them function properly, this data signifies that the route of injection is able to effectively trigger an immune response as protein or vaccine is injected at the site.

On the other hand, it would be obvious that there are other factors contributing to the lack of antibodies produced. Similar to the first observation, such a result is unlikely to occur. There is also the possibility that no antibody binded to the plate in ELISA. However, since there are multiple serums of mice being tested, it is quite unlikely that no antibody attached to any of the receptors in the plates.

Other possible observations of the presence of produced antibodies against CPB are much more likely to occur in reality. If a general trend is discovered in the difference between each combination of antigen administrations, specifically the difference between a high production and a low production, it is possible that the observation is a result of the efficient connection between the antigen receiving B cells and the activated B cells that are responsible for the production of antibodies. However, each mammal may have different features of the immune system. It is not possible to test an individual mammal multiple times on the specific vaccines since the immune system would already have memory of the antigen. Therefore, antibody production using a separate vaccine on the same mammal can be observed to discover if the low production of anti-CPB is a result of ineffective delivery of antigen information or of injection sites.

5 CONCLUSION

This study explores the influences of different injection administration on the production of antibodies against Clostridium perfringens beta toxin. It is predicted that intradermal injection will produce the most amount of antibodies, followed by intramuscular injection having the second most production and intraperitoneal being the least effective. The possible results predict the potential relationship between the identification of antigen after initial injection and the delivery of information in the immune system. Since the hypothesis made in this study can only be directly supported or rejected with numbers, Table 1 suggests that results #1, 3, 5, 6, 11, 13, 16, 17, 18, 19, 21, 23, and 24 all partially support the hypothesis and #2, 4, 7, 8. 9. 10. 12. 14, 15, 20, 22, 25, 26, 27 all partially rejected the hypothesis. In addition, since the use of DNA vaccine has been prevalent on livestock to prevent economic losses, the result of the study will be able to advance the efficiency of vaccination. However, since there are more than three injection administrations and different animals support different injection methods, further comparison between all the routes is necessary before concluding the most efficient method for the vaccination of Clostridium perfringens beta toxin on different mammal species.

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