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Research Article

The Decryption of Bab1 Brucella Abortus Antigen- Bcap31 Receptor Binding in Oral Lactococcus Lactis Vaccine and Its Effect on Activation of Casp-8 And Il-10 For Immunogenicity

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Abstract

Vaccination provides a proactive approach to controlling brucella infection in livestock. To; identify potential immunogenicity for brucella abortus vaccine candidates, in this study, we investigated the effect of the BAB1 Brucella Abortus antigen on casp-8 and IL-10 expression. Using GROMACS simulation and docking, we studied the BCAP31 receptor has a role in the immune response that is activated by BAB1 antigen. Then; Oral vaccinations with L. lactis transformed with pNZ8148 variants encoding for BAB1 (pNZ8148:BAB1) and Free-pNZ8148 were administered to mice.

Our results demonstrated an increased transcription levels of both the BCAP31, casp-8 and IL-1, supporting its potential as an efficient, safe, and effective immunogenicity vaccine candidate.

The findings from this research could someday lead to improved vaccines against illnesses caused by this organism, and also increased immunogenicity when it comes to treating related diseases. It could also mean a better understanding how organisms like B. abortus interact with their hosts at a molecular level. The; effects discovered here in regarding to casp-8 and IL-1 levels may also lead to further research into other products that BAB1 has on immunity and disease. Results of this article, we showed the role of Casp-8 and IL-1 activation through the effect of BAB1 antigen on BCAP31 receptor in Lactococcus Lactis vaccine immunogenicity and its potential for Brucella Abortus antigen detection.

Keywords: casp-8; Il-10; BCAP31 receptor; BAB1 antigen; vaccine

Introduction

Brucellosis is a highly contagious zoonotic disease caused by the bacteria Brucella Abortus. This; disease poses a significant threat to livestock and humans, leading to severe economic losses in the livestock industry and a considerable health risk to humans who come into contact with infected animals or their products [1].

Brucella is a facultative intracellular pathogen capable of surviving and replicating in professional and non-professional phagocytes. Brucella; is small in size, currently classified based on host preference and differences in phenotype, phage sensitivity, and cell envelope structural features. The; genus Brucella includes presently 12 species [2, 3].

Like other pathogenic intracellular bacteria, Brucella infection requires four steps: attachment, invasion, establishment and spread within the host. This; infection eventually leads to chronic and long-term disease. Previous; ultrastructural work in the morphological characterization of B. abortus-infected cells has shown that Brucella proliferate in an intracellular multi-membrane compartment that shares similarities with the rough endoplasmic reticulum. [4, 5].

Specific antibodies play an important role in reducing the initial stage of Brucella infection. They; have a limited role in the intracellular establishment. B lymphocytes can benefit the organism by creating a proliferative niche, or B. cells can produce the interleukin IL-10 cytokine, thereby producing IL-12 and interferon-, which are essential in modulating

the CMI response. Provide. It; has been shown that mice with B-cell deficiency are highly resistant to Brucella infection. Several; investigators have pointed to the importance of the Th1 immune response in controlling Brucella infection, as and the involvement of IL-12 and INF -γ. Therefore, designing vaccines that can increase Th1 response while reducing Th2 response is logical [4, 6].

L-10, also known as interleukin-10, is a cytokine that is crucial in regulating the immune system's response to pathogens. Recent; research has shown that IL-10 activation can stimulate the immune system and produce an immune response against Brucella Abortus antigens. This; approach has shown promising results in animal models, with increased immunogenicity and effectiveness against the bacteria. IL-10; activation works by inducing an immune regulatory response that helps to control inflammation and prevent tissue damage. This; approach is critical in Brucella Abortus vaccine development, as the bacteria's ability to evade the immune system is partly due to its ability to manipulate the immune response. IL-10; activation can help to overcome this barrier and produce a more robust immune response against the bacteria [7].

The B-cell receptor-associated protein 31 was deciphered by Michael Reth's group in 1994. It; is named for the co-purification with the immunoglobulin D component of the B-cell receptor and for visualization. The apparent molecular weight is 31 kDa on the denatured gel electrophoresis. Usually, the protein is called BAP31 and the gene is BCAP31. BCAP31; was also independently cloned in two other studies, where it was referred to by its alternate but now obsolete name DXS1357E and CDM or antigen 6C6. BAP31 is a specific transmembrane protein primarily found in the endoplasmic reticulum, where it acts as a chaperone for several transmembrane proteins and as a regulator of apoptosis. [8].

Several antigens in Brucella have been identified as potential protective antigens. Among them, BAB1 encoding the putative GcrA protein was considered a possible candidate for the production of a probiotic oral subunit vaccine (Lactococcus lactis) against Brucella.

Some strains of lactic acid bacteria LAB are known as probiotics and stimulate the immune system. As a harmless agent, Lactococcus lactis stimulates the immune system in humans and animals, which may make it an exciting system for oral vaccination. L. lactis gene expression is controlled by Nisin, a robust and highly regulated protein production system that depends on the autoregulatory mechanism of the bacteriocin nisin. Currently; This gene expression system is widely used to express exogenous proteins in Gram-positive bacteria. L. lactis, combined with an easy-to-use and NICE-regulated system, has various applications, including the expression of pathogenic antigens for immunization across mucosal surfaces and the production of therapeutic cytokines for medical treatment [9, 10].

In this study the effect of Lactococcus lactis expressed BAB1 Brucella abortus antigen orally on casp-8 was investigated. Using Gene Expression Omnibus (GEO) analysis and a time-lapse simulation, the BCAP31 receptor with BAB1 was studied in striking detail. Through; Gromacs and docking simulations, a complex picture was developed of the effect of this BAB1 antigen on the BCAP31 receptor and the effect it on casp-8 and IL-10 levels in the immune system.

2. Methods and materials

Since the aim of this study was the effect of BAB1 antigen, which is expressed orally and recombinantly in Lactococcus lactis bacteria, it was selected from the data code GSE84949, which is the expression profile of mouse cells that were exposed to this bacterium, that the effect of BAB1 antigen on casp-8 and interleukin 10, and we were looking for a specific receptor in this study, which has the highest expression in treated with Lactococcus lactis bacteria. Therefore, based on Geo analysis, the BCAP31 receptor was identified, in the following, based on the docking approach and the molecular dynamics simulation of the relationship between BAB1 antigen and BCAP31 receptor was investigated [11].

2.1. Identification and functional analysis of DEGs

Expression profiles for the GSE84949 datasets were obtained from the GEO database. All; GSE files were downloaded from the three selected databases using the R language analysis tool from the GCBI website to identify up-regulated or down-regulated DEGs using the cutoff criteria fold change > 2 and p 0.01. The; intersection of the two or three sets of DEGs was obtained by a Venn diagram.

2.2. Construction of the protein-protein coexisting interaction network and central gene identity

We used the Search Engine to retrieve the interaction gene database to generate the PPI network. The STRING Online Database is an online repository for importing PPI data from published literature. Gene-gene interactions with integration score > 0.9 with fold change > 2 were selected to build a coexistence PPI network.

2.3. Molecular Docking

The structure of the BCAP31 receptor and the bab1 antigen was modeled using the ITASSAR server.

We used Patch dock for our molecular docking study [12]. Candidate; transformations were then performed on complementary patched builds. Then, each candidate transformation was compared over the scores obtained by considering the atomic dissolution energy and geometric fit [13]. The set of PDB coordinates for each protein, and the considered ligands were the input parameters for the docking analysis. The clustering RMSD value was set to 4Å. The complex type was changed to this type.

2.4. Analysis of Molecular Dynamic Simulation

Molecular dynamics simulations were performed using the GROMACS program version 2020.1. Gromac g43a1 force field applied to proteins. Receptor-Antigen protein systems with the Spc216 water model were dissolved in a 10 A dodecahedron box and, sufficient neutralizing ions were added to the system, including the Na + and Cl- counter ions required to generate neutralization. All systems were minimized for 10,000 steps using the steepest descent method and stabilized under constant atomic number, volume, and temperature at 300 K for 10 ns in one set. Next; the system was subjected to 40 ns of unrestricted MD simulation in the temperature group with a fixed atomic number, pressure, temperature and pressure. The; LINCS algorithm was used to limit the covalent bonds in the system. Simulations, 1 fs' and the cutoff for Coulomb and Lennard-Jones interactions were adjusted from the initial negative energy state to the equilibrium of 1.2 nm. Finally; the use of Visual Molecular Dynamics software to analyze MD trajectories and check the stability of the systems. The; final build was observed with Discovery Studio 2016.

2.5. Construction of BAB1 antigen expression vector and its animal injection

Female Balb/c mice were purchased from the Biotechnology Research Animal Center.

According to Ethics Committee guidelines, mice were maintained in a specific pathogen-free environment. In accordance with ARRIVE recommendations, the anesthetic/assisted euthanasia techniques used in this publication are PLoS Bio 8 suitable.

Escherichia coli strains TOP10F and L. lactis IBRC-M 11051 were purchased from the Iran Biological Resources Center. L. lactis strains were cultured in M17 medium at 30°C and under anaerobic conditions supplemented with 1% glucose. Escherichia coli TOP10F strains were cultured at 37°C in Luria-Bertani medium. Amitis Gen Med TECH Corporation provided a strain of B. abortus for the study.

Brucella strains were cultured for 72 h at 37°C in tryptone soy broth produced by Difco Laboratories in Detroit, Michigan.

For the expression of BAB1 Brucella protein in L. lactis, the BAB1 gene, together with its promoter, was synthesized and inserted into the lactococcal expression vector pNZ8148 by GENE ray. To transfect the plasmid construct into L. lactis, E. coli was initially used for transformation. The transformed strains were isolated using the matrix method and re-cultivated at 37°C. In this study, an endonuclease-free plasmid extraction kit was then used to purify the plasmid from E. coli.

PCR reactions were conducted to monitor BAB1 using specific primers. A 20-microliter PCR reaction contains 2 microliters of x10 PCR buffer, 2 mM MgCl2, 200 µM dNTPs, 10 pmol of each primer, 100 ng of plasmid DNA and 1 unit of Taq DNA polymerase. The PCR temperature protocol consisted of a first incubation at 95°C for 5 min, followed by 30 replicates at 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min. Finally, final elongation was performed at 72°C for 5 min.

2.6. Real-time PCR assay

Spleen were extracted under sterile conditions and stored in liquid nitrogen at -198°C after rats were anesthetized. Next, RNA extraction and cDNA synthesis from spleen tissue were performed according to the YTA kit instructions. Real-time PCR was performed with the YTA SYBR Green master mix and the GAPDH gene used as internal control. Fifteen microliters of reaction volume consisting of 0.5 µL of cDNA, 0.5 µL of transition primer, 0.5 µL of river primer, 10 µL of primary mix, and 3.5 µL of sterile double distilled water were used for Real-time PCR. The temperature cycle program also includes an initial denaturation at 95°C for 10 minutes, followed by 40 cycles at 95°C for 20 seconds and 60°C for 40 seconds. Relative gene expression of BCAP31, CASP-1 and IL-1.

3. Results and Discussion

3.1. Gene expression microarray analyses

In the present study, we first compared gene expression of seven groups; control, Lactococcus lactis C59 (C59 group and Lactobacillus rhamnosus GG (GG group) administered at a dose of 1 x 107 cells (10^7), 1 x 108 (10^8) cells or 1 x 109 (10^9) cells. With the GEO dataset GSE84949.

A total of 45101 differentially expressed genes (DEGs) with a fold change > 2 and a p-value ≤ 0.01 were found. The differential gene analysis of original chip data was performed using the R language analysis tool from the GCBI website. Principle component analysis (PCA) was applied to visualize the distribution of expressed genes between the two groups and the control group (Fig. 1A). The; research results showed that 1095 receptors or receptor-related factors were identified and caspases had more differential expression in cells treated with these two types of bacteria. Figure 1B shows a volcano plot of the differentially expressed gene Lactococcus lactis C59 (C59 group and Lactobacillus rhamnosus GG (GG group).

To identify the hub genes that might have an essential influence on the formation and development of ALL, DEGs revealed that 20 explicit genes (Casp8ap2, Casp1, Casp12, Aven, Casp6, Cflar, Casp3, Cradd, Casp9, Card14, Caap1, Malt1, Card19, Card10, Casp8, Casp1, Casp2, Card11, Card6, and Casp14) were commonly changed. Next; the co expression networks of the 20 identified genes and receptor-related genes were generated using the protein-protein interaction (PPI) network among the 20 detailed genes was constructed using the STRING online database (Fig. 1C). Among the 1059 explicit genes associated with the receptor, only the BCAP31 receptor genes were directly related and through caspase 1to the expression process related to casp-8 and played a role in the expression of casp-8, Because it was associated with to B Cell receptors and had membrane transmembrane domains, it was chosen as the research target for our purpose, which was to investigate BAB1 antigen with the receptor related to casp-8 stimulation.

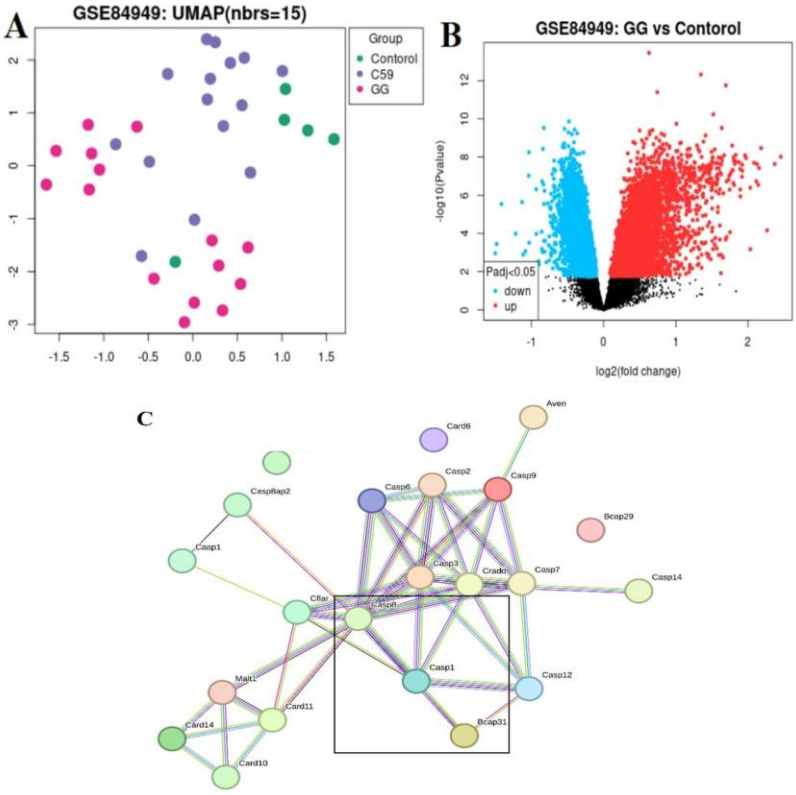

Figure 1: A; Principal component analysis (PCA) shows the clustering of RNA-seq samples by treatment and species. **B;** Volcano plot of differentially expressed gene Lactococcus lactis C59 (C59 group and Lactobacillus rhamnosus GG (GG group). **C;** The protein-protein interaction network of the proteins.

BCAP31 is a ubiquitously expressed transmembrane protein found mainly in the endoplasmic reticulum, including in mitochondria-associated membranes. It acts as a broad-specificity membrane protein chaperone and quality control factor, which can promote different fates for its clients, including ER retention, ER export, ER-associated degradation, or evasion of degradation, and it also acts as a MAM tethered and regulatory protein. It; is involved in several cellular processes – it supports ER and mitochondrial homeostasis, promotes proliferation and migration, plays several roles in metabolism and the immune system, and regulates autophagy and apoptosis. BAP31; loss-of-function mutations cause 'deafness, dystonia, and central hypomyelination' syndrome, characterized by severe neurological symptoms and early death. BAP31 is furthermore implicated in a growing number of cancers and other diseases, and several viruses have been found to target it to promote their survival or life cycle progression. Full-length BAP31 can be antiapoptotic, but it can also mediate casp-8 activation and, be cleaved by caspase-8 to p20-BAP31, which promotes apoptosis by mobilizing ER calcium stores in the MAM [14].

BCAP31 can potentially be used as a prognostic marker for several different cancer forms, and forays have also been made into developing immunotherapies. Early on, an immunotoxin composed of Pseudomonas exotoxin A conjugated to an anti-B31 antibody fragment was effective in killing MDA-231 breast cancer cells in vitro [15]. More recently; vaccination immunotherapy with a plasmid coding for BCAP31 elicited a robust immunogenic response, which suppressed tumor growth in a mouse model for malignant melanoma. In addition; the expression of an intracellular anti-BCAP31 antibody fragment that blocks the interaction with p27Kip1 has been shown to inhibit the growth of mouse gastric cancer [16].

Studies by Niu et al. clarified that BAP31 affects the phosphorylation levels of Zap70/Lck/Lat of upstream members and Akt/GSK/Jnk/Erk of downstream members of the signaling pathway TCR. In addition; BAP31 can regulate the expression of specific markers such as CD3/TCR/TCRβ and specific cytokines such as IL-2/IFN-/IL-6/TNF- which are essential for cell activation T. Taken together, and these results demonstrate that BAP31 may play an essential role in T-cell activation by regulating TCR signaling [17].

Therefore, in this study, we looked for the fact that BAB 1 antigen can play a role in activating the BCAP31 receptor, which can play a role in stimulating immune cells and immune response from a vaccine that can be designed from this antigen by researchers. In; this research, we pursued this goal.

3.2. Identification of BAB1 antigen target to BCAP31 receptor with docking technique

We docked BAB1 Brucella abortus antigen with BCAP31 receptor (Table 1) were analyzed it. According; to our docking analysis, The BAB1 antigen has a relatively high affinity for the BCAP31 receptor (-16.74 Kj/mol).

Protein Name/ID	Global Energy (KJ/mol)	Attractive Van der Wals energy (KJ/mol)	Repulsive Van der Wals energy (KJ/mol)	Atomic contact energy) (ACE)
BCAP31-BAB1	-16.74	-21.99	6.80	3.00

Table 1: Result Docking with patchdock compounds inhibitor BCAP31 receptor and BAB1 Brucella abortus antigen.

The analysis of the complex structure of BAB1 with the BCAP31 with Discovery software showed that the transmembrane part of the BCAP31 receptor, starting from amino acids 7 to 27, can bind with the BAB1 antigen.

So that amino acids Met1, Ser2, Thr10, Phe11, Leu12, Leu22, Lys23, and Ile24 of BCAP 31 receptor can have a binding affinity with amino acids Leu19, Thr20, Ser21, Gly25, Phe26, Arg27, Asp30 and Leu31 of BAB1 antigen that shown in Fig. 2. This; area connecting BAB1 antigen to BCAP31 can be considered a binding area.

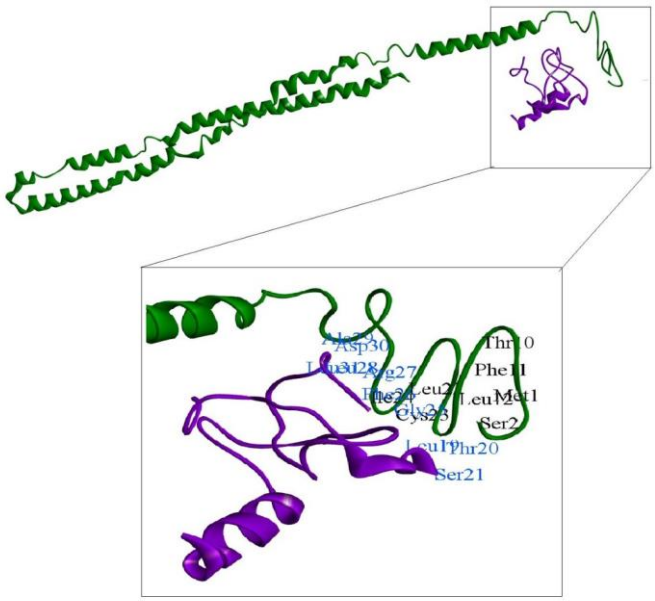

Figure 2: The interaction between BCAP31 receptor (green) and BAB1 antigen (Violet). The inter acting residues are demonstrated by amino acid single letter code and residue numbers.

Chao Jun et al. demonstrate vaccine targeting BCAP31 capable of inducing effective immunity against BCAP31-expressing Malignant melanoma (MM), and the BCAP31, is a promising target for the immunotherapy of malignant melanomas [15].

BAP31 has the potential to be used as a prognostic marker for several different types of cancer, and progress has also been made in the development of immunotherapies. Previously; an immunotoxin consisting of Pseudomonas exotoxin A conjugated to an anti-BAP31 antibody fragment was effective in killing MDA-231 breast cancer cells in vitro [14].

Yu et al. in the 2015 year by vaccination immunotherapy with a plasmid coding for BCAP31 was shown to elicit a robust immunogenic response, which suppressed tumor growth in a mouse model for malignant melanoma [15].

3.3. Structural analysis of BCAP31-BAB1 complex using molecular dynamic simulation (MD)

The final RMSD determines how much the protein changes when its principal coordinates are modified. RMSD; measurements for the protein backbone of the BCAP31 receptor and the BAB1 antigen were evaluated using the basic construction as the starting point (0 to 40 ns). The RMSD values of the BCAP31 receptor alone and the receptor together with the BAB1 antigen are shown in Table 2. The backbone is an essential part of the target structure. Therefore, we have shown the overall backbone simulation concerning time. RMSD values were determined for the free BCAP31 receptor and the BCAP31-BAB1 complex. RMSD values were determined for the free BCAP31 receptor and the BCAP31-BAB1 complex (Fig. 3). Surprisingly; among all the parameters tested, the RMSD values (2.63 nm average) increased rapidly from 0 to 10 ns and remained stable after establishing the equilibrium during the simulation time. BCAP31-BAB1 complexes have higher RMSD values (2.63 nm average) and are roughly comparable to that of the BCAP31 receptor (1.88 nm average). These; this BAB1 antigens have been shown to be stable upon binding to BCAP31, implying that they may serve as a good platform for further studies.

Name	RMSD (nm)	Rg (nm)	RMSF (nm)	H-bond between protein-protein (number)
BCAP31	1.88 ± 0.42	4.36 ± 0.53	0.59 ± 0.89	218.25 ± 51.90
BCAP31-BAB1	2.63 ± 0.85	3.97 ± 0.73	1.00 ± 0.32	225.63 ± 10.90
ave ± SD				

Table 2: The average and standard deviations of Rg, Area per residue, RMSD, RMSF, H-bond between protein-protein H-bond between the BCAP31 receptor and BCAP31 receptor complex with BAB1 Brucella abortus antigen for 50 ns simulation time.

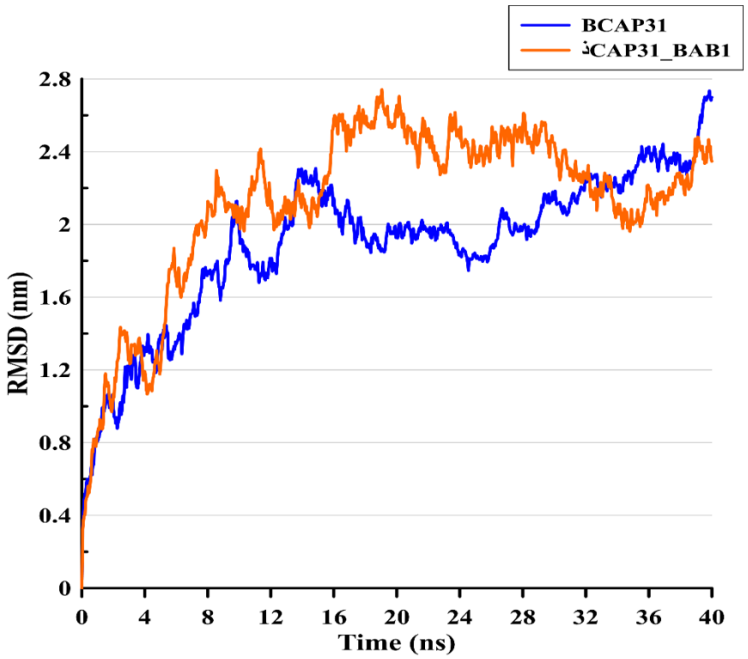

Figure 3: The plot of the root - mean - square deviation (RMSD) of the BCAP31 receptor backbone vs. simulation duration for a solvated BCAP31 receptor complex with BAB1 Brucella abortus antigen.

The backbone of the root means square fluctuation (RMSF) value of the BCAP31 receptor alone was 0.59 nm, and the BCAP31 receptor combined with antigen was 1.00 nm (Table 2). The findings showed the differences at the atomic level. The Fig. 3; shows the RMSF values during the simulation time, the data shows that the BAB1 antigen increases; the structural instability and flexibility of the BCAP31 receptor, which is more in amino acids 120 to 180, which includes the topological domain of the protein. That the RMSF fluctuation of this area shows that the binding of the BAB1 antigen to the BCAP31 receptor can increase the activity of the receptor.

The radius of gyration (Rg) data is utilized in the simulation to determine if the complex is stable in folded or unfolded forms. The BCAP31 receptor and BCAP31 + BAB1 complexes in the first half of the simulation time share similar properties, according to the Rg plot the continuation of the simulation, the BAB1 + BCAP31 receptor complex was more stable and had lower levels (Fig. 4).

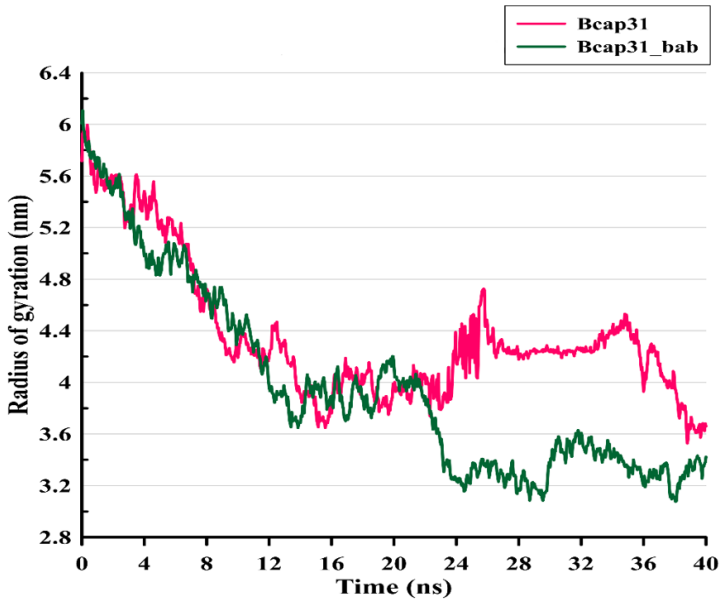

Figure 4: The radius of gyration (Rg) values of the BCAP31 receptor backbone vs. simulation duration for a solvated BCAP31 receptor complex with BAB1 Brucella abortus antigen were plotted against Time (ps) to 40 ns.

Hydrogen bond analysis is used to gain more information about BAB1 antigen molecular interactions, molecular recognition, and selectivity within BCAP31 receptors. Hydrogen bond analysis was used to adjusted receptor-antigen interactions obtained from secondary structure changes during MD simulation (Fig. 5). MD; simulations determine a model for antigen interaction for each receptor orientation. The number of hydrogen bonds formed in MD simulations of all complicated trajectories was investigated. During MD simulations, the BCAP31 + BAB1 complex generated more hydrogen bonds than the BCAP31 alone.

BCAP31 could maintain a regular contact with BAB1 binding pocket throughout the simulation time so that the fluctuations of the BCAP31 receptor alone were higher (standard deviation of 51.90) than the BCAP31 receptor + BAB1 antigen complex (standard deviation of 10.90).

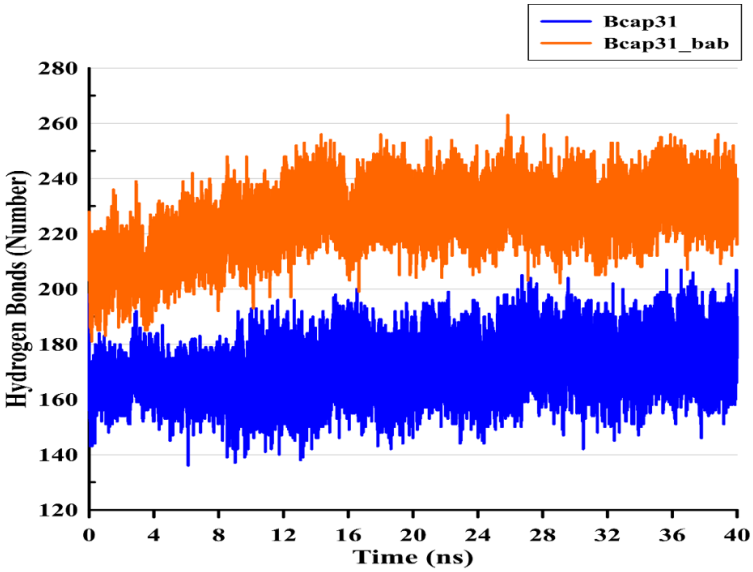

Figure 5: Hydrogen bond patterns of BCAP31 receptor backbone vs. simulation duration for a solvated BCAP31 receptor complex with BAB1 Brucella abortus antigen were plotted against time (ps) to 40 ns.

We then attempted to investigate whether the BAB1 antigen-induced any changes in the secondary structural elements of the BCAP31 receptor during the simulations. Figure 6; shows the classification of the four trajectories in terms of secondary structure elements obtained by the DSSP software tool [18], whose graphs allow a local structural analysis supplementing the characterization of the dynamics abovementioned dynamics characterization. During; the entire period, the stability of the secondary structures was investigated (Fig. 6).

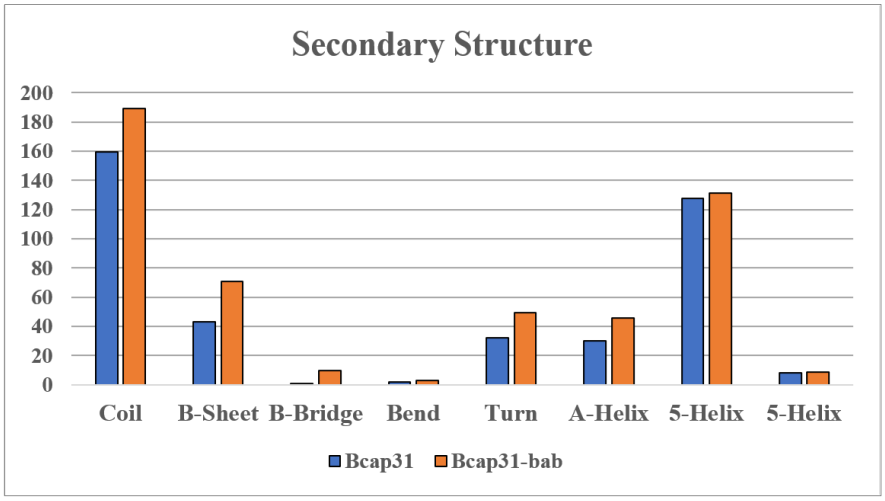

Figure 6: Time evolution of the secondary structural elements along the MD simulation generated by DSSP.

The presence of the BAB1 antigen causes conformational fluctuations in the Bend and Turn and a beta-sheet of the BCAP31 receptor and a small amount of alpha helix.

It can be concluded that the increase of Bend in the receptor structure in the presence of antigen increases the flexibility of the receptor protein. These results of the second structure change are consistent with the results of Rg and RMSF because the Rg and RMSF data showed this increase in the presence of antigen, indicating that the BCAP31 receptor gains a more flexible and open structure in the presence of BAB1 antigen and BCAP31 receptor can be more accessible as a result become more active. As shown in Fig. 1C, the BCAP31 receptor was activated, and as a result, casp-1 and casp-8 activation, and in turn, activation of casp-8 regulates the activation of IL-1.

3.4. Generating and identifying recombinant pNZ8148-Usp45-omp25-L.lactis and expression of it in mRNA and Secretion of BAB1 in L. lactis

The DNA vaccine construct was constructed by inserting the BAB1 gene into the expression vector pNZ8148. The genetic code of the recombinant plasmid served as a measure of cloning efficiency. In addition, DNA analysis showed that the virulence gene sequence of the recombinant plasmid was 100% identical to that of the bacterium B. abortus (Fig .7A).

The product plasmid was digested using KpnI and XbaI. By electrophoretic isolation of the 237 bp digestion fragments, the BAB1 gene, successful production of the recombinant plasmid was confirmed. Blast's findings revealed that recombinant plasmids with an E value of 8e-94, the query covers 100% and Per. The identifier was 99.86% similar to the target bacterium.

The relative amounts of mRNA expression of the BAB1 gene delivery were determined using reverse transcriptase-PCR. The 237 bp band on the agarose gel shows that transcription of the gene has taken place (Fig. 2B).

Western blot analysis by Rojan Azma also revealed that the specific proteins were generated at the protein level.

The electrophoresed L. lactis protein samples from our recombinant bacterium pNZ8148-BAB1-L. lactis and the bacterium transformed with an empty vector are shown in Fig. 7C. The recombinant strain secreted a characteristic protein band that matches the expected size of BAB1 when staining with Coomassie blue gel in cell-free medium. In isolates from cell-free supernatants of the recombinant bacterium pNZ8148-BABA1-L. lactis, Western blot analysis showed distinct single bands that had the predicted size for BAB1. Cultures of L. lactis transformed with the empty vector pNZ8148, lacking the gene coding for rBAB1, do not contain this specific protein band.

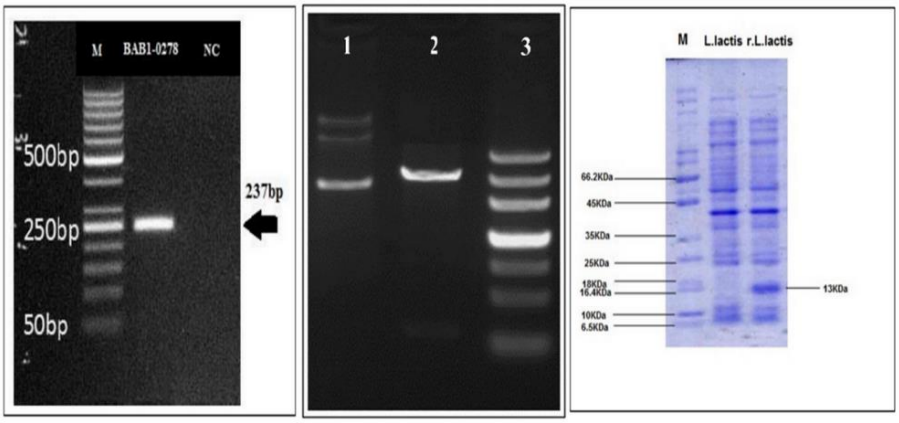

Figure 7: A) Lane 1: Double enzyme digestion shows the bp237 band of the gene along with the signal peptide (693+93bp). Lane 2: recombinant plasmid before enzymatic digestion. **B)** Line 1: expression of BAB1 gene transcription at the mRNA level and the formation of a 237bp band in Lactococcus lactis transformed with a recombinant vector, line 2: Lactococcus lactis transformed with a vector lacking the target gene. **C)** Verify the presence of recombinant protein in transformed Lactococcus lacti

3.5. Gene expression analysis

Examining the expression of the BCAP31 gene in the spleen cells that were immunized by the pNZ8148-BABA1-L. lactis oral vector showed that the expression was higher than the spleen cells that were fed to the mice by the pNZ8148-L. lactis oral vector without the presence of antigen with p-value 0.05 (Fig. 8. A) also showed this difference in expression IL-1 and casp-8 genes were also observed (Fig. 8C and D).

The results of animal experiments with the designed vector showed that BAB1 antigen can increase the expression of the BCAP31 receptor and consequently the expression of caspase 8 and interleukin 1. The causes of this increased expression were discussed and analyzed in the results of the simulation and docking of BCAP31-BAB1 complex, the pathway and protein-protein interactions of the BCAP 31-BAB1 complex.

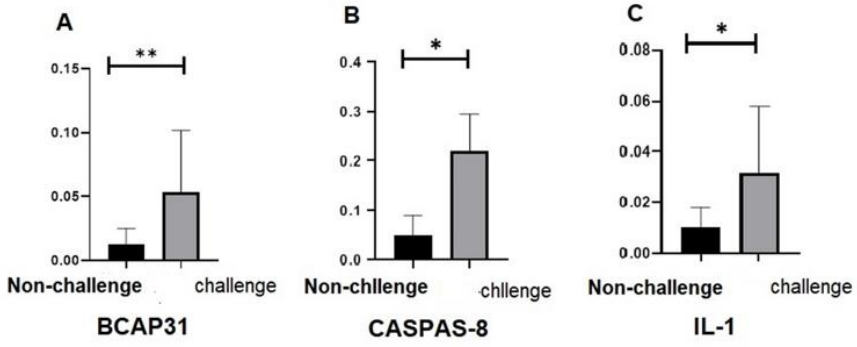

Figure 8: A, B and C show expression levels of BCAP31, CASPAS-8, and IL-1 gene in after oral immunization with pNZ8148-BABA1-L. lactis (Challenge) compared with pNZ8148 A-L. lactis samples (non-challenge) assessed by qPCR, respectively.

Conclusion

Recent research has shown promising results in using Casp-8 and IL-10 activation in Lactococcus Lactis vaccine immunogenicity to detect Brucella Abortus antigens. This; approach could potentially revolutionize Brucella Abortus antigen detection, leading to more accurate and cost-effective diagnostic tools that could significantly reduce the economic and public health impact of the disease. Additionally, using of Lactococcus Lactis as a vaccine delivery platform could lead to the development of more effective and safer vaccines against the bacteria.

This study showed for the first time that oral delivery of BAB1 antigen by Lactococcus Lactis bacteria can create strong structural interactions between the BCAP31 receptor and BAB1 antigen and can activate this BCAP31 receptor and, through this the, cascade pathway of caspases. This; approach of molecular studies and molecular dynamics with the aim of finding mechanisms of immune activation using antigens can be of great help to researchers in this field.

Declarations

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Declaration of Competing Interest

The authors declare that there is no conflict of interests.

Data availability

Data will be made available on request.

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