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Immunohistochemical and pathological changes in BALB/c mice immunized with whole sonicated *Listeria monocytogenes* antigens and the effect of probiotics

Th.J. Shihab and Z.I. Ibrahim

Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq

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Correspondence:
Th.J. Shihab
dr.thamer.vet@tu.edu.iq

Abstract

The current study was undertaken to investigate the role of macrophages as a cellular immune function against immunization with whole sonicated *Listeria monocytogenes* antigens (WSLMAgs) and the effect of probiotics. A preparation of WSLMAgs containing whole *L. monocytogenes* cell, after two subcutaneous immunization of BALB/c mice with 0.5ml WSLMAgs 0.5 mg/ml at an interval of two weeks. The bacterial identification was conducted by a conventional culture method using *Listeria* selective media PALCAM and confirmed by Polymerase Chain Reaction (PCR), As well as, the immunohistochemical and pathological change of it was studied in vivo by inoculating mice with pre-challenge WSLMAgs and post-challenge with virulent *L. monocytogenes* 1×10^8 CFU/mL. The results revealed the cellular immune function against pre- and post-immunization in spleen organ via lymphocytic hyperplasia in white pulp and coalescence of lymphoid follicles and marker $F4/80^+$ show the immune-positive cells in aggregation adjacent to lymphoid follicle or focal aggregation of macrophages between follicles. In conclusion, the effectiveness of sonicated *L. monocytogenes* pre and post-immunization then challenge with virulent *L. monocytogenes* in the induction of cellular immune response, might serve as an immunization platform for applicants.

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Introduction

Listeriosis is a bacterial disease caused by *Listeria monocytogenes* that affects small ruminants (1). *Listeria monocytogenes*, is a Gram-positive facultative rod-shaped bacterium, causes listeriosis, a serious and life-threatening illness caused mostly by ingesting contaminated foods (2), and is a food-borne intracellular pathogen that causes listeriosis also is widespread in the world (3). Listeriosis is characterized through a wide-ranging variety of diseases divided into two types, includes severe invasive listeriosis and non-invasive febrile gastroenteritis (4).

Listeria monocytogenes was utilized as a model organism in our investigations on the immunogenicity of lethally sonicated bacteria, as a result, *Listeria monocytogenes* serves as a rigorous model for evaluating lethal sonicated would retain bacterial immunogenicity and result in a superior vaccine when compared to other ways of pathogen death (5), In addition, the use of a sonicator in this study might have a significant influence on albino mice's cellular immunity (6). *L. monocytogenes* is a prototypic inducer of the cellular adaptive responses, whose antigen-specific effectors are cytotoxic CD8+ T cells (7).

Furthermore, due to the unique process of listeriolysin O pore formation and listerial invasion of host cells, these membrane defects can disrupt phagosome membranes, allowing bacteria to escape into the cytosol and multiply rapidly (8). The administration of prophylactic probiotics could be an important treatment option for preventing infectious complications and modulating immunity (9), also,

the probiotics decrease the severity of clinical signs and increase the immunity respond that can be used as medicinal and remedial method (10). CD8+ present in many local tissue macrophages, comprising the red pulp macrophages in the spleen, microglia in the brain, Kupffer cells in the liver, and Langerhans cells in the skin, are strongly and constitutively expressed (11).

The efficiency of sonicated *L. monocytogenes* pre and post-immunization then challenge with virulent *L. monocytogenes* in the induction of cellular immune response, might serve as an immunization platform for humans and livestock applicants.

Materials and methods

Animal

Adult females BALB/c mice (n=30) were purchased at 6-8 weeks of age from the Animal House/College of Veterinary Medicine, Tikrit University/Iraq, and kept in specific cages at the same animal house at a room temperature of 22±3°C with a 12-hour light/dark cycle, with food and water supplied *ad libitum*.

All experiments were carried out in accordance with Baghdad University's Institutional Animal Care and Use Committee regulations. All animals received humane care. For this study, the mice were divided into three groups at random, ten mice in each groups, 1st Group (control positive) challenge with virulent strain *L. monocytogenes* only $1*10^8$ CFU/ml; 2nd Group Immunized with 0.5ml WSLMAgs 0.5 mg/ml S/C for each mouse in 1st day and repeated after 2 weeks of immunization; 3rd Group immunized with both WSLMAgs as in 2nd group and administration oral probiotics (Vitane Pharmaceuticals, Inc USA) (0.02 mg/ml) for each mouse, twice in week for 4 weeks.

At 28 days from start experimental half animal of each grouped euthanized for histopathological and immunohistochemistry (IHC) investigations immune response change in spleen (GA), and half of the remains animal of each group were challenged with virulent *L. monocytogenes* 1×10^8 CFU/ml, after three day of challenge, all mice were euthanized and samples were collected for histopathology and IHC for immune response change in spleen (GB).

Bacteria

The bacterial sample was achieved from Media Diagnostic Center in Erbil, Iraq. virulent *Listeria monocytogenes* were commonly cultivated at 37°C on Brain heart infusion agar for stimulation of bacteria and after activation on it transported to *Listeria* Identification Agar Base (PALCAM) (HiMedia, India) M1064 with *Listeria* Selective Supplement (PALCAM) (HiMedia, India) to avoid mutation of bacteria also as selective identification of *L. monocytogenes* by providing a grey-green with a black center and a black halo (12).

Polymerase Chain Reaction (PCR)

To check strain in ASCo. lab Baghdad/ Iraq, the primer 27F, 5`-AGAGTTTGATCCTGGCTCAG-3`; and 1492R, 5`-TACGGTTACCTTGTTACGACTT-3` at annealing temperature 60ºC, finally size (bp) 1500 bp (13).

Preparation of WSLMAgs from *Listeria monocytogenes*

The subsequent has been prepared as follow *Listeria monocytogenes* were cultured on Brain Heart Infusion Agar with a 24-hour incubation period at 37°C. For 30 minutes, the precipitate was centrifuged at 3000 rpm at 4°C and extracted with PBS 7.2, at that time washed three times with PBS, resuspended with PBS, and introduced into the universal tube. The universal tube containing *Listeria monocytogenes* suspension was placed in an ultrasonicator (Karl Klob – Germany) at 12 Peak pulse/sec at 2 minute intervals for 30 minutes in a cool atmosphere (ice). For 30 minutes at 25000 rpm, the sonicated suspension was centrifuged. The supernatant fluid was verified through gram stain and culture on blood agar to establish the antigen's sterility.

The total protein concentration of this antigen, which was measured using the Biuret method 9.1 mg/ml then it was watered-down to become 0.5 mg/ml this antigen was considered as Whole Sonicated *Listeria monocytogenes* Antigens (WSLMAgs). In order to prepare the whole soluble Sonicated *Listeria monocytogenes* antigens (WSLMAg), to remove cell debris, the homogenate was centrifuged twice at 14000 rpm for 30 minutes each time. The supernatants went through the 0.22µm Millipore filter and were preserved at-20C previously they were used. The watered-down fluid was examined by gram staining and cultured on nutrient agar and *Listeria* Identification Agar Base (PALCAM) to verify the antigen's sterility.

According to Biuret technique, the total protein concentration of this antigen was 0.5 mg/ml. These antigens were considered to have destroyed soluble whole sonicated *Listeria monocytogenes* antigens (14).

Histopathological study

The organs obtained from each mouse were subjected to the following steps (15). The organs fixed directly in Neutral buffered formalin 10%, dehydrated, and embedded in paraffin for storage. Then, 6-mm sections were cut, de-paraffinized, and rehydrated using a standard protocol.

Immunohistochemistry (IHC) staining

The antibodies used for surface staining included anti-F4/80 antibody (Fine Test); Catalog No.: FNab02922 and Rabbit-DAB(Poly-HRP) Detection IHC Kit (Fine Test) Catalogue No.: IHC0007. It entails using the idea of antibodies (Abs) attaching particularly to antigens in biological tissues to detect antigens (proteins) in cells of a tissue section (16).

IHC was performed on de-paraffinized and rehydrated sections. Briefly, slides were incubated with antigen retrieval solution for 10 minutes. Slides were then incubated in serum block for 60 minutes, followed by rat anti-F4/80 Ab for 60 minutes. Endogenous peroxidase activity was blocked using 3% H_2O_2. Cells were subsequently incubated with a Rabbit-DAB (Poly-HRP) Detection IHC Kit, and all slides were counterstained with hematoxylin.

Results

Polymerase Chain Reaction (PCR)

The molecular results of the 16S bacterial rRNA region showed a positive amplification of 1500 bp amplicon for the *Listeria monocytogenes* bacteria when use sequencing and analyzed with GenBank databases (Table 1).

Table 1: Shows percentage the immunopositive cells of macrophages (F4/80+) antibody Pre- and post-immunization then challenge with *Listeria monocytogenes* in slide microscope of experimental groups

Groups	Spleen % F4/80+	
	Pre-challenge GA	Post-challenge GB
G1	-	45.77%
G2	62.86%	65.01%
G3	65.56%	66.66%

Virulent *Listeria monocytogenes* (Positive control G1B)

In post-challenge G1B group, the prominent microscopic lesions in tissue sections of spleen were infiltration of mononuclear cells mainly lymphocytes and macrophages, with lymphocytic hyperplasia in white pulp and coalescence of lymphoid follicles with histiocytic proliferation (Figures 1 and 2). Also, slimly increase percentage of the immunopositive cells in Table 1 for IHC staining 45.77 in red pulp of spleen also differ than other groups (Figure 3).

Pre Immunization with WSLMAgs G2A and then post Challenged with Virulent *L. monocytogenes* G2B

In pre-challenge G2A group, the tissue sections of spleen represented with moderate histiocytosis in red pulp as dilated vascular sinuses and contained mononuclear cells with proliferation of megakaryocytes and hyperplasia of lymphoid follicles (Figure 4).

Furthermore, in post-challenge G2B group, the spleen presented reactive hyperplasia in white pulp, also proliferation and infiltration of histiocytes mainly mature (basophilic) lymphocytes noted as dark one uniforms and regular rounded cells (Figure 5). Moreover, in post-challenge G2B the percentage of the immunopositive cells for IHC staining in red pulp 65.01 (Figure 6) higher than the pre-challenge G2A 62.86 and G1B 45.77 (Figure 7).

Immunized with WSLMAgs and oral administration of probiotic (Pre G3A and Post G3B challenge)

In pre-challenge G3A group, in spleen shows reactive hyperplasia of lymphoid follicles in white pulp of spleen and moderate thickening capsule with histiocytosis (Figure 8). Moreover, in post-challenge G3B, marked by subcapsular dilation and filled with histiocytes with Malpighian corpuscle (Figures 9 and 10). Besides, in pre-challenge G3A the percentage of the immunopositive cells for IHC staining 65.56 in red pulp (Figure 11) lower from the post-challenge G3B 66.66 (Figure 12), compare to control (Figure 13).

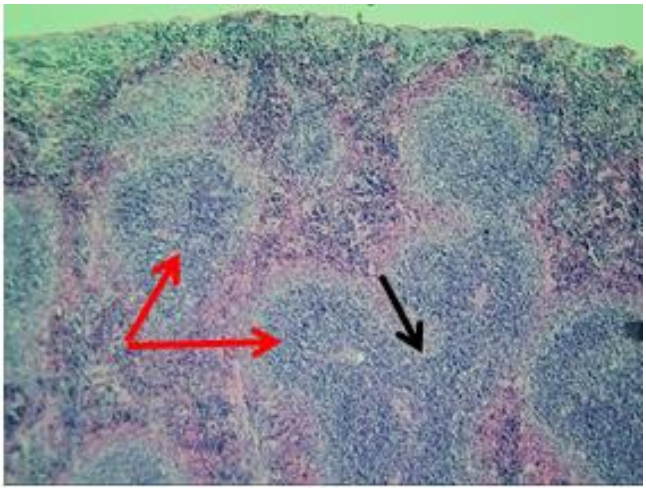

Figure 1: Histopathological section of spleen in post-G1: Shows follicular hyperplasia in white pulp (arrow) and coalescence of lymphoid follicles (arrow). (H&E stain, 40X).

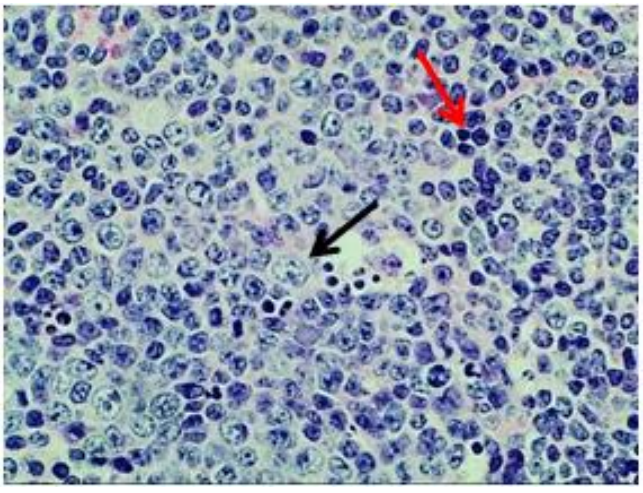

Figure 2: Histopathological section of spleen in post-G1: Shows lymphoblast's proliferation in the center of hyperplastic lymphoid follicle (arrow) and remnant of cellular debris (arrow). (H&E stain, 400X).

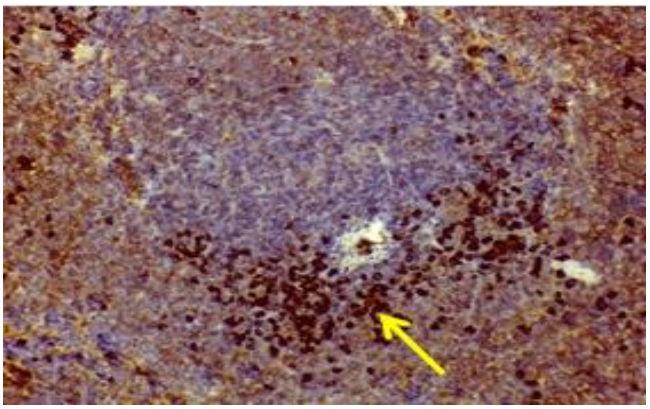

Figure 3: Immunohistological section of spleen in post-G1: Shows histiocytes F4/80+ immunopositive cells (brown color) peripheral to eccentric arteriole (arrow). (DAB Chromogen staining, 200X).

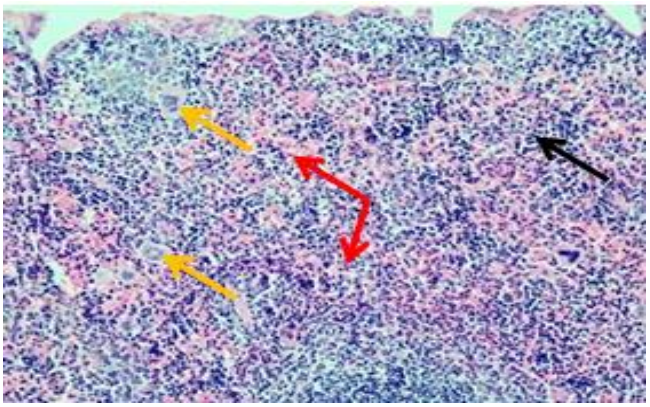

Figure 4: Histopathological section of spleen in pre-G1A: Shows congestion of vascular sinuses in red pulp (arrow); and filled with histiocytes (arrow) with megakaryocyte (arrow). (H&E stain, 200X).

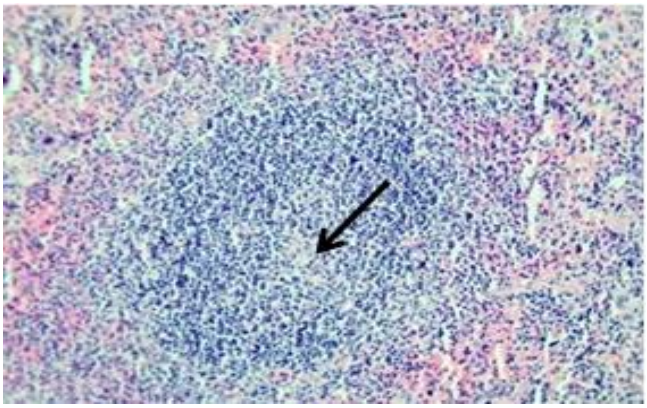

Figure 5: Histopathological section in spleen of post-G2B: shows lymphocytic proliferation (light center) (arrow) in white pulp. (H&E stain, 200X).

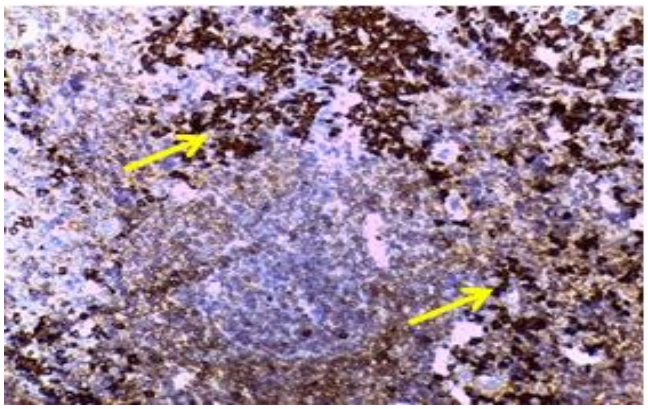

Figure 6: Immunohistological section of spleen in pre-G2A: Shows focal and diffuse immunopositive cells that belong to M1/ F4/80+ (arrow). (DAB Chromogen staining, 200X).

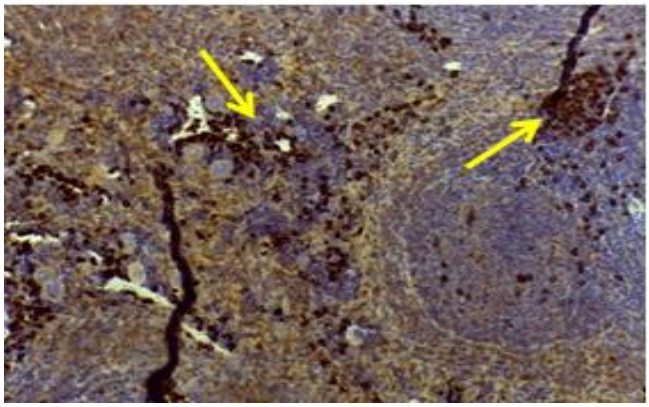

Figure 7: Immunohistological section of the spleen in post-G1B: Shows immunopositive cells (F4/80+/brown color) in peripheral follicular tissue and in red pulp (arrow). (DAB Chromogen staining, 100X).

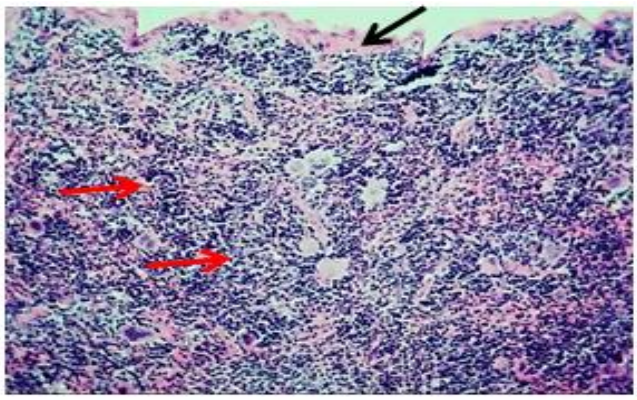

Figure 8: Histopathological section in spleen of pre-G3A: Shows moderate thickening fibrous capsule (arrow) and marked histiocytosis mainly lymphocyte (arrow) in red pulp. (H&E stain, 200X).

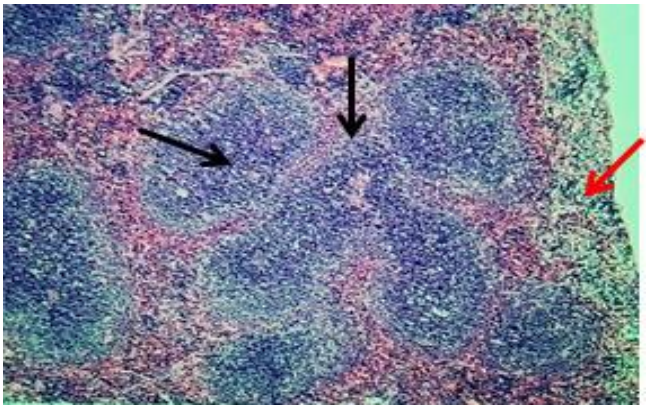

Figure 9: Histopathological section in spleen of post-G3B: shows subcapsular dilation (arrow) and filled with histiocytes and reactive hyperplasia of lymphoid follicles (arrow) in white pulp. (H&E stain, 100X).

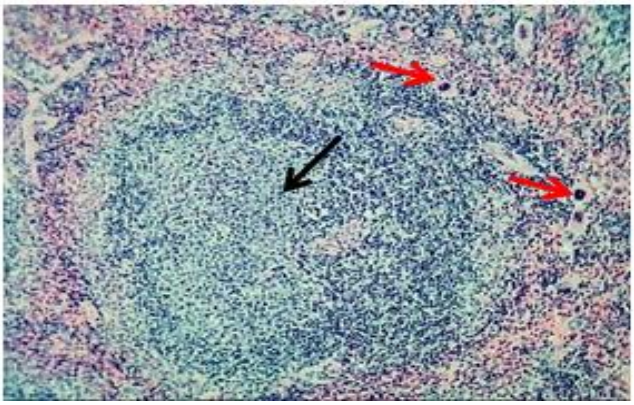

Figure 10: Histopathological section in spleen of post-G3B: shows hyperplasia of malpighian corpuscle (arrow) in white pulp with megakaryocyte (arrow). (H&E stain, 200X).

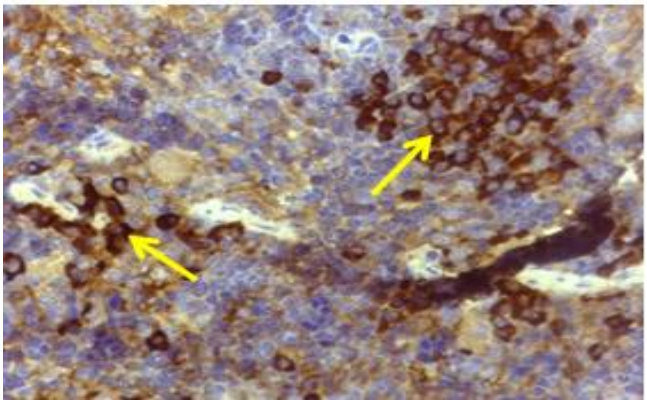

Figure 11: Immunohistological section of spleen in pre-G3A: Shows focal and peri-follicular infiltration of F4/80+ immune-positive cells (brown color) (arrow). (DAB Chromogen staining, 500X).

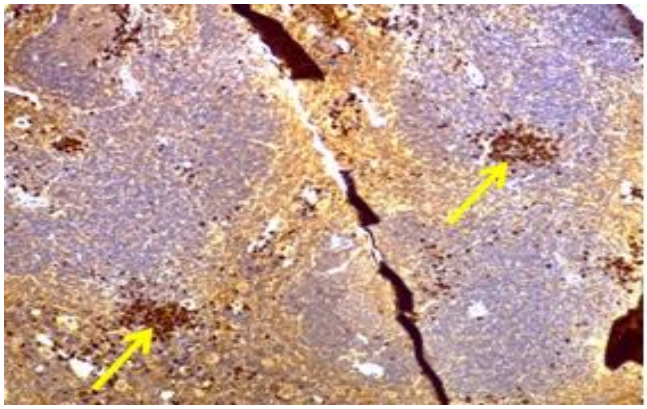

Figure 12: Immunohistological section of spleen in post-G3B: Shows focal F4/80+ immunopositive cells (brown color) in red pulp and near lymphoid follicle (arrow). (DAB Chromogen staining, 100X).

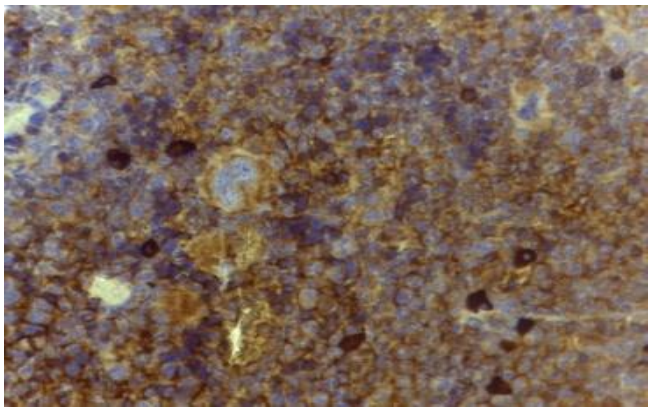

Figure 13: Immunohistological section of mouse spleen paraffin-embedded slide using (F4/80+ Antibody) (DAB Chromogen staining, 500X).

Discussion

The results of the post-challenge G1B group agreed with Yin (17) who described that the virulent strain induced a strong immune response substantial pathological changes in the liver and spleen due to is Listeriolysin O responsible for the development of a pore forming external toxin named LLO, which is necessary for vacuole membrane lysis and cytoplasmic release of *Listeria monocytogenes*, Also, Phosphatidylinositol-specific phospholipase C (PI-PLC) and zinc-dependent broad-spectrum phospholipase C (PC-PLC) are encoded by *plcA* and *plcB*, respectively, which facilitate the escape of the pathogen from single in addition double-membrane bound vacuoles synergistically with LLO Kyoui (18), and the results of IHC for G1B agreed with Kohyama (19) who reported presence of F4/80+ in red pulp of spleen due to infiltration of macrophage. Additionally, The results of Group 2 agreed with Awni (20) who revealed that the

antigens promote cellular immunity in immunized mice and infiltration of innate and adaptive cells in spleen; also, WSLMAgs antigen can trigger both innate and adaptive immune responses, and because WSLMAgs contains all *Listeria monocytogenes* structures that can stimulate innate immune cells, phagocytic cells to secrete pro-inflammatory cytokines such as TNF-α and IL-12, which play an important role in initiating acquired immune responses Al-Bayati (14), in addition to stimulated Toll-like receptors on dendritic cells (DCs) elicited protective T cell responses in mice Datta (5). The IHC of WSLMAg group agreed with Warschkau (21) who told F4/80+ may have a role in the generation of IFN-γ and IL-12 by macrophage cells once they are exposed to WSLMAgs similar to heat-killed *Listeria monocytogenes* (HKL) when infection with facultative intracellular pathogens such as LM requires IFN-γ to limit bacterial growth and which activate NK cells for IFN-γ release and macrophages were triggered once more. Also, The results of Group 3 agreement with Yap (22) who reported that the combined effects of Lactic Acid Bacteria's cell wall structure (polysaccharide) immunomodulatory characteristics and WSLMAgs might generate a stronger immune response at lower vaccination dose and give more effective protection against infection; Besides, increased CD4+ and CD8+ T cells, NK cells and CD8+ DC, and play an important function in *Listeria* protection through the capacity to produce IL-12 and trigger IFN-γ producing CD4+ and CD8+ T cells, additionally, the CD8α+ DCs are essential in the presentation of *Listeria* antigens to defensive CD4+ and CD8+ T cells Mitchell (23). Moreover, accepted with Kearney (24) who noticed large lymphoid aggregation in spleen involved many lymphocyte follicles packed with B and T lymphocyte, B cell proliferation, precursors of plasma cells that production antibody during immune reaction. In addition, The IHC of Group 3 agreed with Dubyak (25) who reported that different forms of G protein-coupled receptors (GPCRs) play a crucial role in host organisms' immune responses to infection or damage to sterile tissue. Also, microbe-induced macrophage/NK cell contact involves direct cell-to-cell communication and provides the first evidence for the functional significance of the macrophage surface glycoprotein F4/80+ Dubyak (25).

Conclusion

Immunization with WSLMAgs was efficient and provides protection against bacterial infection by induction cellular immunity pre and post immunization, might serve as an immunization platform for applicants.

Conflict of interest

We declare that no conflict of interest present with any other published papers.

Acknowledgments

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References

1. Luque-Sastre L, Arroyo C, Fox EM, McMahon BJ, Bai L, Li F, Fanning S. Antimicrobial Resistance in Listeria Species. Microbiol Spectr. 2018; 6(4): 10. DOI: 10.1128/microbiolspec.ARBA-0031-2017
2. Quinn PJ, Markey BK, Leonard FC, Fitzpatrick ES, Fanning F, Hartigan PJ. Veterinary microbiology and microbial disease. 2nd ed. New York: Wiey BlackWell publishing; 2011. 725-848. https://www.wiley.com/en- ISNB- -9781405158237
3. Chen S, Meng F, Sun X, Yao H, Wang Y, Pan Z, Jiao XA. Epidemiology of human listeriosis in China During 2008–2017. Foodborne Pathog Dis. 2020; 17(2): 119-125. DOI: 10.1089/fpd.2019.2683
4. Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control. 2017; 75: 1-13. DOI: 10.4102/ojvr.v87i1.1869
5. Datta SK, Okamoto S, Hayashi T, Shin SS, Mihajlov I, Fermin A, Guiney DG, Fierer J, Raz E. Vaccination with irradiated Listeria induces protective T cell immunity. Immunity. 2006; 25(1): 143–152. Doi.org/10.1016/j.immuni.2006.05.013
6. Ali AA, Ramadhan BB. Effect of ultrasound on protoscoleces of Echinococcus granulosus in vitro and in vivo. Iraqi J Vet Sci. 2021; 35(1): 0-0. DOI: 10.33899/ijvs.2021.126906.1410
7. Pamer EG. Immune responses to *Listeria monocytogenes*. Nat Rev Immunol. 2004; 4(10): 812-823. DOI: 10.1038/nri1461
8. Petrišič N, Kozorog M, Aden S, Podobnik M, Anderluh G. The molecular mechanisms of listeriolysin O-induced lipid membrane damage. Biochim Biophys Acta Biomembr BBA-BIOMEMBRANES. 2021; 1863 (7): 183604. DOI: 10.1016/j.bbamem.2021.183604
9. Shimizu K, Ojima M, Ogura H. Gut Microbiota and Probiotics/Synbiotics for Modulation of Immunity in Critically Ill Patients. Nutrients. 2021; 13(7): 2439. DOI: 10.3390/nu13072439
10. Yousif SY, Ali AA. Effect of probiotic acidophilus plus against infection with secondary hydatid disease in BALB/c mice. Iraqi J Vet Sci. 2020; 34(1): 115-121. DOI: 10.33899/ijvs.2019.125613.1104
11. McKnight AJ, Gordon S. The EGF-TM7 family: unusual structures at the leukocyte surface. J Leukoc Biol. 1998; 63(3): 271–280. DOI: 10.1002/jlb.63.3.271
12. Al-Gburi NM. Detection and pathogenicity of *Listeria monocytogenes* in common carp (Cyprinus carpio) fish in Baghdad, Iraq. Iraqi J Vet Sci. 2020; 34(2): 311-316. DOI: 10.33899/ijvs.2019.125980.1205
13. Mukherjee S, Kumar D, Nanda AK, Chakraborty R. 16S rRNA gene sequence analyses of the metagenome derived from waters of river Mahananda at Siliguri: An approach to understand bacterial diversity. Indian J Biotechnol. 2013; 12(1): 80-87. [available at]
14. Al-bayati HH, Alwan MJ. Immunohistopathological and immunological evaluation of listeria nanoparticle vaccine during the first and third semester in the pregnancy rat model. Plant Arch. 2020; 20(2): 2016-2025. [available at]
15. Al-Mahmood SS. Improving light microscopic detection of collagen by trichrome stain modification. Iraqi J Vet Sci. 2020; 34(2): 273-281. DOI: 10.33899/ijvs.2019.126176.1256
16. Ramos-Vara JA, Miller MA. When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry—the red, brown, and blue technique. Vet Pathol. 2014; 51(1): 42-87. DOI: 10.1177/0300985813505879
17. Yin Y, Tian D, Jiao H, Zhang C, Pan Z, Zhang X, Jiao X. Pathogenicity and immunogenicity of a mutant strain of *Listeria monocytogenes* in the chicken infection model. Clin Vaccine Immunol. 2011; 18(3): 500. DOI: 10.1128/CVI.00445-10

18. Kyoui D, Takahashi H, Miya S, Kuda T, Kimura B. Comparison of the major virulence-related genes of *Listeria monocytogenes* in internalin A truncated strain 36-25-1 and a clinical wild-type strain. BMC Microbiol. 2014; 14(1): 15. DOI: 10.1186/1471-2180-14-15
19. Kohyama M, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, Murphy KM. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. Nature. 2009; 457(7227): 318-321. DOI: 10.1038/nature07472
20. Awni KJ, Alwan MJ, Yousif AA. Study the effects of Corynebacterium pseudotuberculosis antigens in the improvement of fertility in female mice against the virulent pathogen with virulent type of same pathogen. J Entomol Zool Stud. 2017; 5(6): 1138-1144. [available at]
21. Warschkau H, Kiderlen AF. A monoclonal antibody directed against the murine macrophage surface molecule F4/80 modulates natural immune response to *Listeria monocytogenes*. J Immunol. 1999; 163(6): 3409-3416. [available at]
22. Yap PC, MatRahim NA, AbuBakar S, Lee HY. Antilisterial Potential of Lactic Acid Bacteria in Eliminating *Listeria monocytogenes* in Host and Ready-To-Eat Food Application. Microbiol Res. 2021; 12(1): 234-257. DOI: 10.3390/microbiolres12010017
23. Mitchell LM, Brzoza-Lewis KL, Henry CJ, Grayson JM, Westcott MM, Hiltbold EM. Distinct responses of splenic dendritic cell subsets to infection with *Listeria monocytogenes*: maturation phenotype, level of infection, and T cell priming capacity ex vivo. Cell Immunol. 2011; 268(2): 79–86. DOI: 10.1016/j.cellimm.2011.03.001
24. Kearney SM, Gibbons SM. Designing synbiotics for improved human health. Microb Biotechnol. 2018; 11(1): 141. DOI: 10.1111/1751-7915.12885
25. Dubyak GR. GPCRs in innate and adaptive immune responses. In: Jastrzebska B, Park PS-H, editors. GPCRs Structure, Function, and Drug Discovery. San Diego: Academic Press; 2020. 429-461. DOI: 10.1016/B978-0-12-816228-6.00021-0

التغيرات المناعية الكيميائية والمرضية النسيجية في الفئران البيضاء الممنعة بمستضدات الليستريا المستوحدة الكاملة الصوتية وتأثير المعزز الحيوي

ثامر جدوع شهاب و زينب إسماعيل إبراهيم

فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة تكريت، تكريت، العراق

الخلاصة

أجريت الدراسة الحالية للتحقق من دور الخلايا البلعمية كوظيفة مناعية خلوية ضد التمنيع بمستضدات الليستريا المستوحدة الكاملة الصوتية وتأثير المعزز الحيوي. حضر مستضد الليستريا المستوحدة الكامل الصوتي الذي يحتوي على جميع تركيبات لهذه البكتريا الليستريا المستوحدة، وبعد التمنيع تحت الجلد لمرتين في الفئران البيضاء بمقدار ٠,٠٥ مل من مستضد الليستريا المستوحدة الكامل الصوتي ٠,٥ ملغم/مل بفاصل أسبوعين. شخصت البكتريا بالطرق التقليدية والزرع على الوسط الانتقائي الخاص، تم تأكيدها بتفاعل البلمرة المتسلسل، بالإضافة إلى ذلك، تمت دراسة التغيرات المناعية الكيميائية والمرضية في الجسم الحي عن طريق تمنيع الفئران باستخدام مستضد الليستريا المستوحدة الكامل الصوتي قبل وبعد التحدي باستخدام البكتريا الضارية الليستريا المستوحدة ١×١٠^٨ خلية جرثومية/مل. كشفت النتائج عن وظيفة المناعة الخلوية ضد التمنيع قبل وبعد في عضو الطحال عن طريق فرط التنسج الليمفاوي في اللب الأبيض واندماج الجريبات اللمفاوية واظهر المُعَلم المناعي أن الخلايا المناعية الموجبة في تجمع مجاور للجريب اللمفاوي أو تجميع بؤري للخلايا البلعمية بين الجريبات. في الاستنتاج، أن فعالية الليستريا المستوحدة الصوتية قبل وبعد التمنيع ثم التحدي باستخدام بكتريا الليستريا المستوحدة الضارية في تحريض الاستجابة المناعية الخلوية، قد يكون بمثابة قاعدة للتمنيع مستقبلا.