INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

LYMPHOID AND LYMPHOMYELOID CELLS HYPERPLASIA AND IMMUNE PROTECTION

IBRAHIM M S SHNAWA[1], KHALIED Y ALZAMILY[2], RBAB OMRAN[2] MOHAMMED J ALWAN[3].

[1]Department of Biology,College of Science,University of Babylon

Present Address:College of Biotechnology,University of Babylon-Kasim Green University ,Babylon/IRAQ.

[2]Department of Biology,College of Science,University of Babylon.Hilla /IRAQ

[3]Department of Pathology,College of Veterinary Medicine,University of Baghdad.

ABSTRACT

Five groups, each of ten mice were assigned as four test and one control groups. The control mice received saline injections. The test group one and two received several S-LPS F1 and F2 injections of Vibrio fluvalis, while the test groups four and five received several S-LPS F1 and F2 injections of Aeromonas hydrophila. Five from each group were challenged separately with live V.fluvalis and A.hydrophila infectious doses to check for postchallenge immune protection on the cellular levels. Immunized mice from both of the test groups have shown lymphoid cell hyperplasia in spleen and Kupffer cell hyperplasia in liver sections. While challenged groups II-V have shown these cell hyperplasias, but those of A.hydrophila were showing granuloma in spleen and migratory macrophages in other organs. Thus, immune cell hyperplasia is a sign of immune preconditioning in both of the test groups ,as well as in post challenge in V. fluvalis immune mice. Hyperplasia , granuloma and migratory mononuclear cells in A.hydrophila partial immune mice.

Key Words: Hyperplasia,lymphoid cells lymphomyeloid cells ,migratory mononuclear cells immune ,postchallenge immune,preconditioning.

INTRODUCTION

The immune response is actually a series of cellular events mediated by a number of cytokine and cytokine network functions that are eligible for its evolution[1]. In comparison, the inflammatory response is a real vascular phenomenon[2]. Antigens, however, may acts as an injury, provoking an inflammatory response perceeding or along with the immune response[3].Chronic exposure to an antigen(s) in any vertebrate host eventually terminated by chronic inflammation perceeding or parallels the immune response[4]. Bacterial lipopolysaccharide, LPS initiates mitogenic cellular events, activate B lymphocyte to produce antibodies and/or cytokine network as well as immune protection[5]. Such immune protection may be associated with tissue and/or immune tissue injuries besides the normal immune response[6].The objective of the present work was to report on murine immune and non-immune cell responses and their own tissue micro -environment to smooth LPS from Aeromonas hydrophila and Vibrio fluvalis.

MATERIALS AND METHODS

Bacterial strains :- A.hydrophila and V.fluvialis were obtained from Advance genetic engineering and Biotechology lab, Department of Biology, Faculty of Science, University of Babylon. The diagnosis was confirmed through the use of biochemical tests[7].

Method of LPS isolation:- LPS Extraction according to Westphal et al.,[8]

Animals:

Thirty mice, with a weight (20-25gm) are divided into six groups with each group consisting of 5 mice:

Group I- (V.fluvialis F1) include 5 mice inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days gave booster dose, after that challenge by V.fluvialis 1×10^8 at 27days.

Group II-(V.fluvialis F2) include 5 mice inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days gave booster dose, after that challenge by V.fluvialis 1×10^8 at 27days.

Group III-(A.hydrophila F1) include 5 mice inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days gave booster dose, after that challenge by A.hydrophila 5×10^8 at 27days .

Group IV-(A.hydrophila F2) include 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days gave booster dose, after that challenge by A.hydrophila 5×10^8 at 27days.

Group V and VI: - were saline control groups respectively.

Histopathology: After sacrificing the animals, specimens of immune precondioned mice groups I-IV and from post challenged mice groups I-IV internal organ were collected and preserved in 10 % of formalin solution and stored at 4 C until preparation of sectioning according to Luna et al[9].

RESULTS

1-Lipopolysacchride Immunized:

Lymphocyte hyperplasia was noted in the tissue sections of spleen from LPS immunized group for both Vibrio fluvalis and Aeromonas hydrophila in this mouse model. Likewise, Kupfur cell hyperplasia were noted in the liver sections of the immunized mice

2-Vibrio.fluvialis post challenge: The histopathological changes in 7 days post challenged mice immunized group with LPS fraction 1, 2 of V.fluvialis were characterized by no clear lesion in non lymphoid tissues like heart. Hyperplasia of lymphocyte, lymphocyte aggregation in the lymphoid tissues and proliferation of kupffer cell in the immune organ, the liver. Figures 1,2,3,4,5,6,7,8.

3-.Aeromonas.hydrophila postchallenge:

The histopathological changes at 7 days post challenged in mice immunized group with LPS fraction 1, 2 of A.hydrophila were characterized. Such changes were found as lymphoid cell hyperplasia, hyperplasia of the peri arteriolar sheaths lymphocytes in spleens. Granulomatous lesion consist from aggregation of active macrophage & lymphocyte in liver parenchymal, proliferation of Kupffer cell and congested of central vien. Figures 9,10,11,12,13,14,15.

4-Cell infiltrates;

Lymphoid and lymphomyeloid cell infiltrates were noted in lungs,liver and kidneys in postchallenge A .hydrophila model and to lesser extent in the V.fluvalis postchallenge mice model.

DISCUSSION

Shnawa et al[10] have proved that A.hydrophila S-LPS fractions 1 and 2 were mitogenic,immunogenic and partial immune protective.While he and his coworkers[11] have shown that V.fluvalis S-LPS fractions 1 and 2 were mitogenic ,immunogenic,and full immunoprotective. Based on these findings immune protection can be evaluated at gross level through observing the survivors percentages in the preconditioned, postchallenged mice models. In the present work, cellular responses with their own tissue microenvironmental changes are being used to characterize partial and full immune protectivity.

Mice preconditioned with the smooth F1 and F2 LPS and postchallenged with live infectious dose of A.hydrophila were shown to be with lymphoid cell, Kupffur cell hyper plasia in spleen and liver repectively in preconditioned. While, in postchallenge mice there were lymphoid cell, Kupffer cell hyperplasia and grnuloma in spleen .In comparison, these cellular responses in case of V. fluvalis were hyperplasias of lymphoid cells in spleen and Kupffer cells in liver, both in preconditioned as well as postchallenged models. No evident microlesions in spleen, liver or in other non lyhmphoid organs. Figures 1 – 15. Pathologic cell hyperplasia may bear numbers of indications like Antigenic stimulation, immune recognition, immune effector production, chronic inflammatory response and/or emergence of hyperplastic disease conditions[6]. These results were coincidental with the pathological feature of pre and post infectious and may indicate that immune response can be a limiting and destructing potential in most of pathogen at the site of

inoculation. While in the animal immunized with LPS of fraction 1and 2 of A.hydrophila showed granulomatous lesion, haemorrhge and neutrophil infiltration in the examine organ. This may be indicated that antigens stimulated immune response with partial protection potential that destroyed some bacteria at the site of inoculation but large number of these organism reach to internal organs and proliferate, meanwhile, the body attempted to localize of this organism by granulomatous reaction[12,13].

The immune status of mouse were detected by the microscopic changes in infected internal organs. The best protective status with less significant changes in parameters of challenged mice was performed by LPS fraction 1 and fraction 2 of V.fluvialis which induce higher humoral and cellular immune response than in mice which immunized by LPS fraction 1and fraction 2 of A.hydrophilla .

Elucidation of the mechanism by which gram negative bacteria induce inflammation in mouse as that these germs after inoculated to body ,it grows up and multiply and liberated LPS which in turn stimulate leukocytes to activate the pro enzyme (matrix metalloproteinase MMP) by removal of their NH2-terminal domain ,and that MMP destroyed basal membrane and all interstitial tissue proteins as in blood vessels and lead to increase in vascular permeability as well as in chemo attractants. Thus, the result was elevated recruitment of blood derived neutrophils and monocytes into the site of infection (that explain of high infltration of neutrophils and macrophage as well as blood vessels congestion in histopathlogic section). Recruited lymphocytes produce many cytokines especially IL-1 and TNF-α which they were induced programmed cell death (PCD) in endothelial cells in site of infection (apoptosis). At the same time, the cell proliferation increased to serve as a mechanism to ameliorate tissue damage (hyerplasia could be noticeable in histopathological examination)[14].

The reduction in number of gram negative bacteria in internal organs of LPS immunized group was occurred due to that CD14-LPS complexes activate epithelial cells of internal organ by binding to Toll like receptor–4 (Toll-LR4)on epithelial cells. Such binding may lead them to secrete IL-8(a potent chemo attractant of neutrophils) and in resultant early recruitment of neutrophils is crucial to clearance of gram negative bacteria in internal organs[15] . These results were in agreement with those that had been reported on enteric Salmonella typhimurium[16,17].

CONCLUSION

The lymphoid and lymphomyeloid cell hyperplasia parallels immune preconditioning with smooth LPS fractions from both A. hydrophila and V. fluvalis. While in post challenge models, these hyperplasia were noted in V.fluvalis and hyperplasia granuloma and mobile mononuclear phagocyte in A. hydrophila case. Thus, cell hyperplasia is pathognomic with full protection while hyperplasia and granuloma are pathognomic with partial protection.

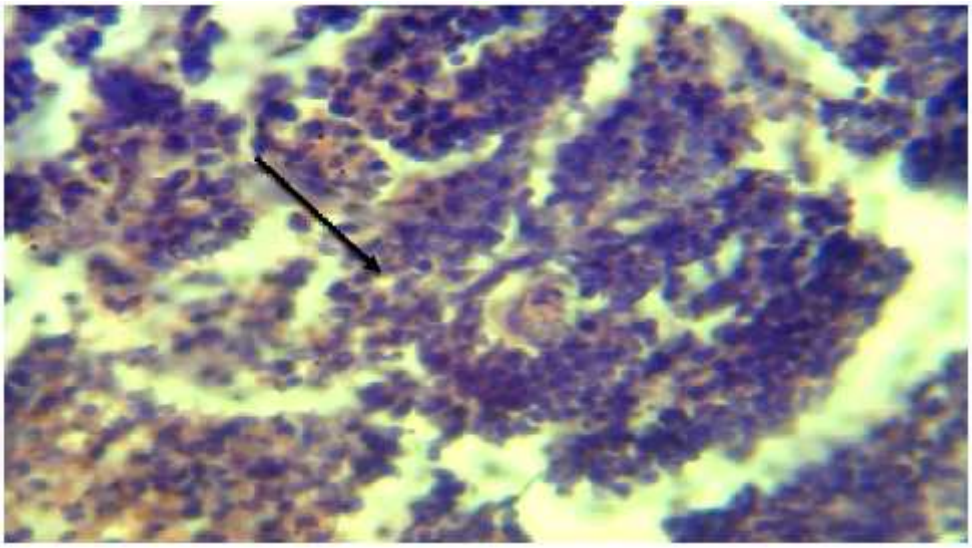

Fig 1

.Histopathological section in the spleen of immunized animal with LPS fraction 1of *V.fluvialis* at 7 days post-challenge shows marked hyperplasia of white plup (H&E stain 40x).

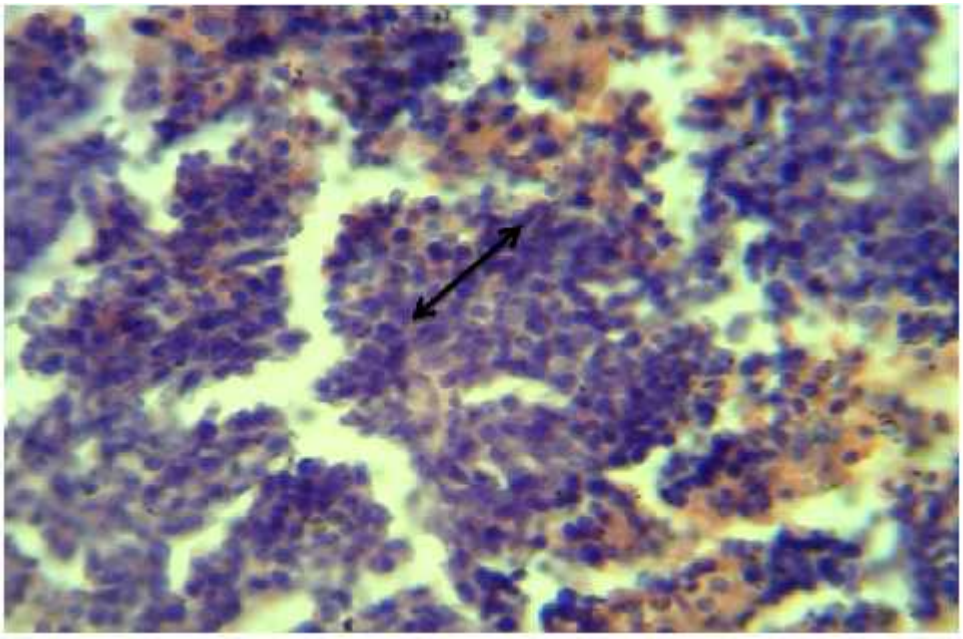

Fig 2.
Histopathological section in lung of immunized animal with LPS fraction 1of *V.fluvialis* at 7 days post-challenge shows marked hyperplasia of lymphoid tissue in the wall of bronchiole and in the interstitial tissue. (H&E stain 40x).

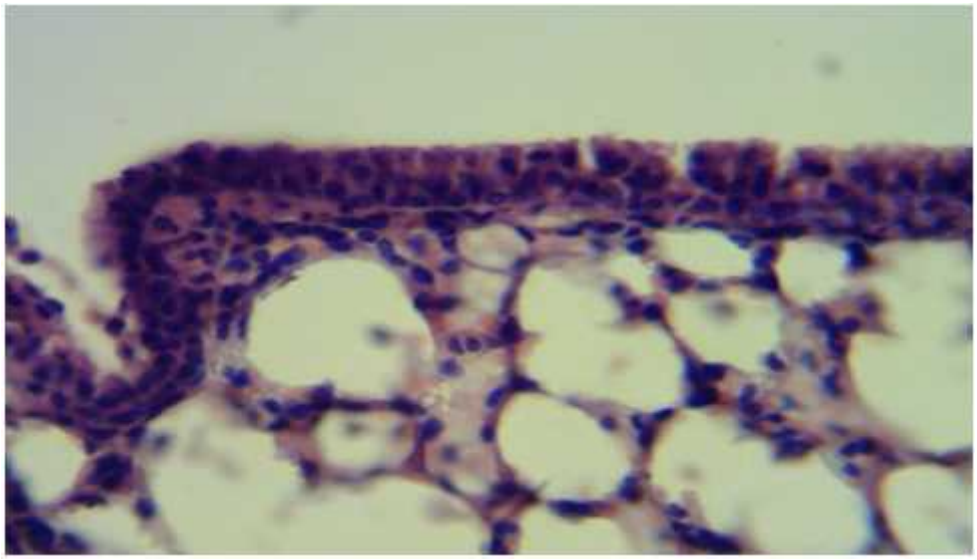

Fig 3.
Histopathological section in the lung of immunized animal with LPS fraction 1of *V.fluvialis* at 7 days post-challenge shows no clear lesions (H&E stain 40x).

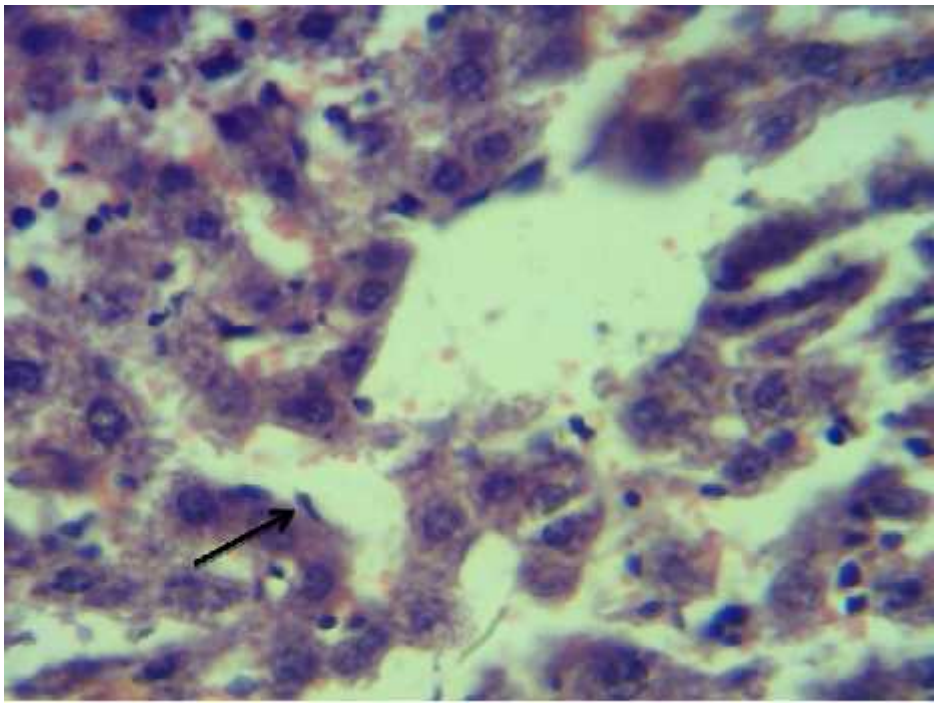

Fig 4

Histopathological section in liver of immunized animal with LPS fraction 1of *V.fluvialis* at 7 days post-challenge shows proliferation of kuffer cell. (H&E stain 40x).

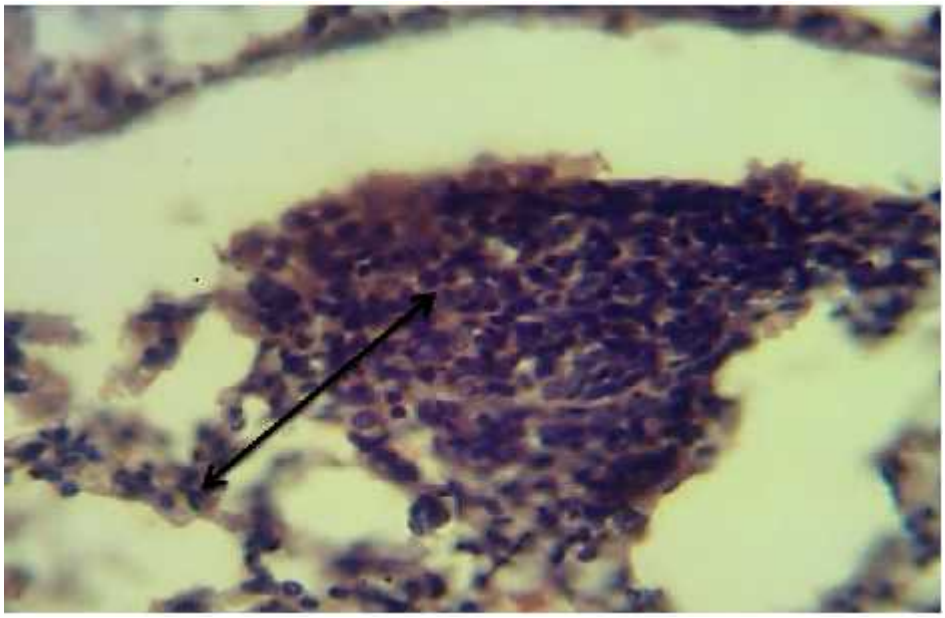

Fig 5

.Histopathological section in lung of immunized animal with LPS fraction 2of *V.fluvialis* at 7 days post-challenge shows proliferation of lymphocyte in the wall of blood vessiles and bronchiole in addion increase thickness of intralviolar septa due to proliferation mesenchymal cell. (H&E stain 40x).

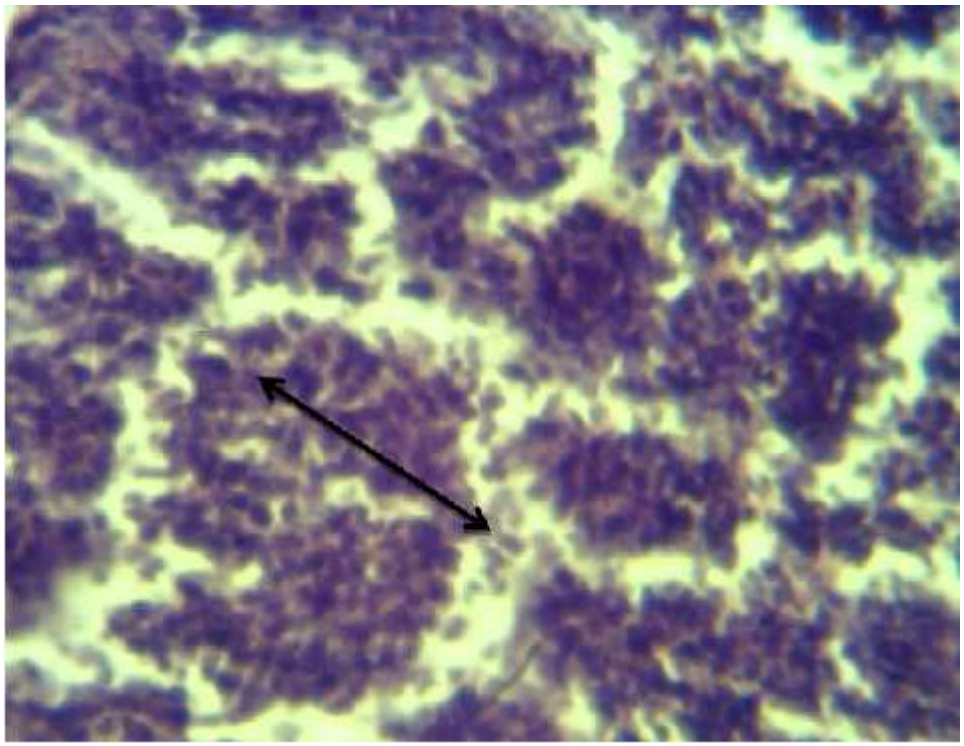

Fig 6

.Histopathological section in spleen of immunized animal with LPS fraction 2of *V.fluvialis* at 7 days post-challenge shows diplation of white pulp and congestion of of red pulp (H&E stain 40x)

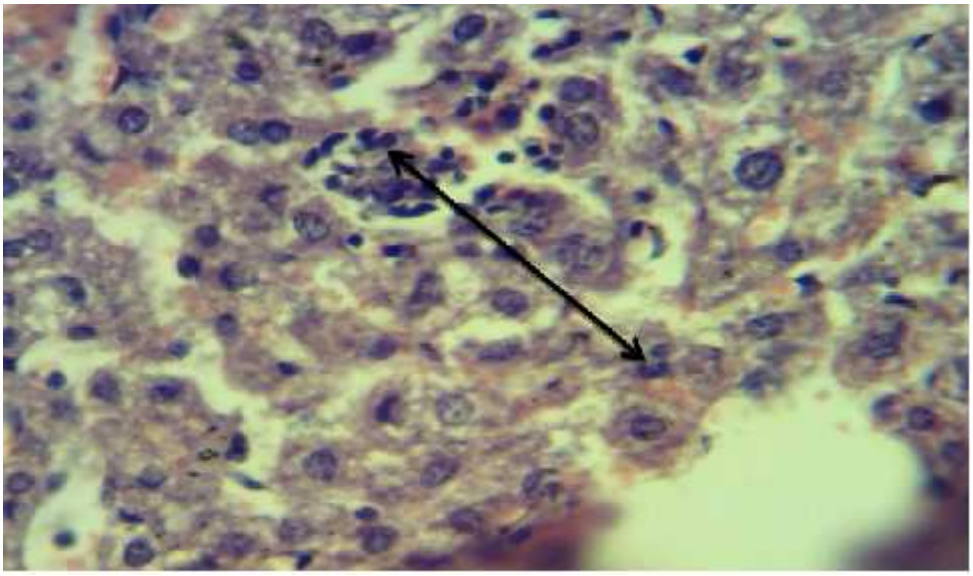

Fig7.

Histopathological section in liver of immunized animal with LPS fraction 2of V.fluvialis at 7 days post-challenge shows proliferation of kupffer cell and mononuclear cell aggregation in the liver parenchymal. (H&E stain 40x)

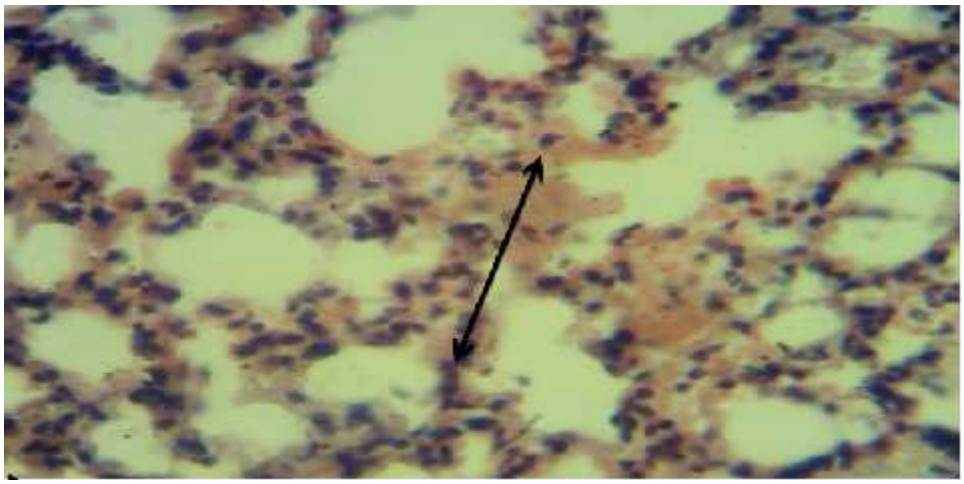

Fig 8.

Histopathological section in lung of immunized animal with LPS fraction 2of *V.fluvialis* at 7 days post-challenge shows RBCs infiltration cell in the alveolar space with thickness of interalveolar septa (H&E stain 40X)

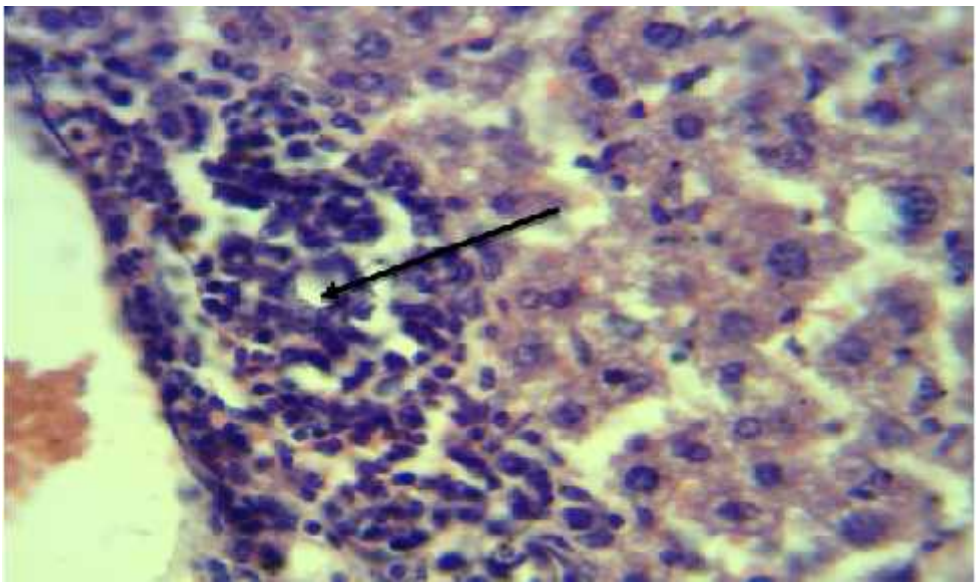

Fig 9.

Histopathological section in the liver of immunized animal with LPS fraction 1of *A.hydrophila* at 7 days post-challenge shows mononuclear cell particulars lymphocyte and macrophage portal area around bile duct and blood vessels (H&E stain 40x)..

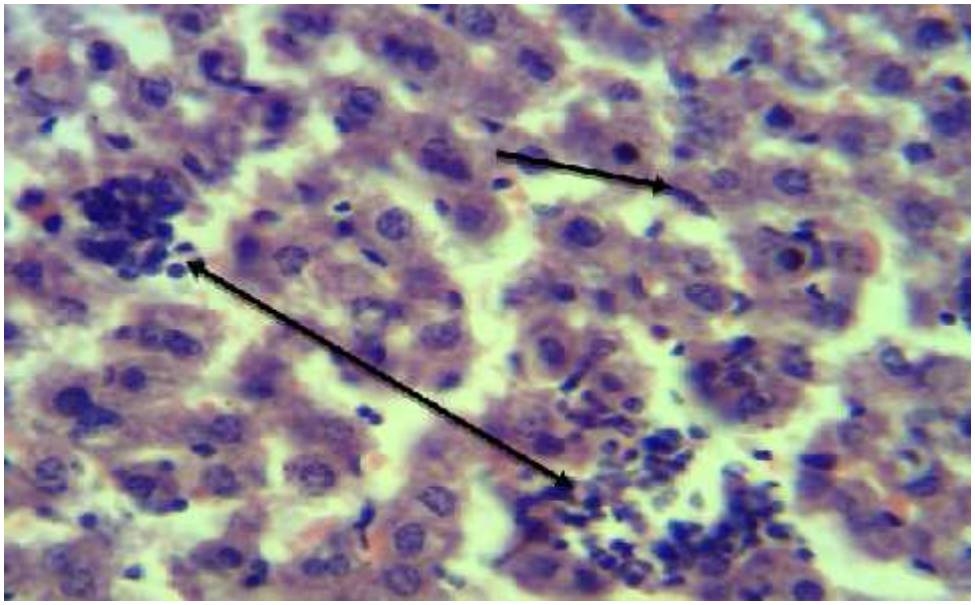

Fig 10.
Histopathological section in the liver of immunized animal with LPS fraction 1of *A.hydrophila* at 7 days post-challenge shows mononuclear cell particulars lymphocyte and macrophage in liver parenchyma and proliferation of kupffer cells. (H&E stain 40x).

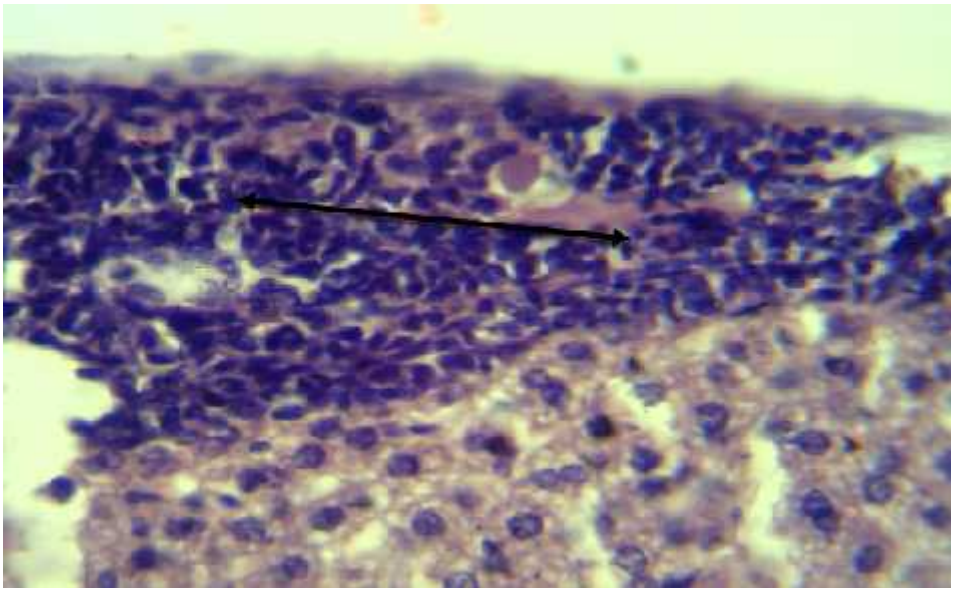

Fig 11
.Histopathological section in the liver of immunized animal with LPS fraction 1of *A.hydrophila* at 7 days post-challenge shows marked mononuclear cell particulars lymphocyte and macrophage portal area around bile duct and blood vessels. (H&E stain 40x)

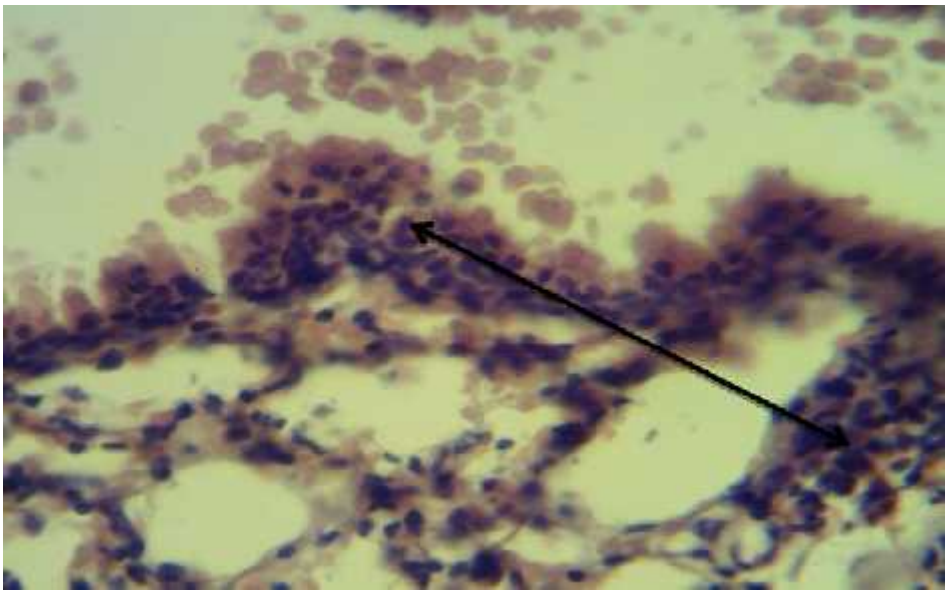

Fig 12.

Histopathological section in the lung of immunized animal with LPS fraction 2of A.hydrophila at 7 days post-challenge shows hyperplasia of epithelial lung cell of bronchiole with moderate thickness of intralviolar septa due to proliferation mesenchymal cell (H&E stain 40x)

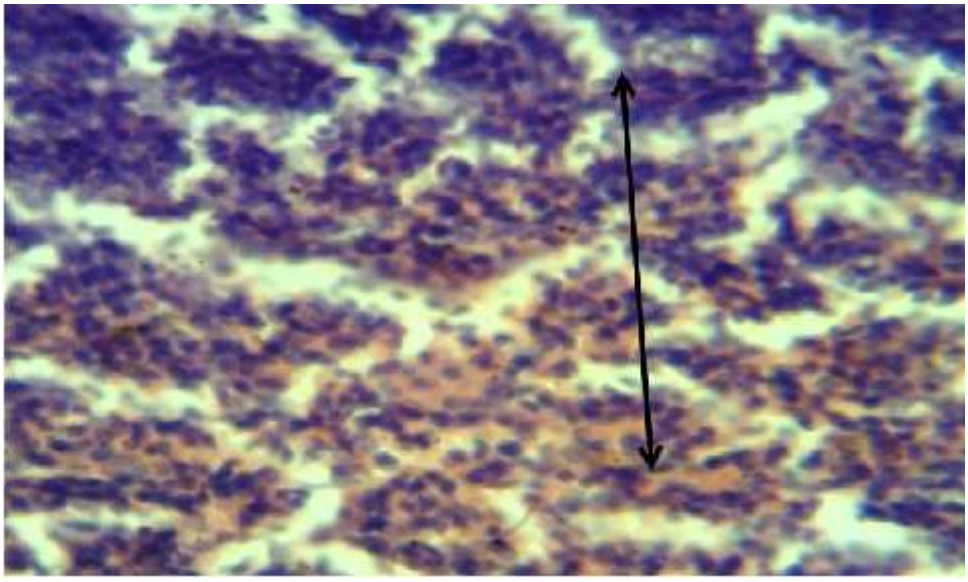

Fig 13.

Histopathological section in the spleen of immunized animal with LPS fraction 2of A.hydrophila at 7 days post-challenge shows proliferation of peri arteriols sheeth with proliferation of mononuclear cell around sinus form cord like appearance (H&E stain)

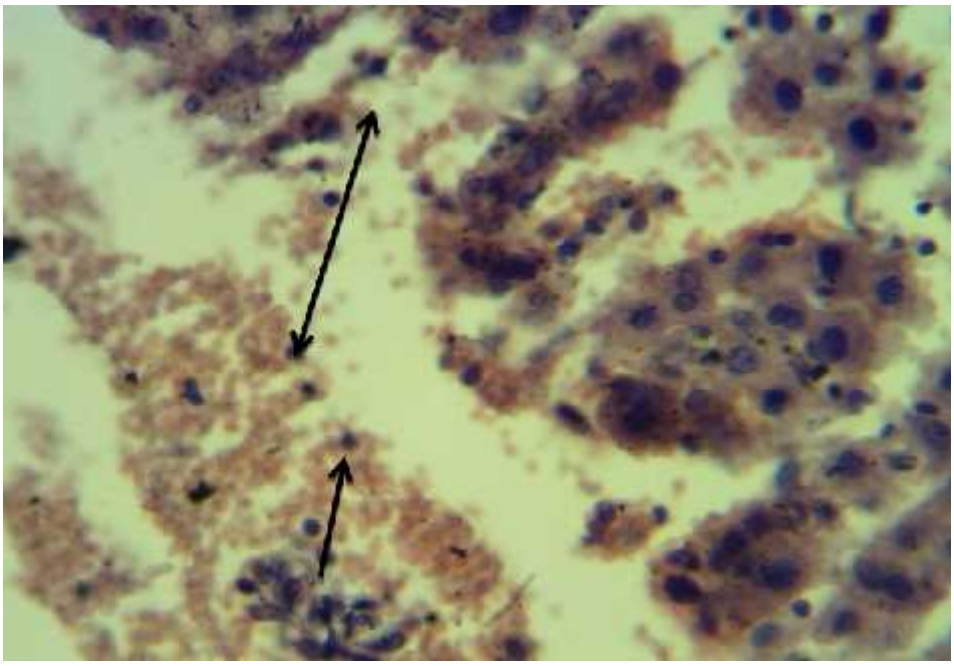

Fig 14

Histopathological section in the spleen of immunized animal with LPS fraction 2of A.hydrophila at 7 days post-challenge shows necrotic area replacement by RBCs and neutrophils.(H&E stain 40x).

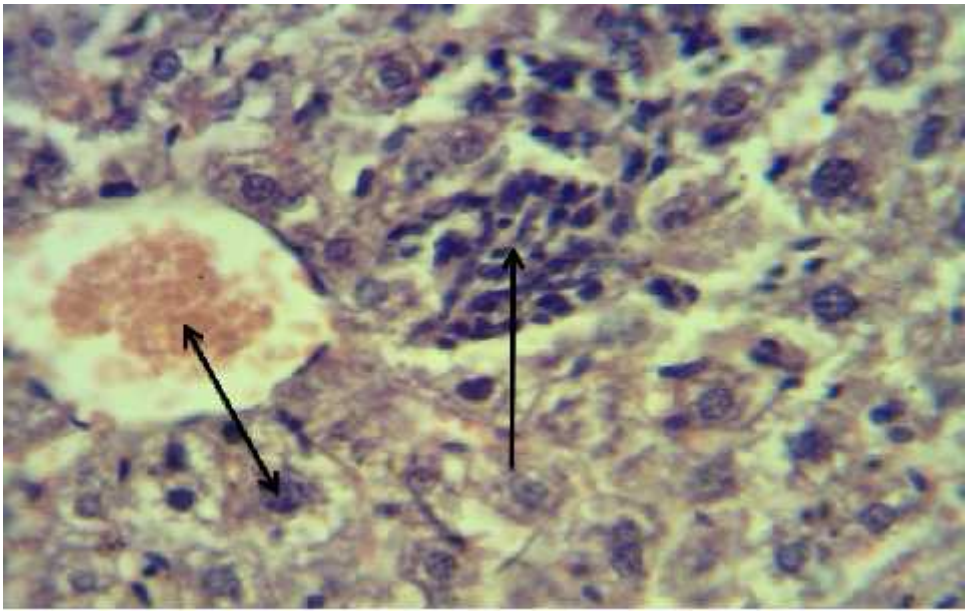

Fig 15

Histopathological section in liver of immunized animal with LPS fraction 2of *A.hydrophila* at 7 days post-challenge shows granulomatous lesion consist of aggregation of active macrophage & lymphocyte in liver parenchymal ,proliferation of kupffer cell and congested of central vien (H&E stain 40x).

REFERENCES

1. Abbas AK, Lichtman AH, Pober JS. Cell and Molecular Immunology W.B.Saunders USA 2000,233-340.
2. Playfair JHL, Chain BM, Immunology At Glance 7th ed, Blackwell Scientific, Iran edition,18-19.
3. Doan T, Melvold R, Viselli S, Waltenbaugh C, Immunology, Lippincott ,Wiley and Wilkins,USA,79-82.
4. Male D, Brostoff J, Roth DP, Roitt,I, Immunology,7th ed, Mosby, USA, 235.
5. Galanos C, Endotoxin/Lipopolysachaaride(LPS), Encyclopedia of Immunology Vol.II, Academic Press London,806 – 808.
6. JanewayCAJr.,Travers P, Walport M, Sholomchik MI, Immunobiology 6th ed, Chuchill-Livingstone,USA,430-431.
7. Macfaddin 2000,Biochemical Identification of Medical Bacteria
8. Westphal O, Luderitz Oand BisterF. (1952). Uberdieextracktion von bacterienmitphenole – water .Z.Naturforsch .TeilB.,1952; 7:148-155.
9. Luna LG.(1968).Manual of Histological Staining Methods .The armed forces institute of pathology .3rd (eds.). McGrow-Hill Book Co .New York., P:12-31.
10. Shnawa IMS, Khalied Y ALZamily, Rabab OMran.The postchallenge partial immune protection of smooth Aeromonas hydrophila lipopolysaccharide in a mouse model IJAPBC,2014:3(3):693-697.
11. Shnawa IMS, Khalied Y ALZamily, Rbab Omran. The postchallenged immune protection mediated by smooth lipopolysaccharide of Vibrio fluvalis in a mouse model, WJPR 2014;3(7):14-23.
12. AL-Zamily, K.Y.2007.The role of *Listeria monocytogenes* antigens immune response against Pseudomonas *aeruginosa* infection in mice. Msc.Thesis .Vet.College.Baghdad University,Iraq.
13. Alwan, M. J. (1996).*Nocardia asteroids* studies on some aspects of Pathogenesis, Ph.D. Thesis, Baghdad University, Iraq.
14. Lee JW, Max JP and Xin Z. Recombinant bovine soluble CD14 reduce severity of experimental *E.coli* mastitis in mice. Vet.Res., 2003:307- 316.
15. Wang Y, Zarlenga DS, Paape MJ and Ahl GE. Recombinant bovine soluble CD_{14} sensitizes the mammary gland to lipopolysaccharide .Vet.Immunopathol.,2002; 86:115-124.
16. UdhayaKumar, V. and Muthukkaruppan, V. R. (1987). Protective immunity induced by outer membrane proteins of *salmonella typhimurium*in mice .Infect.Immun.,55(3):816-821.
17. Kshash QH(2008). The role of some antigens of *E-coli* isolated from bovine mastitis in the protection of mammary gland in mice .Ph.D.Thesis .Vet.College.Baghdad University ,Iraq.