INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 2, p. 160-165, 2019

RESEARCH PAPER OPEN ACCESS

Infection and immunization of mice with *Pseudomonas aeruginosa* antigens

Noor M. Mahmood*, Mohammed J. Alwan

Zoonosis Unit, Collage of Veterinary Medicine, University of Baghdad, Iraq

Key words: P.A: Pseudomonas aeruginosa, P12: Pseudomonas aeruginosa strain 12, P15: Pseudomonas Aeruginosa strain 15. Sonicator MSE-UK: device to break the bacteria to get broken antigen. PBS:Phosphate-buffered saline.IL: Interleukin. IFNγ: Interferon gamma. I G: Immunoglobulin.

http://dx.doi.org/10.12692/ijb/15.2.160-165 Article published on August 09, 2019

Abstract

The aim of this is preparation of different antigens, we prepare these types of antigens to compare which one is the best for uses in future in another animals (broken antigen, floating antigen, double antigen, dissolved antigen, killed antigen) from the *Pseudomonas Aeruginosa* to Immunize mice and measure the humoral and cellular immune response in immunized and control mice. Finally note the clinical symptoms and conduct the bacterial isolation of the internal organs. (95) Swiss laboratory white mice were taken from the National Center for Drug Control / Ministry of Health. First group: consisted of (15) animals prevented by a dose of (0.5) mL of the broken antigen subcutaneously, protein concentration is (4.7) mg / ml and the same dose was returned after (2) weeks. Second group contains (15) animals were treated as in the first group, but the use of floating antigen. Third group have (15) animals were injected with (0.5) ml of the subcutaneous antigen and after one week were treated as in the fourth group with the floating antigen and dissolved antigen (the double antigen). Fourth group consist from (15) animals treated as in the first group but using the dissolved antigen. Fifth group it includes (15) animals treated as in the first group, but using the killed germ antigen. Six group includes (10) animals as a positive control. Seven group contain (10) animals as a negative control. After that all mice have Delayed type hypersensitivity test and Humoral immune response (indirect blood test). I advice to do a study on use of a combination of floating antigen and broken antigen to immunize the laboratory animals after exposure to burns contaminated with vesicles.

* **Corresponding Author:** Noor M. Mahmood ✉ Noormahmood23@yahoo.com

Introduction

P.A form bacilli (0.5-0.7) microns and their lengths (1.5-3) microns and appear in swabs taken from the broth and media in single or pairs formations, and may sometimes appear in short chains (Jawetz *et al.*;1987). Govan (1997) noted that the *P.A* colonies are large colonies with a diameter of (3-4) mm, convex, its edge not arrangement take three shapes (Rough forms), soft colonies and mucus forms (Mucoid forms). These microbial media have been producing bluecyanidic dye and pyopridine, respectively (Quinn *et al.*, 2006). The bacteria produce many pigments such as radioactive Pyoverdin dye, Pyocyanine blue dye, red pyrobinin pigment, Brown Pyomelanin dye, Fluoresence, Phenazine and phytoplankton Oxyphenizin, a biocyanine dye with bacteriosin, which kills other germs (Knight *et al.*, 1979). Another distinguishing characteristic of the cyanobacteria is its ability to produce a distinctive odor similar to that of grapes or fermented apples when they grow at different temperatures. This smell is due to amino acetophenone (Jawetz *et al.*, 1987; Quinn *et al.*, 2006). The aims of this study are preparation of different antigens from the *P.A*, Immunization of mice with prepared antigens, measure the humoral and cellular immune response in controlled and control mice, at the end observe clinical symptoms and conduct bacterial isolation of internal organs and study pathological changes on the inhibited mice after giving a challenge dose.

Material and methods

(95) Of the Swiss laboratory white mice were used by the National Center for Drug Control / Ministry of Health. There age are from (60-75) days and there weight from (22-30) g, and provided adequate hygienic conditions in terms of cleanliness, sterilization, food, clean water, proper temperature and ventilation. The animals were placed in clean plastic cages and fitted with a metal cover with a water bottle and a food place. The floor was sprinkled with sawdust and changed from time to time to keep the place clean. The mice were left for a week without treatment to adapt to the new situation. Preparation of antigens: The germ of bacteriophageal strains (p12) isolated from the skin burns of the activated and purified on Nutrient broth and incubated at (37) ° C for (24) hours. Examine the bacterial growth to ensure purity by preparing a glass slide and Gram stain. Transfer (0.5) ml of nutritious broth to Nutrient agar medium and distribute this amount evenly on the surface of the plant medium by stirring the dishes, then leave to dry. Transfer the dishes to the incubator and leave for (24) hours. Take the non-contaminated dishes by adding (5) mL of PBS solution to each dish and use a sterile glass diffuser to harvest the germs and place them in sterile test tubes. The centrifuges were centrifuged at (3000) cycles / min for (15) minutes and retained the floating fluid representing the antigen. The protein concentration was measured and was (42) mg / ml according to the Bayoret method. Wash the germ cells three times using PBS and each time the floating liquid is neglected. Add an appropriate amount of PBS to the bacterial precipitate. Bacterial cells were subjected to cracking using a (12) peak-peak by using Sonicator MSE-UK for (30) minutes using ice. Break the broken cells using the centrifuge at (3000) rpm and keep the drip. To verify the cracking efficiency, a sample of the precipitate was taken and Gram stain was used. Add a fraction of the liquid containing the broken cells to the precipitate. The cracker was retracted for (30) minutes. A sample of this liquid was also taken and Gram stain was doing and tested under the microscope to confirm the cracking. The fractured cells were separated using a centrifuge with a speed of (3000) cycles / min. The floating fluid was separated from the precipitate and dissolved antigen was found. The concentration of the protein was measured at (142) mg / ml and the floating liquid was stored in the refrigerator at (-20 °) C. The same steps (1-8) were performed to prepare the total broken antigen and the protein concentration of this nutrient was measured at (149) mg / ml. The same steps were taken from (1-9) but using the cooled centrifuge (4) at (10,000) cycles / minute and taking the floating fluid to examine the delayed skin sensitivity and the concentration of the protein was (133) mg / ml and was diluted to (3.2) mg / ml.

Preparation of Killing Antigen of the germinated germ cells of the P.A:

Attended by Hiallibartioto and Blazkovec (1975) as follows, the germinated (beaten) 2 germinated on the nourishing broth was incubated and incubated at 37 ° C for 24 hours. Examine bacterial growth to ensure its purity by Gram stain. Transfer (5) ml of nutrient broth and culture on nutrient agar and plant this amount evenly on the surface of the media by stirring the dishes and then leave to dry. Then transfer the dishes to the incubator and leave for 24 hours. Non-contaminated dishes were harvested germs as follows: Harvested using PBS solution and put into test tubes. Comparison of total number of bacterial harvesting in MacFarlane tube. After washing the microbial growth by centrifugation at (3000) cycles / minute for (30) minutes and for two consecutive times and the floating liquid is neglected. Add formalin with a (0.05%) concentration to the original volume and leave the plant for (48) hours to ensure killing.Wash bacterial growth three times with PBS solution and using centrifuge at (3000) rpm for (15) minutes. To ensure the bacteria is killing, take bacteria and culture on agar media to notice there is no bacterial growth. Suspend the precipitate with PBS solution and keep in sterilized bottles until use.

Preparation of the challenge dose

The germ was planted on nutrient agar medium and incubated at (37) ° C for (24) hours. Separated bacterial growth using the centrifuge at (3000) cycles / minute for (15) minutes and neglected the floating fluid. PBS was added to the original volume level and the bacterial count was (1 × 10^9) by MacFarlane tube and both.

Design Experience

First group: consisted of (15) animals prevented by a dose of (0.5) mL of the subcutaneous total antigen and a protein concentration of 4.7 mg / ml and the same dose was returned after 2 weeks. Second group: Contains (15) animals were treated as in the first group, but the use of floating antigen. Third group: - (15) animals were injected with (0.5) ml of the subcutaneous antigen and after one week were treated as in the fourth group with the floating antigen and dissolved antigen (the double antigen). Fourth group: It includes (15) animals treated as in the first group but using the dissolved antigen. Fifth group: It includes (15) animals treated as in the first group, but using the killed germ antigen. Six group: - includes (10) animals as a positive control. Seven group: - Includes (10) animals as a negative control. After (27) days of the first immunization, the delayed sensitivity examination of the vaccinated animals was carried out, and (5) animals from the blood withdrawal groups were screened for the humoral immune response. After (30) days of immunization, the first, second, third, fourth, fifth and sixth groups were infected with (0.5) ml the bacterial suspension contains (1 × 10^9) cfu / ml of the *P.A* (P12). The seventh group was injected under the skin with (0.5) ml of sterile sterile saline solution. Twenty days after the challenge dose, (6) animals from the first, second, third and fourth groups were killed and the remaining animals from these groups (4 animals) were infected in the same way as the challenge dose, but in the (P15) strain of *P.A* and positive control group.

Delayed type hypersensitivity test

This test was approved according to the method (Ohta *et al.*, 1983) to examine the cellular immune response in the experimental animals. The left foot pad was injected with (0.1) ml of the forbidden animals as well as the negative control animals of the antigen. The right foot pillow was injected with the same dose of the equivalent saline solution. The thickness of the foot pad was measured before the injection and after (24) and (48) hours of the examination using the vernea to measure the thickness of the skin.

Collection of serum samples

The blood was withdrawn by capital of plastic syringes with a capacity of (1) ml of controlle and immune mice. The blood was slowly emptied from the syringe after the needle was removed from sterile test tubes and left for an hour at (37) ° C in the incubator. The clot was separated on the wall of the tube by a

sterile iron rod the samples were discarded at a speed of (2000) cycles / min for (5) minutes, followed by serum, placed in sterile small containers and kept at (-20) ° C until the examination.

Measurement of Humoral Immune Response: - by indirect blood test

Sampling Collecting

Herbert (1978) was examined by taking the blood from the jugular vein of a sheep using medical syringes and placed in a sterile glass tube containing the same amount of AL sever solution as anticoagulant and keeping this mixture in the refrigerator for (3) days and then separating the plasma and solution Al sever for red blood cells by the device at (1500) cycles / minute for (10) minutes and washed the blood cells three times using PBS solution (7.2 = PH) using the alarm device (1500) cycles / minutes for (5) minutes after which we get rid of the stink using pasture pipette) and red blood cells were concentrated at (3%) concentration.

Dice Red blood corpuscles with Tannic acid

The tank acid is prepared at a concentration of (20000/1) by dissolving (2.5) mg of acid in (50) ml of PBS solution and well mixing. After that, equal amounts of tank acid and RBC (3%) are mixed for (20) minutes and at (37) ° C then remove the tank acid by washing the RBC with PBS solution three times in the dispenser and then re-suspend the RBC with the same size as the previous PBS solution.

Raising red blood cells with soluble antigen

Take (2) ml of the antigen and dissolve it in three sterile tubes in sterile test tubes containing PBS solution and add (2) ml of the red blood cell buffer to each tube. Place in the incubator at (37) ° C for (30) min. And the red blood cells are separated from the antigen residue by deposition of (1,500) cycles / min for (5) minutes. The red blood cells are then washed three times with PBS solution and the sensitized blood cells with the PBS solution are (2) ml.

Optimal concentration testing of antigens

The microtiter plate, which contains (96) small holes, is distributed and the rabbit serum solution (0.01) ml is distributed in each hole. After that, a double-acting animal serum is activated, preventing the supplement by heating it to (56) ° C for half an hour in the bath. (0.02) of the serum in the first hole by the microtiter pipette and mix and transfer (0.02) ml to the next hole and follow the same method for each hole is discarded another (0.02) ml of the last hole added (0.02) ml of Each antigen is optimized for red blood cells that are sensitive to each dilution of one row of holes. Of red blood, which gives the highest positive reading, and the optimal reduction of the antigen of *Pseudomonas Aeruginosa* was (50/1).

Control

Control reagents are used to compare with the results that have emerged

1. (0.02) ml of non-sensitized blood with (0.02) ml of neutral PBS solution.
2. (0.02) ml of non-sensitized blood with (0.02) ml of natural rabbit serum at (1%) concentration.
3. (0.02) ml of non-sensitized blood with (0.02) ml of known anti-serum.
4. (0.02) ml of non-sensitized blood with (0.02) ml with first dilution of non-vaccinated mice as negative control.

Results

Cellular immune response (delayed skin sensitivity test)

The average thickness and error scores in the foot pads of experimental animals after (27) days of the first immunization and after (24-48) hours of skin examination against the soluble antigen of the bacteria. The results showed that the standard thickening and error rate in the pillow by double antigen (0.19 ± 1.88) was higher than in the floating antigen (0.14 ± 1.39) and the total antigen (0.11 ± 1.3) and 48 hours after the examination was decreased Standardization and standard error in animals with double antigen (0.12 ± 0.9), floating antigen (0.09 ± 0.8) and total antigen (0.15 ± 0.77) and dissolved antigen (0.12 ± 0.45) and killed antigen (0.07 ± 0.38). The results indicated that there is a significant statistical difference between the totals level of P

<0.05).

Humoral immune response (indirect blood test)

Results showed that mice with total antigen inhibitor showed the highest rate of total antigen (31.3 ± 179) compared with dissoluble antigen (15.67 ± 102.4); float antigen (17.5 ± 64), double antigen (783 ± 35.2) and killed antigen (4.8 ± 20.8).

Discuss

The results showed that the antigen used in the immunization stimulated a cellular and humoral immune response. Cellular immune response (delayed skin sensitivity test) showed the animals with the double antigen was the highest rate of thickening in the foot comfort (0.19 ± 1.88) followed by the animals that were immune by the floating antigen (0.09 ± 0.8), then the total antigen (0.15 ± 0.77), finally the killed antigen, this result showed that there was a difference in the standard of antibodies in protected animals according to the type of immunization antigen, where the highest rate of antibody standard was recorded in animals with a total antigen and the lowest rate in immunized animals by killed antigen. The reason for this disparity in the cellular immune response between the immune groups may be due to the difference in the components of these antigens as well as in the area of interaction with immune cells. These results coincided with what was stated by Dannenberg (1968), where he explained that the difference in the composition and proportions of antigens affect the size Immune response in skin examination and types and numbers of sensitive lymphocytes. The thickening of the area injected with the dissolved antigen indicates the accumulation of pharyngeal and lymphocytes as a result of the cellular immune response. This observation is consistent with what Tizard (1982) stated. We believe that the antigens used in this study stimulated the cellular and humoral response, which is essential in the resistance of *P.A* because the microbial takes two phases in its morbidity: the extracellular stage and intracellular phase. This result is consistent with what some researchers mentioned, (Gregory *et al.*, 2002; Gocke *et al.*, 2003). Our results showed that animals immunized with total antigen (31.3 ± 179) and floating antigen (17.5 ± 64) and double antigen (783 ± 35.2) are the highest values in humoral immune responses compared to the antigen group with significant difference between them (P <0.05). In this finding, we believe that there is synergistic action between T lymphocytes Tomohiro (1997). T lymphocytes have synergistic action of B lymphocytes (there function is produce antigen)to multiply and differentiate them into affective cells and immune cells B lymphocytes have the ability to devour antigens, process them and present them to MHC II (molecules are a class of major histocompatibility complex), and Th2 lymphocytes (a kind of T cell that do an main role in the immune system) , sensitize these antigens introduced by B lymphocytes, multiply and produce cellular orbits IL.10, IL.4 and IL.12 These lymphocytes activate the differentiation of active B lymphocytes into immune cells and antibody-producing plasma cells. IL.12 stimulates B lymphocytes to produce IFN.y, which regulates the response of IgE, IgG1, and IgG2a antibodies, activating the IgE / IgG1 approved production on IL.4.

Conclusions

The double antigen stimulates the best cellular immune response and stimulates the broken antigens in the immune response. The broken antigen, dissoluble antigen, double antigen, and the floating antigen are protect the mice against the dose of challenge with Pseudomonas aeruginosa (P12) after the challenge dose of (P15) and the longest stay a life of mice which immune with double antigen. The cellular and humoral immune response works together to counteract the infection of vesicular spores.

Recommendations

Doing a study on use of a combination of floating antigen and broken antigen to immunize the laboratory animals after exposure to burns contaminated with vesicles, use a combination of bacterial antigens to immunize against other

serotypes, finally conducting a study of the pathogenicity of *Pseudomonas Aeruginosa* in field animals.

References

Jawetz E, Melnick JL, Adeberg EA. 1987. In Medical Microbiology. Appleton and 17th ed auge, Norwalk connec ticut. Los. Atlos. California, p 247-250.

Govan JR. 1997. Pseudomonas and non-fermenter. In : Medical Microbiology, By: Greenwood, D.; Slack, R. and Peutherer, J., 15th Ed. Vol.1, Cjurchill Livivg Stone, New York, p 413-422.

Quinn PJB, Carter ME, Markey PK, Carter JR. (2006). Clinical veterinary microbiology. London Mosby-Wolf., P 284-286.

Knight MPE, Hartman ZH, Young VM. 1979. A new method of preparation of pyocinin and demonstration of unusual bacterial sensitivity. Analytical Biochemistry **95,** 19-23.

Hiallibarton BL, Blazkovec AA. 1975. Delayed hypersensitivity and acquired cellular resistance in guinea pigs infected with Listeria monocytogenes. Infect. Immun. **11,** 1-7.

Ohta V, Saeki K, Yoneyema F, Tuzuka M, Yagoi Y. 1983. Immunomodulating activity of thymisin fruction-5 and Thymosine α-1 in immunosuppressed mice. Cancer Immunol. Immunother **15,** 108-113.

Herbert WJ. 1978. Passive haemagglutination with special reference to the tanned cell technique In: "Handbook of Experimental Immunology". (Weir. P.M.). 2nd ed. **3,** 20.1-20.20, Blackwell Scientific.

Dannenberg A. 1968. Cellular hypersensitivity and Cellular immunity in the pathogenesis of tuberculosis: specifically systemic and local nature and associated macrophages enzymes. Bacteriological Reviews.
32, 85-102.

Tizard L. 1982. An introduction to veterinary immunology 2nd (ed.) W. B. saunders. Co. Canada. p 300.

Gregory PP, Bringing MM, Hatano K, Coleman FT, Pier GB, Goldberg JB. 2002. Constructional characterization of a live, attenuated aro A delenom mutant of *Pseudomonas aeruginosa* as a caudicate intranasal vaccine. Infect. Immun **70,** 1507-1517.

Gocke K, Baumann U, Hagemann H, Gabelsberger J, Hahn H, Freihorst J, Von Specht BU. 2003. Mucosal vaccination with a recombinant Oprf-I vaccine of *Pseudomonas aeruginosa* in healthy volunteers: comparison of a systemic vs. a mucosal booster schedule. FEMS. Immunol. Med. Microbiol. **37,** 167-171.