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Histopathological Changes of Two Antigens Prepared from Methicillin-Resistant *Staphylococcus aureus* in Rats

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***Abstract*: The aim of the present study was to determine the effect of two prepared antigens fromMethicillin-resistantStaphylococcus aureus (MRSA), whichincluded crude alpha toxin (Hla) and heat inactivate bacterial suspension (HIBS) in rats through the examination of the inflammatory response of both antigens on primary and secondary lymphoid organs (thymus gland and spleen), respectively. Examination of sections from spleen of rats treated with HIBS antigen induced high immune response compared with rats treated with Hla antigen which induce mild immune response by established changes. The histological observations on sections from thymus gland of rats treated with HIBS had the ability to induce different changes than Hla antigen compared with control group.**

Keywords: MRSA, Hla, HIBS, spleen, thymus, histopathology changes

I. Introduction

Staphylococcus aureus is a widespread Gram-positive bacterium that colonizes the skin and anterior nares of about 20–30% of the healthy human population. Although mainly a harmless colonizer, *S. aureus* can cause invasive diseases like skin and soft tissue infections, and can be responsible for severe infections in humans like *pneumonia*, endocarditis and osteomyelitis (Wertheim *et al.*, 2005), which are frequently associated with *S. aureus* bacteremia (Klevens *et al.*, 2007).

Staphylococcus aureus is a unique microorganism as compared with other clinically relevant bacteria in three respects; that the organism expresses a variety of virulence factors, continues to demonstrate the ability to develop resistance including a broad array of antimicrobial classes (Jacobsson, 2009).*S. aureus* in its methicillin resistant form (MRSA) is the most important cause of antibiotic-resistant health care-associated infections worldwide (Cosgrove, 2006).

(Garcia *et al.*, 2015) revealed that immunization with heat-inactivated *S. aureus* elicited a significant antibody response characterized by production of IgG antibodies. (Bubeck and Schneewind, 2008) used an inactive form of alpha toxin to immunize mice and evaluate the protection against *S. aureus* ina murine pneumonia model, compared to animals immunized with phosphate buffer saline, mice immunized with the H35L protein and then challenged intranasally with *S. aureus* showed reduced mortality.Effective protection against pathogenic bacteria requires both mucosal and systemic immune responses (Mansour, 2005).S. aureus strains that are deficient in Hla have significantly reduced virulence in animal infection models (Kennedy *et al.*, 2008). The role of Hla in the pathogenesis of MRSA infections was unknown until recently, many studies have demonstrated that Hlaelicits production of CXC chemokines by host cells during experimental *S. aureus pneumonia*, thereby promoting severe lung inflammation (Bartlett *et al.*, 2008). Determining the immune changes duringS. aureus infection and the factors leading to such changes will make it possible to prevent the spread of the infection and to eliminate the pathogens resistant to many antimicrobial agents (Gomez *et al.*, 2002).The aim of this study was to prepare two antigens from MRSA bacteria: crude alpha toxin (Hla) and heat inactivate bacteria suspension(HIBS) in rats through the examination the effect of both antigens on primary and secondary lymphoid organs (thymus gland and spleen), respectively.

II. Materialsand Methods

Bacterial isolates and Culture Media

In this study, S. aureus isolated from burn patients in burn unit of AL-Hussein Teaching Hospital, Thi-Qar province, Iraq. S. aureus subjected to routinediagnostic tests, such as culturing on mannitol salt agar, blood agar, biochemical tests (catalase test, coagulase and DNase tests) (MacFaddin, 2000).All isolates were confirmed by API staph system(BioMerieux / France), and finally subjected to Staphylo Monotec kit Plus(Fluka Analytical, Switzerland) as serodiagnosis assay.

Preparation of crude alpha hemolysin antigen

MRSA isolate was selected for preparation of crude alpha hemolysin, expressed hla gene, that detected by PCR (Mehrotra *et al.*,2000), and it was given alpha hemolysis on blood agar, this antigen was prepared according to (Siritool and Makonkawkeyoon, 1978).

Heat-inactivated bacterial suspensions antigen of MRSA

MRSA isolate was selected for preparation this antigen had sea gene that detected by PCR (Betley and Mekalanos, 1988) with modification, and this antigen prepared by using the method of (Lawrence *et al.*, 2012; Degaim *et al.*, 2016).

Cytotoxicity Test

The cytotoxicity activity of the both antigens was determined against human red blood cells using the method described by (Nair *et al.*,1989).

Immunization schedule

For evaluation of Hla and HIBS antigens efficiency in immunization, white albino female rats (170-230) gr. were obtained from the animal house at Science College in Thi-Qar University, Iraq. The rats were housed in standard metal cages (5 rats/cage). The rats were divided into three groups comprising ten animals in each group (20 of them were immunized of each antigen and 10 were treated as control group). Antigens emulsified with an equal volume (v/v) of complete Freund's adjuvant and two booster injection performed for 20 rats, by using the method of (Wang *et al.*, 2015) with modification.

Control group were injected with Phosphate Buffer Saline(PBS) only in same periods and volumes described in immunization schedule of immunized rats.Seven days after thelast injection all rats were bled, and organs were collected from each group that include (spleen and thymus gland).

III. Histological study

Histological changes were studies comparing between primary and secondary lymphoid organs (thymus gland and spleen) of immunized rats with those of control group, according to method of (Luna, 1968).

IV. Results and Discussion

Cytotoxicity test

The results of cytotoxicity test showed that both prepared antigens not cause any lysis for human RBCs, and these cells were not susceptible to effect of those antigens.

The non-susceptibility of human RBCs may be associated with the erythrocyte types of diverse animal species. The variation of sensitivity to lysis of these toxinsdisplayed by RBCs of diverse animal species, such as a nearly 400-fold-higher concentration of this toxin was needed to lyse erythrocytes of human compared with erythrocytes of rabbits (McCartney and Arbuthnott, 1978).

The inherent resistance of RBCs of human against alpha toxin action fostered the impression that the pathogenic role of this toxin could be limited against to specific animal species. Also,(Bhakdil and Tranum,1991) revealed that only rabbit erythrocytes, but not human, have a restricted number of surface receptors for this toxin. The binding of those receptors and alpha toxin occurs irreversible, and according to high susceptibility of erythrocytes of rabbit; so they concluded that only high concentrations of this toxin might be bound by alternative, unspecific interaction against resistant cells like RBCs of human.

Histological study

The present study indicated that both antigens had the ability to induce varied immune response in spleen and thymus gland of immunized rats compared with rats of controlled group. According to examination of tested organs, the HIBS had the ability to induce high immune response than Hla antigen, depending on changes in both organs.

The results of the present study revealed that the immunization with Hla antigen induce mild immune response (Fig 2A and B), while the HIBS antigen can induce high immune response through observed changes in spleen sections (Fig 3A,B and C) such as heavy infiltration of lymphocytes, inflammatory cells, and macrophages, compared with control group (Fig 1A and B). The investigated results were in agreement with (Narita *et al.*,2010), whom showed that infiltration of neutrophils and macrophage in spleen of immunized mice with clumping factor A antigen, and the reduction of bacterial loads in organs of the immunized mice including kidneys and spleens, and the immunization with ClfA antigen prompts the decreasing of the bacterial loads in mice organs which were infected with S. aureus, so that this effect may be important in protection by immunization against sepsis-induced death.

The other changes of spleen such as some active lymph nodules with large number of macrophages, some macrophages with debris and remainents of RBC, area of lysis within red pulp, large number of lymphocytes and macrophages,and showed deposition of haemosidrin pigment. Also area of hemorrhage distributed through red pulp. The other immune responsiveness of this antigen (HIBS) on rat spleen by appearance of fibroid trabecullae and increased their thickness, bundles of collagenous fibers, sheathed arteries are seen. The results of current study were relatively agreed with the results of (Mansour, 2005) recorded that the spleen of mice treated with S. aureus lysat (formalin-inactivated bacterial lysates) showed congested sinusoidal spaces filled with erythrocytes and early signs of inflammation with mononuclear and polymorphonuclear leucocytes, stagnation of blood within splenic vessels that infiltrated the red pulp.

The influence of both antigens on thymus gland was clarified through indicating recorded changes. Also HIBS antigen must induce high immune response on thymus gland (Fig 6A, B and C), compared with Hla antigen and control group (Fig 5A and B) and (Fig 4), respectively. HIBS antigen can cause change in thymus gland such as an irregular incomplete lobules, heavy infiltration of lymphocytes, neutrophils, infiltrated regions included macrophage with debris and proliferation of epithelial reticular cells with visible nucleoli and large number of lymphocytes. These present results were in a likeliness with the results of (Mansour, 2005), who verified that thymus gland treated with S. aureus lysat caused heavy lymphocytic infiltration.

The immunization with these antigens may provide protection against virulence S. aureus, according to their ability to induce the production of antibodies and show high immune response in identified organs especially Hla. The current results were agreed with results of (Zhang *et al.*, 2015), whom showed that passive immunization with polyclonal antibodies against three antigens of S. aureus, SEB, wild-type manganese transport protein C (MntC) and Hla afterward challenge with lethal dose of S. aureus which affected decreasing of bacterial counts, infiltration of inflammatory cell, reduced the pathology, and was capable to provide closely complete protection in a murine sepsis model.

The use of heat inactivated bacterial suspension as an antigen had induced humoral and cellular response in addition to induce high immune response in identified organs. These findings were in agreement with study of (Vinod*et al.*, 2015), whom showed that immunization with

S. aureus ghostsvaccine (SAGs) injected via the subcutaneous, oral and intravenous routes prompted immune responses and those rats were protected from a virulent S. aureus challenge. In this respect,(Capparelli *et al.*, 2011) showed that mice were immunized with peptidoglycan by the intravenous, intramuscular and aerosol routes decreased the bacterial colonization in the spleen, liver, kidneys and lungs, and this antigen stimulated a significant protection against the lethal dose of S. aureus. Also, vaccine of UV-irradiated genetically attenuated bacteria reduced the bacterial loads and affords protection from a succeeding challenge with S. aureus (Burnside *et al.*, 2012; Neverthelesse*t al.*, 2011), reported that vaccination with heat-killed S. aureus was unsuccessful in decreasing bacterial counts in organs. In the same field, (Chen *et al.*, 2011), documented that mice immunized with multiple antigen peptide improved the clearance of bacteria in mice challenged with viable S. aureus ATCC 25923. Also,(Wang *et al.*, 2015), showed that the immunization with MAP27 reduced the bacterial counts in spleen and lung of mice after challenge with lethal S. aureus. The injection routes of antigens or vaccine may be effective to induce immune response in organs. The subcutaneous immunization with Hla and HIBS antigens induced diverse immune response in spleen and thymus gland.

The current results were agreed with results of (Brown *et al.*, 2009) whom showed that mice subcutaneously vaccinated with Luk-PV were protected against an intradermal infection, but not against a pulmonary infection.

The inactivated bacteria suspension had more activity than Hla antigen on both organs, that may be due to this suspension, consist of complete inactive bacteria which contain variety of effective epitopes of functional antigens, while the other reasons of Hla that induce mild immune response in these organs, may be associated with differences of protein concentration of each prepared antigens.

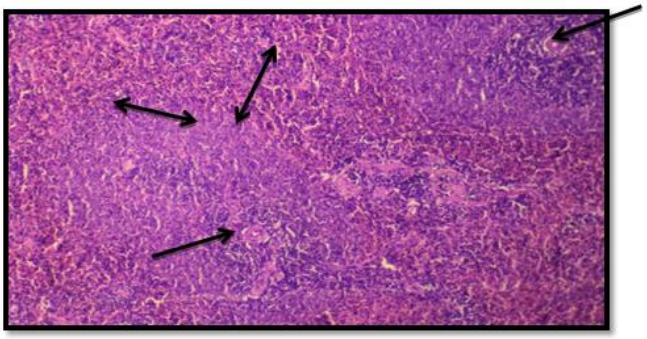

Fig. (1 A): Section on control spleen showed normal lymph nodules (→) and vascularized red pulp (↔) stain (H and E), 10X.

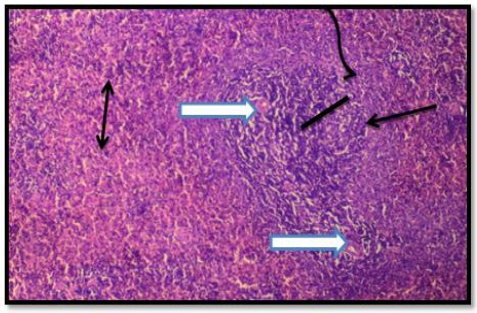

Fig. (1 B): Section on spleen from control rats showed two grouped lymph nodules ( ) with eccentric central arteriole ( ), rich red pulp ( ). Also cortex ( ) and germinal central visible ( ), stain (H and E), 10X.

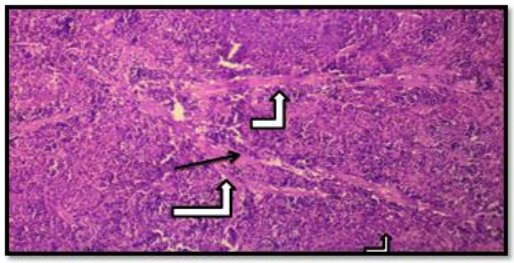

Fig. (2 A): Section on spleen from treated rats with Hla antigen showed irregular splenic cords ( ) separated by sinusoid lined with endothelial cells ( ), stain (H and E), 10X.

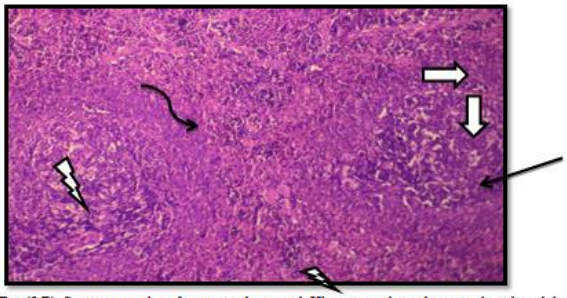

Fig. (2 B): Section on spleen from treated rats with Hla antigen showed reactive lymph nodules ( ), vascular red pulp ( ), macrophage with debris ( ) germinal center with macrophages a deposition of haemosidrin pigment ( ), stain (H and E), 10X.

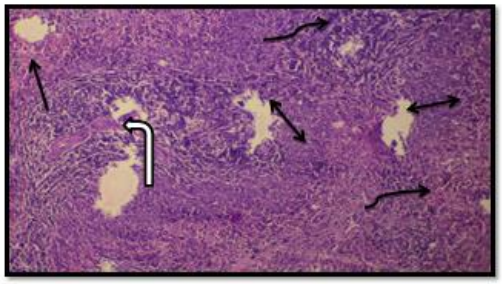

Fig. (3 A): Section on spleen from treated rats with HIBS antigen showed more than one lymph nodules aggregated ( ) with lysis regions ( ) inflammatory cells ( ) and congested blood vessels ( ) stain (H and E), 10X

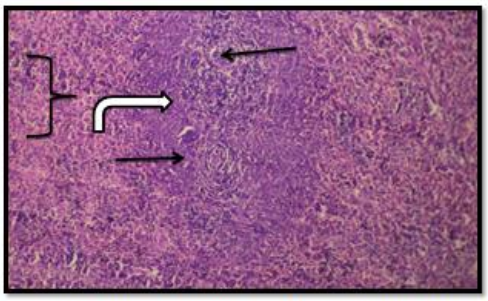

Fig (3 B): Section on spleen from treated rats with HIBS antigen showed active lymphoid nodules ( ) with sheathed arterioles ( ), more vascularized red pulp ( ), stain (H and E), 10X.

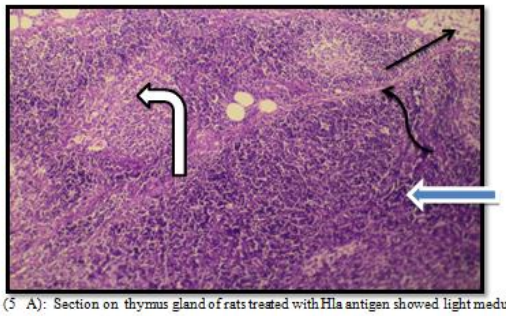

Fig. (5 A): Section on thymus gland of rats treated with Hla antigen showed light medullary zone ( ), cortex ( ), fatty tissues ( ), trabeculae ( ), stain (H and E), 10X.

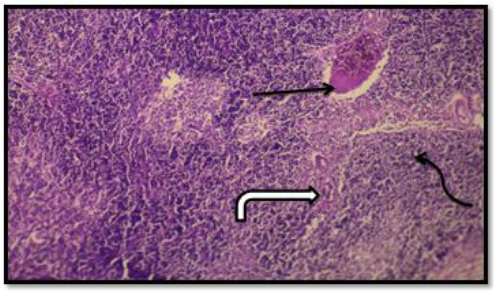

Fig. (5 B): Section on thymus gland of rats treated with Hla antigen showed blood vessel ( ), congested blood vessel ( ), trabeculae ( ), stain (H and E), 10X.

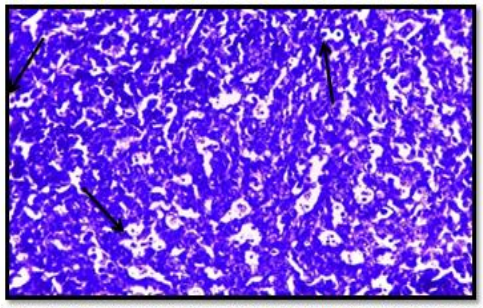

Fig. (6 B): Section on thymus gland from rats treated with HIBS antigen showed reactive lobule cortex had macrophage with debris ( ), stain (H and E), 40X.

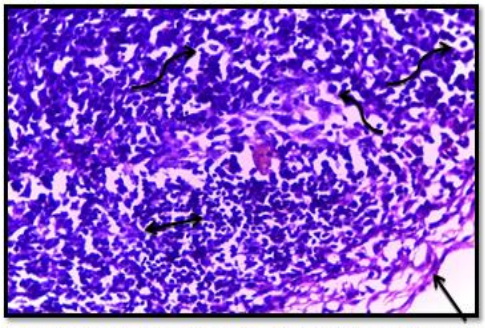

Fig. (6 C): Section on thymus gland from rats treated with HIBS antigen showed epithelial reticular cells ( ), large number of lymphocytes ( ), capsule ( ), stain (H and E), 40X.

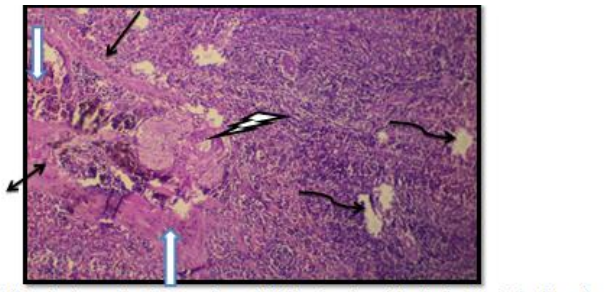

Fig. (3 C): Section on spleen from treated rats with HIBS antigen showed fibroid trabecullae ( ), collagen fiber bundles ( ), sheathed arteries ( ), lysis regions ( ), haemorrhage ( ) stain (H and E), 10X.

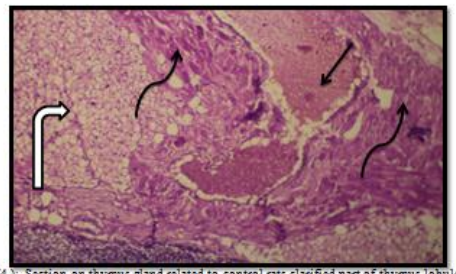

Fig (4): Section on thymus gland related to control rats clarified part of thymus lobules( ), dense trabecullae ( ) and fatty tissue extended from capsule ( ), stain (H and E), 10X.

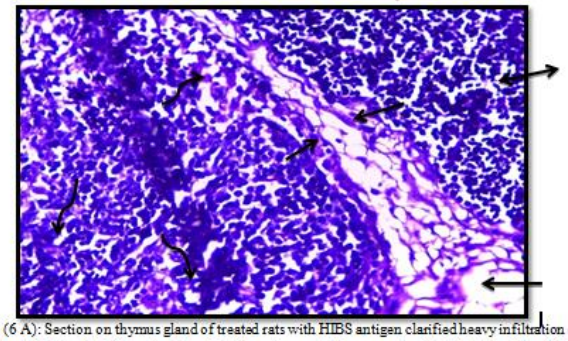

Fig. (6 A): Section on thymus gland of treated rats with HIBS antigen clarified heavy infiltration of neutrophils ( ), thick trabecullae ( ), active part of thymus gland ( ), stain (H and E), 40X.

V. Conclusions

We concluded that the HIBS antigen had the ability to induce higherimmune response than Hla antigen.

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