Journal of Biotechnology & Biomaterials

Mini Review Open Access

As a Candidate for a Vaccine against Acne and its Immunisation in an Animal Model, the CAMP-Sialidase Chimaera Protein was Created and Purified

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Abstract

Introduction: Approximately 85% of young individuals between the ages of 12 and 25 have acne vulgaris. It is important to create effective acne vaccines due to *Propionibacterium acnes*' crucial involvement in the aetiology of acne and existing treatment failures. As a result, the goal of this work was to create a chimeric protein from the CAMP and sialidase portions of *Propionibacterium acnes* and assess its immunogenicity in a mouse model as a potential acne vaccine candidate.

Literature Review: The pET28a vector was used to clone the CAMP-sialidase recombinant gene and transfer it to the *E. coli* BL21DE3 strain. Utilizing a Ni-NTA column, the protein was purified, and its concentration was assessed. Both the test and control mouse groups received injections of the recombinant protein. To ascertain immunogenicity, antibody titration and challenge tests were conducted.

Discussion: The protein was successfully expressed and purified, and a band with a molecular weight of 65 kDa was seen. The isolated protein was verified by western blotting. The serum ELISA findings showed that the IgG titer against the recombinant protein and inactivated *P. acne* was 1:204800 and 1:1600, respectively. Although test mice showed no change, 50% of the control group had inflammation.

Conclusion: The recent work shown that the recombinant CAMP-Sialidase protein may effectively produce humoral antibodies. It still need additional testing before it can be considered a contender for an acne vaccination.

Keywords: Acne; Propionibacterium acnes; CAMP-Sialidase; Recombinant protein; Acne vaccine

Introduction

One of the top three most prevalent skin conditions worldwide is acne [1]. A whopping 85% of adults between the ages of 12 and 25 have acne at some point in their lives [2]. Acne is a multi-factorial condition that is primarily brought on by excessive follicular keratinization, which blocks the pilosebaceous canal and leads to the spread of acne scars, and abnormal bacterial function. Sebum production in the skin is increased, which contributes to the formation of comedowns and other acne-causing substrates (*Propionibacterium acnes*, a Gram positive anaerobic bacterium that resides as a member in the human skin normal flora). Creating a suitable acne vaccination is welcomed given the burden of the condition and increased therapy method failure. In this context, research have shown that *P. acnes* plays a crucial role in the aetiology of acne illness, making the pathogen an excellent target for an anti-acne vaccination as a preventive measure. The *P. acnes* extracellular enzymes, cell wall or membrane fragments, and proteins linked to cell membranes have been found to be the best vaccine targets thus far. Kim showed in a study conducted in 2008 that some antibodies made against *P. acnes* caused tissue damage [2]. He came to the conclusion that all antibodies made against *P. acnes* are useless and can potentially make the condition worse. Recombinant sialidase-vaccinated mice developed antibodies against acne infection. In a different investigation, at the prevalence root causes, and interactions between *P. acnes* and toll-like receptors of the acne disease (TLRs). The CAMP factor-vaccinated mice that produced antibodies had less inflammation brought on by *P. acnes* [3]. As an alternative to topical antibiotics, new therapies should be developed using molecules implicated in immune system activation, such as TLRs, protease receptors, and microbial peptides [4]. In this experiment, we attempted to employ a chimeric construct (a combination of both sialidase protein and CAMP factor) and delivery in a mouse model as a vaccination candidate for acne in light of the significant frequency of this illness, antibiotic resistance, and absence of an effective vaccine.

Literature Review

Culture of *P. acnes*

The Pasteur Institute provided lyophilized *P. acnes* (PTCC 6919), which was purchased. 1 ml of sterilised BHI was added after being moved to the research lab, and the mixture was incubated there for an hour at 37°C. Following that, 150 l of the material was grown linearly on blood agar under anaerobic conditions for three days at 37°C using a gas-pak.

Design and Construction of Recombinant Chimeric Structure

230 and 300 carboxyl terminal amino acids, respectively, were

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Received: 03-Jun-2022, Manuscript No. JBTBM-22-70473; **Editor assigned:** 06-Jun-2022, PreQC No. JBTBM-22-70473(PQ); **Reviewed:** 20-Jun-2022, QC No. JBTBM-22-70473; **Revised:** 22-Jun-2022, Manuscript No. JBTBM-22-70473(R); **Published:** 29-Jun-2022, DOI: 10.4172/2155-952X.1000282

Citation: Mansouri K, Ahmadi A, Salimian J (2022) As a Candidate for a Vaccine against Acne and its Immunisation in an Animal Model, the CAMP-Sialidase Chimaera Protein was Created and Purified. J Biotechnol Biomater, 12: 282.

encoded by two sections of the sialidase and CAMP genes, respectively. For greater flexibility and effective separation, a third component was used as an interface between the two parts, consisting of four copies of the EAAAK linker sequence. The protparam online server, proccaleexpas software, proccaleexpas software for flexibility and polarity, and clustalW software for detecting conserved sequences were used to estimate the physicochemical properties of the chimeric structure. The *Escherichia coli* bacterial host was used to clone and express the chimeric gene. The construct was extended with the His-tag to streamline the purification process.

Expression of the CAMP-Sialidase Recombinant Protein

The recombinant plasmid pET28a-carrying BL21DE3 strain of *E. coli* colonies was used to express the CAMP-Sialidase gene. First, kanamycin (40 g/ml) was added to 5 ml of LB containing 50 l of bacteria, and the mixture was incubated at 37°C with overnight shaking. Then, 100 ml of fresh culture media was mixed with kanamycin (40 g/ml) and a 5 ml sample of the bacterial culture, and the mixture was shaken for 3 hours at 37°C and 150 rpm until the absorbance at 600 nm reached around 0.7. In order to express the target gene, 1 ml of IPTG at a concentration of 1 mM was added to 100 ml of culture medium, and the culture was shaken overnight in an incubator [5, 6].

Purification of Recombinant Proteins

The 6His-tagged sequence required the use of a Ni-NTA column for purification. At 4°C for 10 minutes, the medium was centrifuged at 6000 rpm. After the precipitate was separated, the supernatant was drained, and the sediment was then mixed with lysis buffer and denaturation B buffer in a 3:1 ratio before being sonicated. After sonication, the material was centrifuged for 15 min. at 13000 rpm to separate the supernatant. The column was fed the supernatant, and the result was collected. The output was then collected after the column had been washed with 20 and 250 mM imidazole and 20 mM MES buffer.

Gene Expression Analysis Using Polyacrylamide Gel

The samples were first produced and purified, after which they were electrophoresed on a 12% SDS-PAGE gel, and the Bradford method was used to gauge the protein concentration.

Confirming Expressed Protein with Western Blot

Each sample was placed into the SDS-PAGE 12% gel electrophoresis container with 10 µl, along with the loading buffer, and the electrophoresis was carried out at 100 voltages. The gel was taken off after electrophoresis, and the Wattman filter paper was then put on the sponge. The nitrocellulose paper was placed on the gel after spending at least 30 minutes being submerged in an electroblotting buffer, and was then followed by another wattman filter paper on nitrocellulose paper. The sandwich was put inside the tank, the transfer operation operated for 2 hours at 100 V, and then the sandwich floated in blocking buffer 5% (milk powder in PBST) for the night. Each nitrocellulose paper cut was washed with PBST before being soaked in a 1:1000 dilution of the anti-histidine solution, which was then stacked for an hour at 37°C. After washing, DAB solution was applied to nitrocellulose paper, and the reaction was stopped when bands appeared.

Mice Immunization

Balb/c mice were split into two groups, each with five animals, for the delivery of recombinant CAMP-Sialidase protein. Twenty micrograms of the recombinant CAMP-Sialidase protein were combined with both complete and incomplete Freund's adjuvants in the initial injection. For the control group, PBS was injected. Every two weeks, three injections were given, and blood samples were taken and stored at -20°C after each injection.

Immunization of Mice with Heat-killed *P. acnes*

Five mice were used as positive control groups, and they received the *P. acnes* vaccine after being exposed to heat-inactivated *P. acnes*. colonies were cultivated and dissolved in PBS until the absorbance reached 0.3 in order to render *P. acnes* inactive (600 nm). The sediment was then dissolved in 500 ml of PBS and incubated at 70°C for 45 minutes using 5 ml of the culture that had been centrifuged at 5000 rpm for 10 min. Each mouse received 200 µl of the solution, which was combined with complete Freund's adjuvant in the first injection and incomplete Freund adjuvant in the following injections. Three injections were given every two weeks, one after each blood sample.

Determination of IgG Responses to Recombinant Proteins Using Indirect ELISA

The ELISA wells were coated with five micrograms of CAMP-Sialidase recombinant protein. Additionally, all wells received 100 µl of blocking buffer (5% skim milk/PBST). The conjugated mice antibody was added (diluted 1/800) after the serum titration in the wells was completed. After adding the TMB substrate, the reaction was stopped with 2M H_2SO_4 and the absorbance was measured at a wavelength of 450 nm. After finishing each ELISA stage, the washing procedure with PBST buffer was repeated three times.

Bacterial Challenge

One month following the last injection, 107 CFU of *P. acnes* were diluted in a PBS buffer and injected into the ear of the inoculated mice and the control group to study vaccination in mice. The other ear received an injection of PBS as the control. The thickness (measured using a millimetre ruler) and ear inflammations in mice were then monitored daily for the following 40 days.

Statistical Analysis

The test and control groups were analysed using the t test. P values under 0.05 were deemed significant.

Discussion

The best target for the vaccine that can develop protective antibodies is currently the main issue with the P. acnes vaccination [5]. 24 h after the inactivated bacterial injection, the mice's ears were enlarged and their skin was fried [3]. Tissue infusion in this study revealed that the *P. acnes* injection significantly increased the number of inflammatory cells. Mice were vaccinated with injections of heat-killed *P. acnes* three times over the course of a one-week period in order to generate a sufficient antibody against *P. acnes*. Serum was taken a week after the last immunisation. Two main *P. acnes* combinations (about 64 and 250 kDa) had a greater immune response than antibodies isolated from heat-treated entire bacteria, according to data from SDS-PAGE and western blot. Additionally, nasal delivery shows that *P. acnes*-based inactivated vaccines are appropriate for mucosal immunity. The inoculated mice were challenged intraperitoneally with *P. acnes* to test the level of immunity produced by vaccination with the heat-killed *P. acnes*, and the increase in ear thickness was noted. When the mice received an immunisation with inactivated *P. acnes* in both periods, this increased ear thickness drastically decreased (days 1 and 7). After 22 days of the challenge, the *P. acnes*-immunized mice's rise in ear thickness totally disappeared, indicating that immunisation with inactivated P. acnes slows down bacterial growth and lowers inflammation. Acne

vaccinations would result in new treatments for acne-related disorders. The results of this work demonstrated that purified recombinant sialidase degrades sialoglycoconjugates and releases sialic acid. *P. acnes* was subcutaneously administered into the left ear of mice that had received recombinant sialidase or GFP vaccinations afterward, while PBS was put into the right ear as a control. The thickness of the ear was tested every 71 days until it totally shrank. The sebocyte cells' ability to survive after two hours of treatment with pure sialidase was unaffected. Sebocytes were subjected to *P. acnes* culture overnight after receiving sialidase treatment for the first two hours. *P. acnes* caused 20% of the treated sebocytes in the control group to die, but the sialidase-treated sebocytes experienced much higher levels of cell death (about 34%). This shows that sialidase therapy improves the sebocytes' selectivity and susceptibility to *P. acnes*. These findings, along with the fact that sialidase is a peripheral protein with the LPXTG pattern, suggest that sialidase may be a useful candidate for an acne vulgaris vaccine. Mice were given a nine-week vaccination with dead *P. acnes* to test the immunogenicity of sialidase. The *P. acnes* cell lysate, recombinant sialidase, and GFP were all analysed using a western blot. The mice serum, which was taken from the immunisation treated mice with heat-killed *P. acnes*, demonstrated an immune response to a significant number of proteins with a molecular weight larger than 50 kDa [6]. In this experiment, mice were immunised using Freund's adjuvant and either recombinant sialidase or GFP. Two weeks after the final vaccination, antibody production was seen in the serum of immunised mice during the western blot examination. A distinct band of 53 kDa was seen when the pure sialidase reacted with the serum of sialidase-immunized mice, indicating that sialidase induced an immunological response in the immunised mice. In this investigation, recombinant sialidase was added to the inactivated P. acnes vaccination of the mice. The findings demonstrated that sialidase should not be immunogenic when administered with deceased P. acnes, but is immunogenic when administered with recombinant protein. Because it causes a potent antibody response against *P. acnes* sialidase, it can successfully stop the progression and recurrence of the disease in acne patients. Instead of using dead *P. acnes* as an immunogenic agent, acne vaccines use *P. acnes* sialidase, which lowers the risk of negative side effects. Consequently, a sialidase-based vaccine for acne may be more targeted while minimising side effects. The CAMP factor plays a role in the *P. acnes*-induced inflammation by immunising mice with the CAMP factor, which confers protective immunity against *P. acnes*. CAMP factor intranasally immunised mice with UV-deactivated *E. coli* were compared to a control group in order to gauge the immunogenicity of the CAMP factor (GFP control protein) [7-9]. Raised IgG titers were seen fourteen days following immunisation, however they weren't seen in mice that had been inoculated with GFP. The antibody titer significantly increased at 14 and 21 days following inoculation, according to an ELISA analysis. The IgG antibody titer in the serum of the inoculated mice was greater than 100,000 in the twenty-one days following vaccination, but it was less than 100 in the control group. Recombinant CAMP factor and GFP were injected subcutaneously into the mice's ears in order to assess the immunogenicity of the CAMP factor in *P. acnes* in vivo [9-12]. The thickness of the ears significantly increased after 24 hours of CAMP factor injection, demonstrating the CAMP factor's contribution to inflammation. In the test and control mouse groups of our study, we used a mixture of two components of the CAMP protein and *P. acnes* sialidase as a vaccination candidate. The intervention group received three subcutaneous injections of the recombinant protein. After that, both the test and control groups received injections of live bacteria into their ears. The ultimate immunogenicity was evaluated by measuring the antibody titer in the intervention group. We also looked for any inflammation in the mice's ears. The vaccine group, for which the inactivated bacteria were injected in three stages and the antibody titer was assessed, was also taken into consideration for additional research and comparison. The intervention group's antibody titer was 1.204800, while the vaccine group's was 1.1600. This striking and significant change in antibody titer suggested that the recombinant protein was more selective and effective. The mice's ears were reportedly irritated in 50% of the control group. The mice's ears did not get inflamed after receiving a live bacterial infection in the recombinant protein immunised group, and the antibody titer rose after each blood draw. As a result, it would seem that this recombinant protein is a perfect option for a safe vaccine. To better assess the recombinant protein's therapeutic efficacy for acne illness, more research on this protein may be required [11].

Conclusion

In general, dead bacteria have non-specific immunity, but the CAMP and sialidase factors as well as the chimeric protein CAMP-sialidase function in a particular way. In our investigation, the specificity of the CAMP-sialidase protein was improved by combining portions of the genome that support disease.

Author's Contributions

The project was conceived and overseen by JA, who also carried out the analysis and designed the figures. KM participated in the planning phase of the study and wrote the article. AA and JS also contributed to the planning phase of the study and proofread the paper. Each author contributed to the manuscript's critical editing.

Conflict of Interest

The authors declare that they have no conflicts interest.

Acknowledgement

Financial funding for this study came from Baqiyatallah University of Medical Sciences. Additionally, the project was the outcome of an MSc student's thesis.

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