Iraqi Journal of Veterinary Sciences, Vol. 36, Supplement I, 2022 (77-84)
Proceedings of the 7th (1st International) Scientific Conference, College of Veterinary Medicine, University of Mosul

Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Evaluation of immunogenicity and efficacy of *Borrelia burgdorferi* derived peptide (PepB) antigen formulated in a protein scaffold in mice against Lyme disease

W.S. Hassan[1], C.M. Brock[2] and M. Esteve-Gasent[2]

[1]Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, [2]Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, TX, USA

Article information

Article history:
Received September 14, 2022
Accepted November 09, 2022
Available online November 29, 2022

Keywords:
Lyme disease vaccine
Borrelia burgdorferi
Protein scaffold
Peptide antigen

Correspondence:
W.S. Hassan
wissamsaleem@uomosul.edu.iq

Abstract

Lyme disease (LD) is the most prevalent tick-borne disease in both Europe and North America. The development of effective Lyme disease vaccines is complicated by the complex biology of *Borrelia* species and alterations in the expression of outer surface membranes. In this work, PepB, which is *Borrelia burgdorferi* BB0172-derived peptide was evaluated in scaffolded formulation as a vaccine candidate in murine model of LD. In brief, four groups of 6-8 weeks old C3H/HeN breed of mice (n=6 per group) were immunized subcutaneously with BBA34: PepB (BP), outer surface protein C (OspC), BBA34 and a naïve control group. In 8 weeks, post-priming blood samples were collected, and specific IgG titers were evaluated by ELISA. After that, A dose of 10^5 *B. burgdorferi* /mouse was administered subcutaneously to all animals as a challenge. The mice were euthanized at 4 weeks post-challenge and then blood and tissue samples were collected to evaluate bacterial burden by real time qPCR, and bacterial recovery from tissues. Taken together, and considering both bacterial burden and bacterial recovery, immunization with BP was not able to confer protection against borreliosis in this experiment, and consequently it was not considered as a potential vaccine formulation in subsequent studies. It was concluded, that other alternative antigen platforms and delivery methods might be considered to improve the immunogenicity of the PepB-based vaccine candidate.

DOI: 10.33899/ijvs.2022.135519.2487,

Introduction

Lyme disease (LD) is a zoonotic tick-borne disease caused by bacterial spirochetes within the *Borrelia burgdorferi* sensu lato complex. In humans, the disease manifests as the emergence of the recognizable skin rash (erythema migrans), arthritis, cardiac, and neurological involvements. The disease is mainly transmitted by hard ticks of *Ixodes* spp (1). The most successful preventive strategy to reduce the prevalence of LD worldwide is vaccination; unfortunately, no vaccine is currently accessible for human use. To date, a lot of Scientific works has been applied into creating a vaccine that will protect humans. Finding a conserved antigen to combat the variety of Lyme spirochetes is difficult, though. Protein scaffold is an alternative method used to generate immunogenicity of poorly or non-immunogenic antigens (2,3). Naturally synthesized proteins with rigid, well-defined structure and intrinsic stability are considered effective scaffold that can be used for antigen presentation (4,5). Some studies revealed that inserted epitopes into an unrelated scaffold protein structure was a feasible strategy to display the HIV-

1 gp41 epitopes 2F5 and 4E10. In such experiments, scientists observed that after immunization, the scaffolded antigens produced significant high-binding affinity antibodies compared to the epitope peptide alone (4,6). Suitable scaffolds are molecules with sufficiently broad molecular surface areas that can withstand replacements or insertions without compromising the integrity of the overall three-dimensional structure (7). Also, scaffolds are considered efficient immunogens in many respects to other antigen presentation platforms, including peptide conjugates (3).

Reverse vaccinology has enabled us to discover and characterize the antigenic maps of different infectious agents, and the structural vaccinology, which tremendously assist in identifying the three-dimensional structure of pathogen-specific components, and potentially recognize pathogen-specific antigens as well (8). Therefore, the epitope-based vaccination can be used to mainly target critical neutralizing epitopes, and avoid inducing non-protective antibodies in vaccinated individuals (9).

There are a number of scaffolding alternatives such as Virus-Like Particles (VLPs) and backbone grafting. The virus-like particles (VLPs) have been used as vaccine development platforms for years. Furthermore, VLP scaffolds have been used to present immunogenic antigens from various viral, bacterial, or parasitic pathogens, known as chimeric VLPs (10). Viral capsid proteins can be synthesized *in vitro* through exploiting the advancing biotechnology, and creating self-assembled, empty virus-like particles (11-13). Furthermore, VLPs can be presented by Antigen Presenting Cells (APC) and stimulate B and T-lymphocytes. Therefore, scientists have taken advantage of these immunogenic viral particles and used them as platforms to display different antigens with the objective of inducing a strong immune response (12). Backbone grafting is a strategy that implies using protein scaffolds as a presentation platform, in which a protein backbone can be engrafted with different epitopes (14,15). Correia and collaborators showed that a viral epitope, derived from respiratory syncytial virus and inserted in a protein scaffold-based structure was able to elicit potent neutralizing antibodies (16).

Borrelia burgdorferi has a single peptide transport system of the ABC transporters family, which resembles oligopeptide permease (Opp) and dipeptide permease (Dpp) transport systems that are found in other bacteria (17,18). BBA34 is a 61 kDa surface lipoprotein, and one of five oligopeptide permease A homologs present in *B. burgdorferi*, and is designated OppA5 (19). Furthermore, BBA34 participates in the transport of solutes like acetate and bicarbonate. Moreover, BBA34 is a lipoprotein exposed to the periplasmic environment of the *B. burgdorferi*, and on linear plasmid 54 (lp54) resides the *bba34* gene. In addition, BBA34 is upregulated when the spirochetes are growing under tick feeding and mammalian host conditions (20).

Based on the evidences described above, the borrelial membrane protein BBA34 was considered as a potential protein scaffolding molecule. Consequently, we hypothesized that by engrafting PepB into an exposed region of the borrelial protein BBA34, known as solute binding domain, It could be able to improve the presentation of the PepB to the immune system, and therefore stimulate the generation of protective specific antibodies against *B. burgdorferi*.

Materials and methods

Ethics statement

The Institutional Animal Care and Use Committee (IACUC) of Texas A&M University granted approval to all animal-related treatments. The mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility at Texas A&M University (AAALAC).

Cloning and expression of scaffolded antigen

The scaffolded construct BBA34:PepB (BP) was synthesized in the expression vector pET23a by Genscript (Piscataway, NJ, USA) with optimized codon usage for expression in *Escherichia coli*. The plasmid was transformed into Rosetta™ (DE3) pLysS *E. coli* strain (Novagen, Madison, WI, USA). Clones were verified by PCR and protein expression, and kept at -80°C for further use. Transformed cells were recuperated in LB medium containing appropriate antibiotics, and overexpression of BP was done at 37ºC using 1mM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 hours. After overexpression, cells were harvested and lysed via sonication in lysis buffer (20 mM imidazole; pH 7.4) with addition of the protease inhibitor cocktail, HALT™ (Thermo Scientific, Inc., USA). The sonicated suspension was centrifuged, and the supernatant was run through affinity purification column using a His60 Ni Superflow resin (Clontech, Mountain View, USA) according to manufacturer's recommendations. Following binding of proteins, the affinity column was washed with 30ml of wash buffer (40 mM imidazole; pH 7.4). Finally, the bound 6×His-tagged proteins was released by elution buffer (300 mM imidazole; pH 7.4), and the collected protein fractions were aliquoted and stored at -80ºC. Samples were separated and analyzed under SDS-12.5% PAGE conditions (21).

Purification of BBA34: PepB (BP) construct

The highest protein-containing fractions were combined and concentrated with Spin-X™ centrifugal filters (Corning, Lowell, USA) with a 10 kDa MWCO. Then, concentrated proteins were passed through a column of

Sephadex G-75 resin for size exclusion chromatography. Pierce™ BCA Protein Assay (Thermo Scientific, Inc., Rockford, USA) was used to quantify protein concentration of the collected fractions. Then, fractions with relatively high concentration were pooled and centrifuged as mentioned previously, and used on a desalting column PD-10 (GE Healthcare, Piscataway, USA). SDS-12.5% PAGE was used to evaluate the collected fractions, and the fractions with the highest protein content were combined and concentrated using a Spin-X centrifugal filters. A 61.5-KDa BBA34: PepB was purified and kept at 4°C until further use in animal experiments (21) (Figure 1).

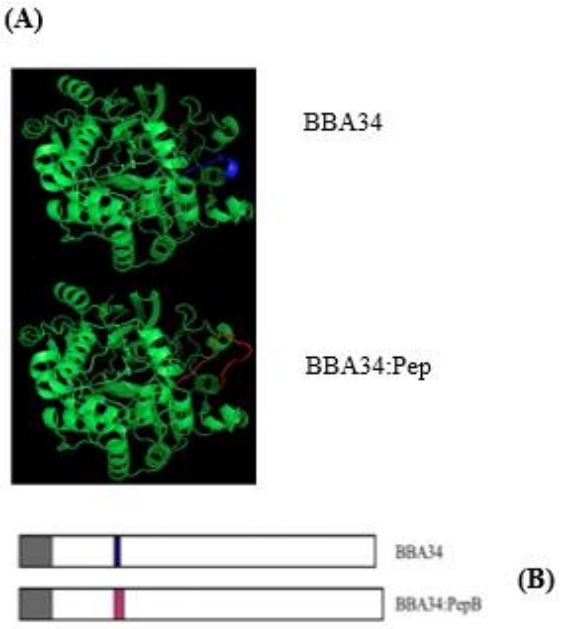

Figure 1: Illustration of scaffolding protein BBA34. (A) The models depict BBA34:PepB and the expected structural modifications to the protein as a result. (B) The lipoprotein signal peptide, which directs surface localization (gray part). The PepB scaffolding molecule BBA34, in which the solute-binding domain has been substituted by PepB (pink part) (22).

Expression and purification of control proteins

Control proteins OspC and BBA34 were cloned into the cloning vector (pET23a) and then transformed into Rosetta™ (DE3) pLysS *Escherichia coli* (Novagen, Madison, USA), and kept at -80ºC for further use. Transformed cells were recovered in LB medium containing appropriate antibiotics, and overexpression of BBA34:PepB was done at 37ºC using 1mM isopropyl-□-D-thiogalactopyranoside (IPTG) for 3 hours. After overexpression, cells were harvested, washed, and sonicated for 10 minutes in lysis buffer (20 mM imidazole; pH 7.4) with addition of the protease inhibitor (Thermo Scientific, Rockford, USA). The sonicated suspension was centrifuged, and the supernatants were applied to a His60 Ni Superflow resin (Clontech, Mountain View, USA), for affinity purification following the protocol provided by the manufacturer. Proteins were processed after binding with 30ml of wash buffer (40 mM imidazole; pH 7.4). The 6xHis-tagged proteins were then extracted using an elution buffer (300 mM imidazole; pH 7.4), and the obtained fractions were subsequently subjected to SDS-PAGE analysis (22).

Purification of the control proteins was started with dialysis of the concentrated fractions of protein into dialysis buffer (50 mM Sodium Phosphate, 300 mM Sodium Chloride, 0.1% Triton X-100; pH 7.4) by using dialysis cassettes (Thermo Scientific, Rockford, USA). Dialyzed proteins were then concentrated with a 10 kDa MWCO (EMD Millipore, Billerica, USA). After that, Using BCA Protein Assay, the control proteins were measured and they were then kept at 4°C.

Immunization

Six to eight weeks old C3H/HeN mice (n=6 per group) were immunized subcutaneously with decreasing antigen dosages of 50µg/mouse for priming followed by 10µg/mouse at 14-days post-priming, and a final booster dose of 5µg/mouse at 28-days post-priming in combination with 5% Adjuplex™ adjuvant (Advanced Bio-Adjuvants LLC, USA). In this study we included the following groups: BP, BBA34, OspC and a naïve control group. Eight weeks post-priming blood samples were collected, and specific IgG titers were evaluated by using ELISA methods previously described by others (22).

Infection

To evaluate protection induced by the scaffolded antigen BP, mice (n=6) were infected subcutaneously ten weeks post-priming with a dose of 10^5 Borrelial cells per mouse. First, PCR screening was conducted to detect the presence of essential plasmids required for the survival and infection of *B. burgdorferi* strain that was used in the challenge studies. The primers for PCR screening method were previously described (23). Mice were euthanized four weeks after the challenge, and blood and tissue samples were taken to evaluate the level of protection. Skin, spleen, inguinal lymph nodes, heart, bladder, and tibiotarsal joints were among the tissues that were sampled.

At 32°C and 1% CO2, tissues were grown in (Barbour-Stoenner- Kelly) BSK-II media supplemented with 6% inactivated naïve rabbit serum. After 5 days from the original inoculation, the cultures were blindly transferred to new BSK-II media, where they were cultured for up to 21

days at 32°C and 1% CO2. At 14 and 21 days after inoculation, the original and blind passaged cultures were examined for bacterial growth under dark field microscopy (24). By using real-time qPCR, the number of bacteria were assessed in the skin, spleen, inguinal lymph node, and tibiotarsal joint as mentioned in (25).

Statistical analysis

Two-way ANOVA was used to analyze antibody titers in order to determine whether there were any significant differences between the various immunized groups (26). The Mann Whitney U test was used in quantitative real-time PCR data to statistically compare between groups. Using Prism 7.0 (GraphPad Software, Inc., USA), all graphs and analyses were produced.

Results

Plasmid profile of *B. burgdorferi*

The results of PCR screening showed that all essential plasmids were detected in the *B. burgdorferi* B31 strain that was used in challenge protocol to confirm the ability of that strain to colonize and induce infection in a host (Figure 2).

Efficacy of BBA34: PepB construct

It was demonstrated that the immunization schedule described above was able to induce specific IgG antibodies in immunized animals using BBA34: PepB. Furthermore, it was showed the presence of comparable antibody titers in both BBA34 and BP immunized mice. Hence, in this study the presence of protective antibodies was evaluated in the immunized animals. To this end, both control and immunized groups were infected with 10^5 spirochetes/mouse via needle inoculation (Figures 3 and 4) immunized animals were able to induce high specific antibody titers 8-weeks post-priming. In addition, all groups developed specific antibodies against *B. burgdorferi* post-challenge.

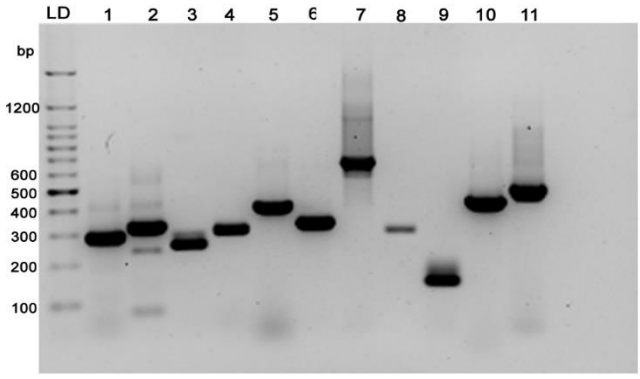

Figure 2: Plasmid profile of Borrelia burgdorferi B31 strain. Conventional PCR screening on 1% agarose gel revealed presence of all essential plasmids. Ld: DNA Ladder (LD) weight is represented in base pairs (bp) on the left of the image. 1: cp26; 2: lp5; 3: lp17; 4: lp25; 5: lp28-1; 6: lp28-2; 7: lp28-3; 8: lp28-4; 9: lp36; 10: lp38; 11: lp56.

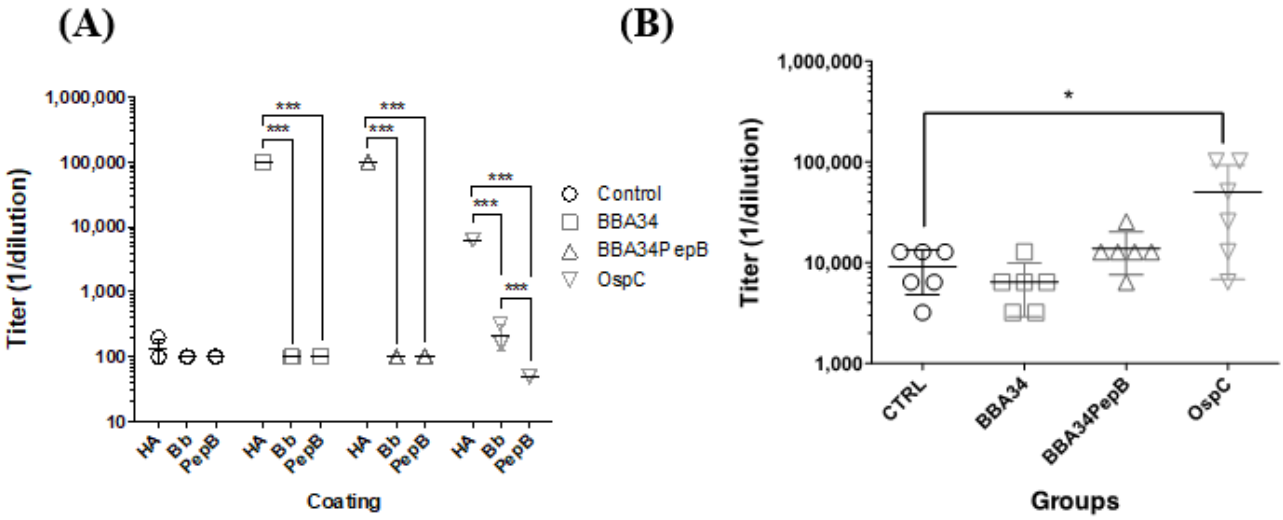

Figure 3: BBA34, BP and OspC induce specific antibodies in immunized C3H/HeN mice. (A) Specific IgG titers in the immunized groups. HA denotes Homologous Antigen; Bb denotes Borrelia burgdorferi whole cell lysates; PepB denotes the specific peptide B antigen used as coating agents in the ELISA. (B) anti-Borrelia burgdorferi IgG levels in the different groups 4 weeks post challenge. * Denotes significant differences: * P value < 0.05; *** P value < 0.001.

On the other hand, and as shown in Figure 4 immunized mice were not able to clear the infection when tissues were inoculated in BSK-II medium and incubated at 32ºC and 1%CO_2. As shown in this figure, even though there is significantly lower bacteria recovery from tissues isolated from immunized animals, most tissues were able to grow viable bacteria in media, and therefore, we considered that immunized animals were not protected.

Furthermore, the bacterial burden was evaluated in the collected tissues by means of qPCR. As shown in figure 5, there was significantly low bacterial burden in all examined tissues of BBA34: PepB compared with the control group. Interestingly, BBA34 and OspC groups generally had lower bacterial load in all tissues compared with the control group and the BP group. Taken together, and considering both bacterial burden and bacterial recovery from tissues, immunization with BP was not able to clear the infection in this experiment, and consequently we did not consider this alternative as a potential vaccine formulation in subsequent studies.

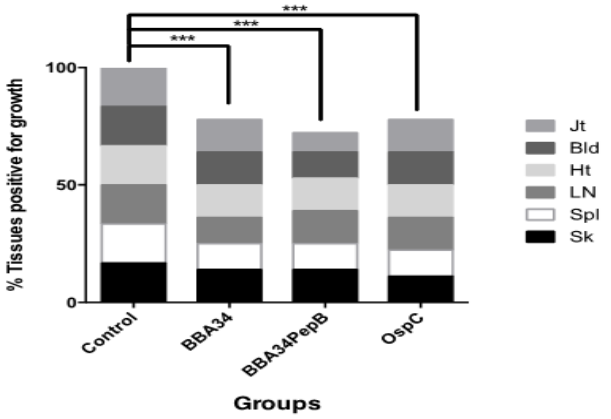

Figure 4: Bacterial restoration. In BSK-II specific media for Borrelia spp. In vaccinated groups 1/6 animals showed clean tissues. In BBA34: PepB immunized animals 2/6 spleens and 3/6 joints showed clearance. * Denotes significant differences: * P value < 0.05; *** P value < 0.001.

Discussion

The increasing of geographic distribution and prevalence of LD cases has been associated with expanding of public health concern since the disease was identified. Vaccination is an effective approach that can be used efficiently to control the LD cases in humans and reduce the economic burden of the disease (27). Different studies have evaluated several vaccine candidates against LD including outer surface lipoproteins and other antigens (28,29). In previous study, PepB has shown promising results as a vaccine candidate against LD, providing 50% protective efficacy in tick challenge model when the peptide was conjugated to KLH (22).

Scaffolding molecule BBA34 in conjunction with PepB was used in this study to enhance the efficacy of the selected peptide in the murine model of LD. The use of scaffold protein BBA34 excludes introducing foreign immunostimulants that might induce adverse reactions, after administration in a host. Evaluation of immune response in immunized mice revealed presence of significant high antibody titers compared to the control group, however, PepB specific antibody titer was very low. Therefore, it may be revealed that the immune response was not enhanced against the PepB, this might be due to the fact that the scaffolding molecule impeded the appropriate exposure of the peptide to the immune system. The scaffolding molecule with inserted PepB might require additional optimization in order to be effective. This outcome is similar to the study of McLellan *et al.* (15) in which authors showed that the use of scaffolding structure was unable to induce antibody response in immunized mice against an antigen derived from respiratory syncytial virus (RSV).

Bacterial burden in tissues was evaluated to determine a protective efficiency of the scaffolded PepB antigen in immunized mice. The BBA34: PepB construct, and the BBA34 and OspC proteins showed a reduction in the bacterial load in the examined tissues compared with the control group. In addition, viable spirochetes were grown in the cultured tissues in BSK-II media from all immunized groups. Hence, the PepB was not considered protective in the scaffolding context, and other alternatives could be investigated to formulate an efficient PepB-based vaccine candidate. The 12-mer peptide (PepB) is unable to induce an immune response by itself, because of its small size, therefore it is essential to increase its size to render it immunogenic. The PepB design can be further explored in the context of a multiple-copy peptide antigen approach. In fact, multiple-copy peptide method has been shown that it induced a more efficient immunological response than a single copy peptide linked to KLH did (30).

In the multiple-copy peptide design the selected antigen is exposed in a well-defined orientation, and it excludes any unnecessary structural components that may include suppressor epitopes that might be found in scaffolding, and/or carrier molecules. In addition, a multiple-copy peptide is considered highly immunogenic, and can induce both humoral and cellular immune responses against HIV infection in human (31). Furthermore, previous studies showed that a multiple-copy peptide antigen derived from the circumsporozoite protein (CSP) of *Plasmodium yoelii* was able to confer protection in mice against malaria infection (32). Also, other antigen

delivery systems can be used with PepB to improve its protective efficacy such as use of liposomes, archaeosomes, polymersomes, and immuno-stimulating complexes (ISCOMs) (33,34). These methods can be used to improve antigen persistence, uptake, and presentation, and thus able to effectively potentiate the immune response.

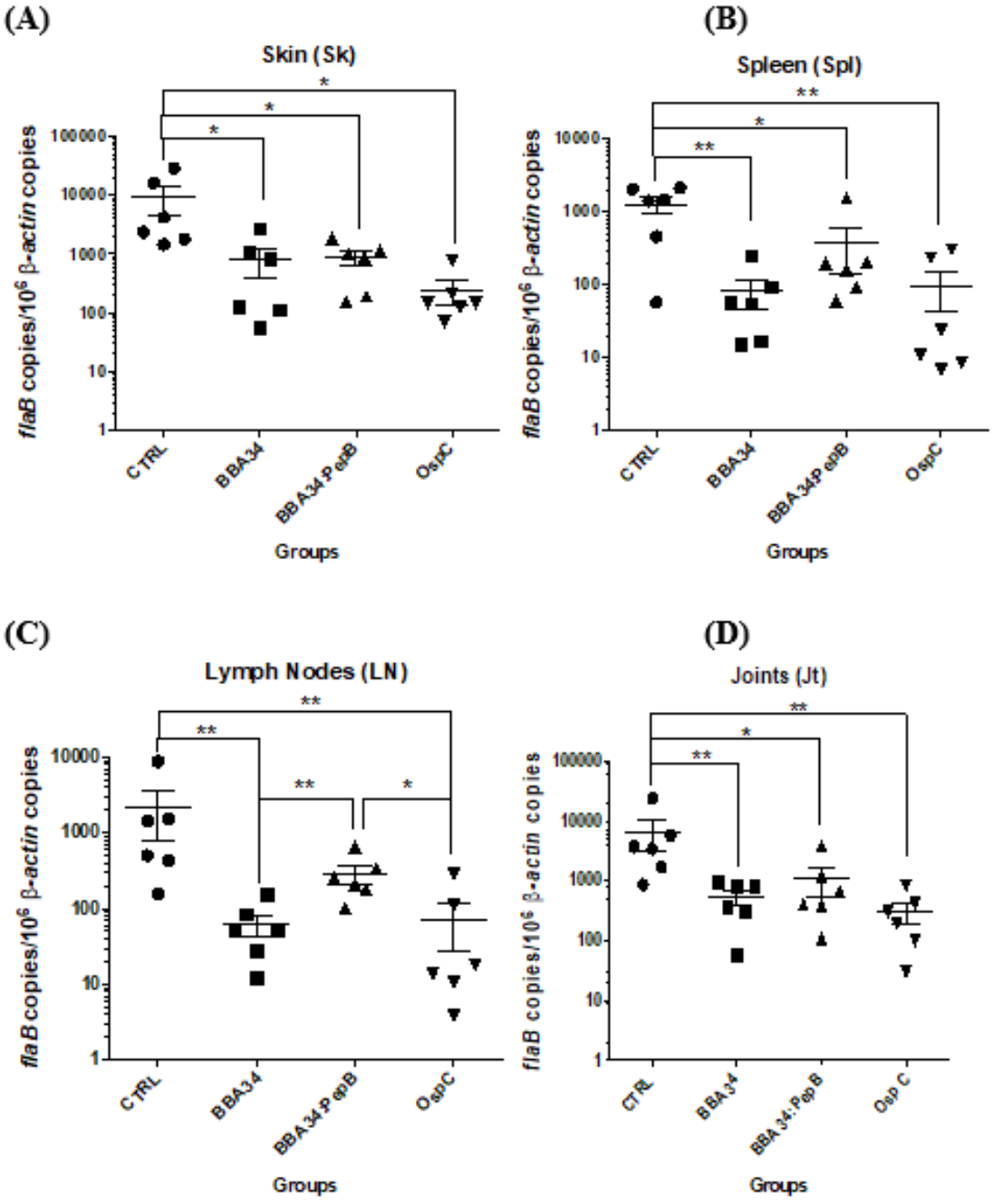

Figure 5: BBA34: PepB did not confer protection in immunized mice inoculated with B. burgdorferi. Real time qPCR revealed the bacterial load was significantly decreased in mice immunized with BBA34: PepB, BBA34, and OspC in all examined tissues including skin (A), spleen (B), lymph nodes (C), and joints (D) compared with control group (* P value < 0.05; ** P value < 0.01).

Conclusion

In the present study, the scaffolding of PepB approach did not yield promising results, therefore, this formulation was not considered for further vaccine studies. Hence, other novel methods were explored in terms of reformulation of PepB design, and/or conjugation with carrier molecules to improve the immunogenicity and protective efficacy of the PepB-based vaccine candidate.

Acknowledgement

The College of Veterinary Medicine and Biomedical Sciences at Texas A&M University, Texas, USA, provided financial support for this work.

Conflict of interest

No conflicts of interest have been acknowledged by the authors.

References

1. Mead PS. Epidemiology of Lyme disease. Infect Dis Clin North Am. 2015;29(2):187-210. DOI: 10.1016/j.idc.2015.02.010
2. Back JW, Langedijk JP. Structure-based design for high-hanging vaccine fruits. Adv Immunol. 2012;114:33-50. DOI: 10.1016/B978-0-12-396548-6.00002-0
3. Burton DR. Scaffolding to build a rational vaccine design strategy. Proc Natl Acad Sci U S A. 2010;107(42):17859-17860. DOI: 10.1073/pnas.1012923107
4. Skerra A. Engineered protein scaffolds for molecular recognition. J Mol Recognit. 2000;13(4):167-187. DOI: 10.1002/1099-1352
5. Wang J, Lisanza S, Juergens D, Tischer D, Anishchenko I, Baek M, Watson JL, Chun JH, Milles LF, Dauparas J, Expòsit M, Yang W. A. Deep learning methods for designing proteins scaffolding functional sites. BioRxiv 2021. DOI: 10.1101/2021.11.10.468128
6. Azoitei ML, Ban YE, Julien JP, Bryson S, Schroeter A, Kalyuzhniy O, Porter JR, Adachi Y, Baker D, Pai EF, Schief WR. Computational design of high-affinity epitope scaffolds by backbone grafting of a linear epitope. J Mol Biol. 2012;415(1):175-192. DOI: 10.1016/j.jmb.2011.10.003
7. Fernandez-Carneado J, Grell D, Durieux P, Hauert J, Kovacsovics T, Tuchscherer G. Surface grafting onto template-assembled synthetic protein scaffolds in molecular recognition. Biopolymers. 2000;55(6):451-458. DOI: 10.1002/1097-0282(2000)55:6<451
8. Cozzi R, Scarselli M, Ferlenghi I. Structural vaccinology: a three-dimensional view for vaccine development. Curr Top Med Chem. 2013;13(20):2629-2637. DOI: 10.2174/15680266113136660187
9. Adhikari RP, Karauzum H, Sarwar J, Abaandou L, Mahmoudieh M, Boroun AR, Vu H, Nguyen T, Devi VS, Shulenin S, Warfield KL, Aman MJ. Novel structurally designed vaccine for *S. aureus* α-hemolysin: protection against bacteremia and pneumonia. PLoS One. 2012;7(6):e38567. DOI: 10.1371/journal.pone.0038567
10. Tan M, Jiang X. Subviral particle as vaccine and vaccine platform. Curr Opin Virol. 2014;6:24-33. DOI: 10.1016/j.coviro.2014.02.009
11. Xiang SD, Selomulya C, Ho J, Apostolopoulos V, Plebanski M. Delivery of DNA vaccines: an overview on the use of biodegradable polymeric and magnetic nanoparticles. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010;2(3):205-218. DOI: 10.1002/wnan.88
12. Plummer EM, Manchester M. Viral nanoparticles and virus-like particles: platforms for contemporary vaccine design. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2011;3(2):174-196. DOI: 10.1002/wnan.119
13. Manayani DJ, Thomas D, Dryden KA, Reddy V, Siladi ME, Marlett JM, Rainey GJ, Pique ME, Scobie HM. A viral nanoparticle with dual function as an anthrax antitoxin and vaccine. PLoS Pathog. 2007;3(10):1422-1431. DOI: 10.1371/journal.ppat.0030142
14. Ofek G, Guenaga FJ, Schief WR, Skinner J, Baker D, Wyatt R, Kwong PD. Elicitation of structure-specific antibodies by epitope scaffolds. Proc Natl Acad Sci U S A. 2010;107(42):17880-17887. DOI: 10.1073/pnas.1004728107
15. McLellan JS, Correia BE, Chen M, Yang Y, Graham BS, Schief WR, Kwong PD. Design and characterization of epitope-scaffold immunogens that present the motavizumab epitope from respiratory syncytial virus. J Mol Biol. 2011;409(5):853-866. DOI: 10.1016/j.jmb.2011.04.044
16. Correia BE, Bates JT, Loomis RJ, Baneyx G, Carrico C, Jardine JG, Rupert P, Correnti C, Kalyuzhniy O, Vittal V, Connell MJ, Stevens E, Schroeter A, Chen M, Macpherson S, Serra AM, Adachi Y, Holmes MA, Li Y, Klevit RE, Schief WR. Proof of principle for epitope-focused vaccine design. Nature. 2014;507(7491):201-206. DOI: 10.1038/nature12966
17. Lin B, Short SA, Eskildsen M, Klempner MS, Hu LT. Functional testing of putative oligopeptide permease (Opp) proteins of *Borrelia burgdorferi*: a complementation model in opp(-) *Escherichia coli*. Biochim Biophys Acta. 2001;1499(3):222-231. DOI: 10.1016/s0167-4889(00)00121-x
18. Manson MD, Blank V, Brade G, Higgins CF. Peptide chemotaxis in *E. coli* involves the Tap signal transducer and the dipeptide permease. Nature. 1986;321(6067):253-256. DOI: 10.1038/321253a0
19. Raju BV, Esteve-Gassent MD, Karna SL, Miller CL, Van Laar TA, Seshu J. Oligopeptide permease A5 modulates vertebrate host-specific adaptation of *Borrelia burgdorferi*. Infect Immun. 2011;79(8):3407-3420. DOI: 10.1128/IAI.05234-11
20. Medrano MS, Ding Y, Wang XG, Lu P, Coburn J, Hu LT. Regulators of expression of the oligopeptide permease A proteins of *Borrelia burgdorferi*. J Bacteriol. 2007;189(7):2653-2659. DOI: 10.1128/JB.01760-06
21. Matsumoto H, Haniu H, Komori N. Determination of Protein Molecular Weights on SDS-PAGE. Methods Mol Biol. 2019;1855:101-105. DOI: 10.1007/978-1-4939-8793-1_10
22. Christina M Brock. Development of a novel, peptide-based vaccine for Lyme disease [Ph.D. dissertation]. College Station: Texas A&M University; 2017. 122 p.
23. Labandeira-Rey M, Skare JT. Decreased infectivity in *Borrelia burgdorferi* strain B31 is associated with loss of linear plasmid 25 or 28-1. Infect Immun. 2001;69(1):446-455. DOI: 10.1128/IAI.69.1.446-455.2001
24. Maruskova M, Esteve-Gassent MD, Sexton VL, Seshu J. Role of the BBA64 locus of *Borrelia burgdorferi* in early stages of infectivity in a murine model of Lyme disease. Infect Immun. 2008;76(1):391-402. DOI: 10.1128/IAI.01118-07
25. Esteve-Gassent MD, Elliott NL, Seshu J. sodA is essential for virulence of *Borrelia burgdorferi* in the murine model of Lyme disease. Mol Microbiol. 2009;71(3):594-612. DOI: 10.1111/j.1365-2958.2008.06549.x
26. Wang D, Huang J, Xie H, Manzella L, Soares MB. A robust two-way semi-linear model for normalization of cDNA microarray data. BMC Bioinformatics. 2005; 6:14. DOI: 10.1186/1471-2105-6-14
27. Hahn BL, Padmore LJ, Ristow LC, Curtis MW, Coburn J. Live Attenuated *Borrelia burgdorferi* Targeted Mutants in an Infectious Strain Background Protect Mice from Challenge Infection. Clin Vaccine Immunol. 2016;23(8):725-731. DOI: 10.1128/CVI.00302-16
28. Earnhart CG, Marconi RT. Construction and analysis of variants of a polyvalent Lyme disease vaccine: approaches for improving the

تقييم الكفاءة والقدرة التمنيعية للمستضد الببتيدي ب المشتق من جراثيم البوريليا وتم تركيبه في تشكيل السقالة البروتينية في الفئران ضد مرض اللايم

وسام سالم حسن[١]، كريستينا بروك[٢] و ماريا استيفي كاسنت[٢]

[١]فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق، [٢]فرع علم الأمراض الإحيائي، كلية الطب البيطري والعلوم الطبية الحياتية، جامعة تكساس أي اند ام، تكساس، الولايات المتحدة الأمريكية

الخلاصة

يعد مرض اللايم من الأمراض المنتقلة عن طريق القراد والذي قد ينتشر في اغلب بقاع العالم وبخاصة في قارتي أوروبا وأمريكا الشمالية، إن التركيب الإحيائي المعقد لجراثيم البوريليا والتغيرات الحاصلة في تركيب الأغشية الخارجية المستضدية يعقد من صعوبة إنتاج لقاحات كفؤة ضد مرض اللايم. في هذا البحث العلمي تم استخدام ببتيد ب وهو سلسلة أحماض أمينية قصيرة مشتقة من بروتينات تدعى (بي بي ٠١٧٢) متواجدة في جراثيم البوريليا، إذ تم تقييمه من خلال استخدامه في تركيب سقالة كلقاح تجريبي في الفئران ضد مرض اللايم. حيث تم تمنيع أربعة مجاميع من الفئران من نوع سي٣ اج/هن وبأعمار تراوحت بين ٦-٨ أسابيع بطريقة الحقن تحت الجلد ببروتين (بي بي اي ٣٤: بيتيد ب)، بروتين الغشاء الخارجي سي (اوسب سي)، وبروتين (بيبي اي ٣٤)، ومجموعة السيطرة السالبة. تم جمع عينات الدم في الأسبوع الثامن بعد التمنيع حيث تم تقييم مستوى الكلوبيولينات المناعية المتخصصة من نوع جي باستخدام اختبار الاليزا. بعد ذلك خضعت حيوانات التجربة جميعها لاختبار التحدي عن طريق حقن جراثيم البوريليا بجرعة 10^5/ حيوان تحت الجلد. لاحقا تم تشريح الحيوانات بعد مرور أربعة أسابيع على اختبار التحدي وتم جمع عينات الدم والأنسجة لغرض تقييم الحمل الجرثومي باختبار تفاعل البلمرة المتسلسل الكمي وكذلك محاولة إعادة تنمية الجراثيم من الأنسجة المصابة. وبالاعتماد على نتائج تفاعل البلمرة المتسلسل الكمي وإعادة تنمية الزرع الجرثومي، في هذه التجربة لوحظ بان التمنيع ببروتين (بي بي اي ٣٤: بيتيد ب) كان غير قادر على حماية الحيوانات ضد الإصابة بمرض البوريليا، وبذلك قد لا يعد هذا التركيب البروتيني مثاليا في تصنيع اللقاحات التجريبية في الدراسات المستقبلية. استنتج من هذه الدراسة أن ضرورة استخدام منصات مستضدية بديلة فضلا عن طرائق إيصال للمستضد قد تساهم في تعزيز الكفاءة والقدرة التمنيعية في اللقاحات التجريبية المعتمدة على الببتيد ب.

immune response to chimeric vaccinogens. Vaccine. 2007;25(17):3419-3427. DOI: 10.1016/j.vaccine.2006.12.051
29. Brown EL, Kim JH, Reisenbichler ES, Höök M. Multicomponent Lyme vaccine: three is not a crowd. Vaccine. 2005;23(28):3687-3696. DOI: 10.1016/j.vaccine.2005.02.006
30. Tam JP. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc Natl Acad Sci U S A. 1988;85(15):5409-5413. DOI: 10.1073/pnas.85.15.5409
31. Gorse GJ, Keefer MC, Belshe RB, Matthews TJ, Forrest BD, Hsieh RH, Koff WC, Hanson CV, Dolin R, Weinhold KJ, Frey SE, Ketter N, Fast PE. A dose-ranging study of a prototype synthetic HIV-1MN V3 branched peptide vaccine. The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. J Infect Dis. 1996;173(2):330-339. DOI: 10.1093/infdis/173.2.330
32. Wang R, Charoenvit Y, Corradin G, Porrozzi R, Hunter RL, Glenn G, Alving CR, Church P, Hoffman SL. Induction of protective polyclonal antibodies by immunization with *Plasmodium yoelii* circumsporozoite protein multiple antigen peptide vaccine. J Immunol. 1995;155(3):1637. PMID: 7636221
33. Smith D, Simon J, Baker Jr J. Applications of nanotechnology for immunology. Nat Rev Immunol. 2013;13:592-605. DOI: 10.1038/nri3488
34. Mazumder S, Maji M, Ali N. Potentiating effects of MPL on DSPC bearing cationic liposomes promote recombinant GP63 vaccine efficacy: high immunogenicity and protection. PLoS Negl Trop Dis. 2011;5(12):e1429. DOI: 10.1371/journal.pntd.0001429