

Study the influence of whole sonicated *Staphylococcus aureus* antigens on septic arthritis in rabbits infected with these microorganism

M.J. Alwan MA. AL-Nueimy Z.J. AL-Shaibani
Coll. of Vet. Med./ Unive. Of Baghdad

Abstract

In order to know the influence of whole sonicated *S.aureus* antigens (WSSAgS) on *S.aureus* septic arthritis, 15, white local rabbits, both sex, their weights ranged between 1.25-1.50 Kg ,were randomly divided into 3 equal groups and treated as follow:
1-the 1st group was immunized with 0.5 ml of WSSAgS (3.3mg/ml protein concentration), two doses,14 days interval, at 28 days post-immunized .skin test was done by used soluble sonicated *S. aureus* antigens and at day 30,Ab titers was recorded ,then the 1st and 2nd groups were challenged with 0.5ml of bacterial suspension containing 1X10⁸CFU/ml ,intra -synovial. the 3 group was inoculated with 0.5 ml of sterile normal saline and served as control negative group. All animals were sacrificed at 30 days post- challenge, sample from knee joint were taken for bacterial isolation and pathological examination. The results showed that WSSAgS elicited both humoral and cellular immune responses against *S.aureus* infection .severe swelling of the knee joint was noticed in the 2 group as well as the animals unable to use their legs. heavy bacterial isolation from infected joints was recorded in the 2nd group with severe histopathological lesions in the infected joints characterized by suppurative inflammation of the perichondral area with inflammatory reaction, necrosis and erosion of the articular cartilage and these changes were extended to subchondral bone trabeculae and bone marrow lead to suppurative inflammation and necrosis of the bone trabeculae .while there was no clinical signs and no bacterial isolation reported in immunized infected animals as well as no clear pathological changes t noticed in joints of immunized-infected animals.

Introduction

Septic arthritis is the purulent invasion of a joint by an infectious agent which produces arthritis(1}The etiology of septic arthritis is bacteria, but viral, and fungal arthritis occur occasionally(2).The most common bacterial agent in septic arthritis is *Staphylococcus aureus* ,which causes a severe ,rapidly progressing erosive disease with high morbidity and mortality (3)).The inflammatory processes during septic arthritis erode articular cartilage,destroy bone and promote joint destruction lead to irreversible loss of joint function in 25-50% of patients(3). However, early administration of antibiotic eradicates the bacteria but does not stop joint destruction resulting in severe sequelae (4).The pathogenicity of *S.aureus* depends on the expression of a variety of virulence factors bacterial products that ,by different means, enable the establishment of infection, these include surface associated adhesions capsular polysaccharides, exoenzymes,and

exotoxins, (5). Clumping factors (CLFs)A and B are 2 structurally related fibrinogen-binding proteins that are expressed on the surface of *S.aureus* , Both proteins mediate the fibrinogen-dependent adhesion and clumping of *S.aureus* cells(6). Fibronectin - binding protein (FnBPs) A and B enable *S.aureus* adherence to and invasion of a range of cell types ,including all epithelial cells, endothelial cells, fibroblasts and osteoblasts (7);such invasion factors might provide a mean by which *S.aureus* evade host defenses and resist antibiotic killing.Septic arthritis in human occur due to hematogenously spread *S.aureus* ,which extravassate into most of parenchymatous organs and within a few days to one week, the innate system of the host typically clears the infection from most of the organs, except the kidney and joints, the persistence of infection lead to a total or subtotal destruction of the cartilage and subchondral bone(9). Increasing ability of *S.aureus* to withstand antibiotic treatment

is alarming (Chastre,2008,10] and calls for the development of new strategies for antistaphylococcal prophylaxis and prevention Staphylococcal infection. vaccination appears to be an interesting

Material and methods:

Media

Blood agar medium, STAPH.110 agar. and brain heart infusion broth, all media were prepared according to the instruction of manufactures.

Strains of *S.aureus* were obtain from the Unit of Zoonotic disease/College of Vet.Medl University of Baghdad. The specific biochemical tests were done to these strain to confirm diagnosis and identification.

Preparation of *S.aureus* antigens:

Whole sonicated *S.aureus* antigens (WSSAg5) were prepared according to Mitove et al.,(11)

soluble sonicated *Staphylococcus aureus* antigens (SSSAGs) were prepared according to Saleh, (12) Determination of the challenge dose. The preparation of bacterial suspension of counting was made according Miles and Misra (13).

Experimental design:

fifteen white bread rabbits ,both sex, their weight ranged between 1.25-1.50 kg were randomly divided into 3 equal groups and treated as following:

1-1st group immunized with WSSAGs 0.5ml,S/C (protein concentration 3.3mg/ml), two dose,14 days interval.

Immune response

The result of skin test showed the mean thickness of skin of immunized animals at 24hrs post test was 1.96 and declined into 1.44 at 48 hrs post test, the mean of antibody titers in immunized animals at day 30 post-immunization was 179.2.

Clinical signs and bacterial isolation:

Infected - non-immunized animals showed swelling of knee joint and the animals were unable to use their legs, ,these clinical signs accompanied with heavy bacterial isolation from the infected joint. While immunized infected animals

alternative to prevent the Staphylococcal infection ,therefore, the objective of the present study was to investigate the influence of WSSAGs on *Staphylococcus aureus* joint infection in rabbits.

2-2nd group used as control positive group.

3-3rd group used as control negative.

At 27 days post-first immunization, cellular immune response was measured by skin test and at day 30 post immunization humoral immune response was detected by passive haemagglutination test and then the 1st and 2nd groups were challenged intra synovial with 0.5ml of bacterial suspension containing 1X10⁸CFU/ML of virulent *S.aureus* , the 3rd group was injected intrasynovial with 0.5ml of syerile normal saline and served as control negative group. All animals were sacrificed at 30days post challenge and postmortem examination was carried to all animals, speiment was taken from joint for bacterial isolation and other specimen for histopathological examination, these specimen 1X1X1cm dimention were decalcification and fixed in 10% buffer formaldehyde solution for 72hrs, then used normal roten tissue section preparation according to Luna.(17).

Delayed type of hypersensitivity test:

These test was done according to the procedure that mentioned by Hudson and Hay(15) Passive haemagglutination test was done according to Herbert(16).

Results

showed no clinical signs and no bacterial isolation was recorded from the joints.

Histopathological examination:

Infected-non-immunized animals

The histopathological examination of the joints revealed necrosis and inflammatory cells infiltration in the perichondrial area (Fig:1) with granulation tissues characterized by dilated congested blood vessels and fibroblast proliferation (fig:2) together with fibrin network deposition and granulation tissue in other section of the perichondral area (Fig:3).in other section, large area of hyaline cartilage necrosis which showed that the matrix between necrotized chondrocytes

was lost its characteristic basophilia and became homogenous eosinophilic color, calcification and erosion of necrotic cartilage with neutrophils infiltration were seen (Fig:4). necrosis of cartilage extended to adjacent subchondral bone, leading to osteonecrosis of trabeculi of cancellous bone which characterized by empty or dilated lacuna space,. sequestrum of necrotic bone fragment surrounded by osteoblast and sclerosis area infiltrated by round macrophages and neutrophils was seen in the bone marrow (Fig:5). The inflammatory cells (round macrophages, neutrophils and osteoclasts) replaced the haemopoietic components of the bone marrow (Fig:6). In another section, several surface cracks extended deeply into the

cartilage reach the underlying subchondral bone trabicul (fibrillation). vascularization fibrous connective tissues extended into the area of crack with inflammatory cells in the lumen of the blood vessels (Fig:7). microscopic section also showed that the increasing of osteoblast activity lead to increase thickness of subchondral bone trabicula at the marging of the bone (osteophytes) (Fig:8). the inflammation extended to the subperiosteal area which investigated extensive fibrous connective tissue proliferation, inflammatory cells infiltration and necrotic debris (Fig:9). Immunized infected animals showed no clear lesion in their joints (Fig:10).

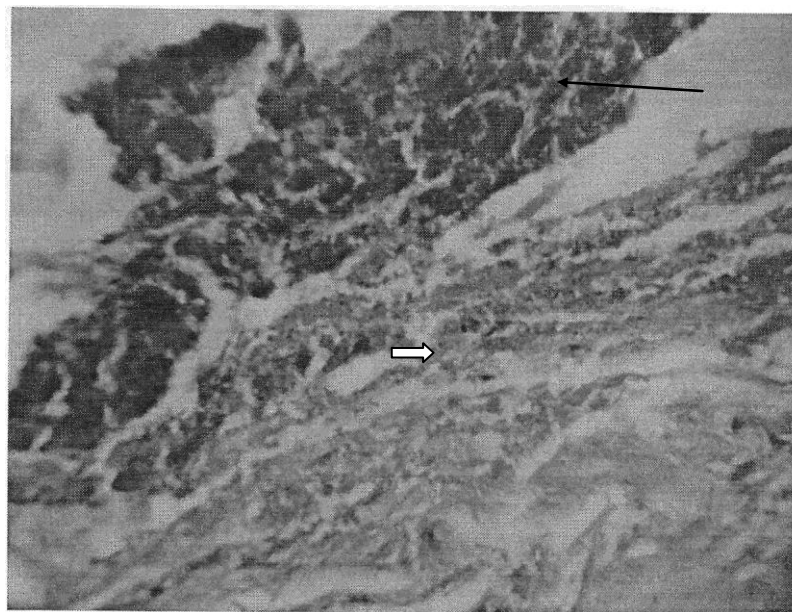


Fig:1 Histological section in joint of one animal of infected non-immunized animals at 30days post-challenge shows inflammatory cells infiltration → , necrotic debris and fibrous connective tissue proliferation in subchondral ⇨ (H& E40X).

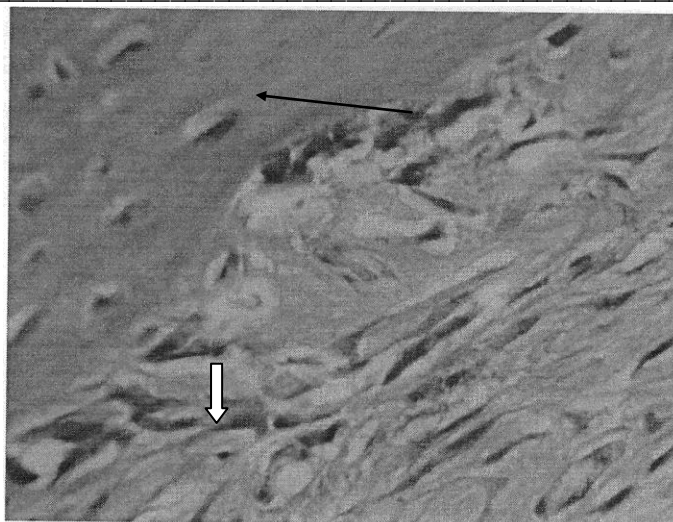


Fig:2 Histological section in joint of one animal of infected non-immunized animals at day 30 post challenge shows granulation tissue with erosion of the cartilage (H&E40X).

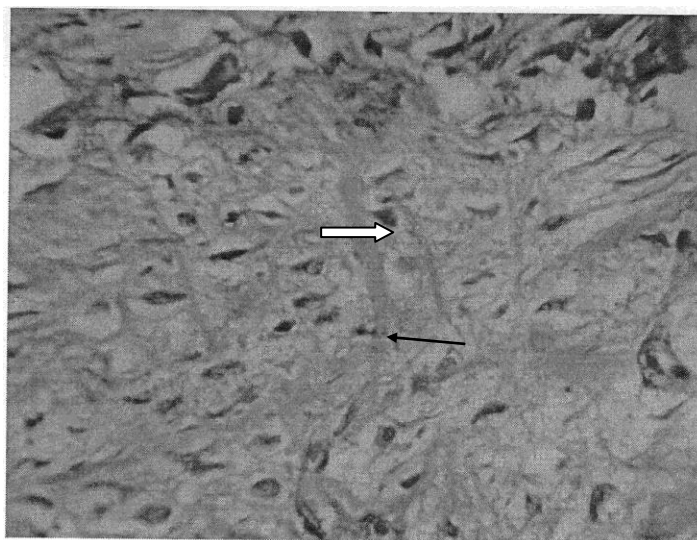


Fig:3. Histological section in the joint of one animal of infected non-immunized animal shows fibrin networks invaded by granulation tissue (H&E40X).

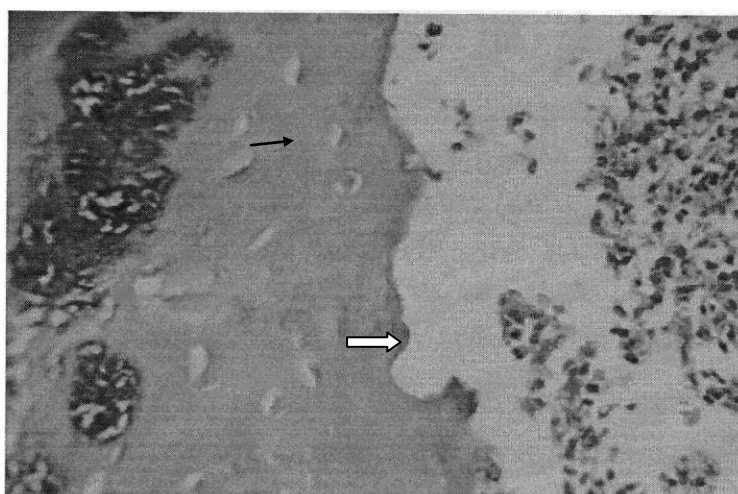


Fig:4. Histological section in joint of one animal of infected non-immunized animals reveals calcification of necrotized cartilage with inflammatory cells infiltration (H&E40X).

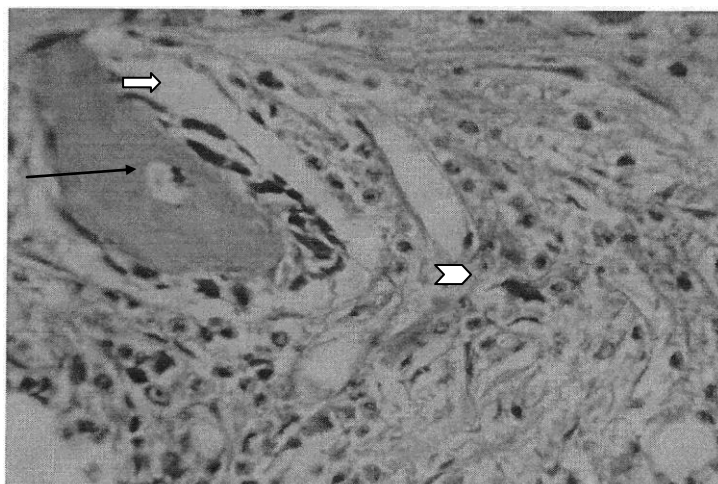


Fig:5. Histological section in joint of bone animal of infected non-immunized animals shows sequestrum of necrotic bone fragment → surrounded by osteoblast ⇨ and sclerosis tissue infiltration with inflammatory cells ⇨ (H&E40X).

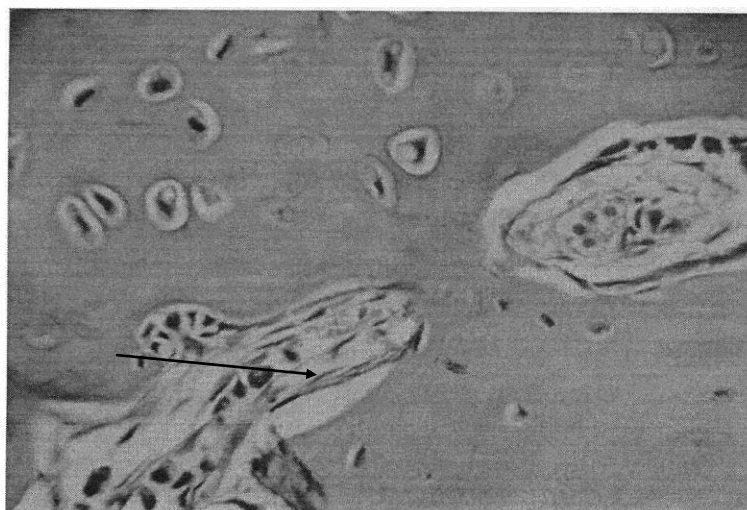


Fig:7. Histological section in joint of one animal of infected non-immunized animals shows surface crack invasion by vascular connective tissue(H&E40x).



Fig:8. Histological section in the joint of one animal of infected non-immunized animals shows proliferation of subchondral bone forming thickness margining bone trabeculi (H&E40X).

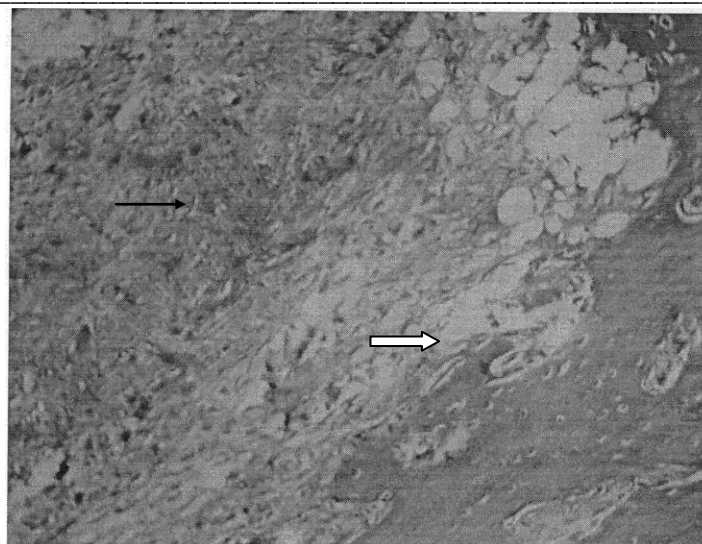


Fig:9.shows proliferation of fibrous connective tissues with inflammatory cells —→ in the peristeal areaan with necrosis and erosion of the bone⇒ (H&E40X)

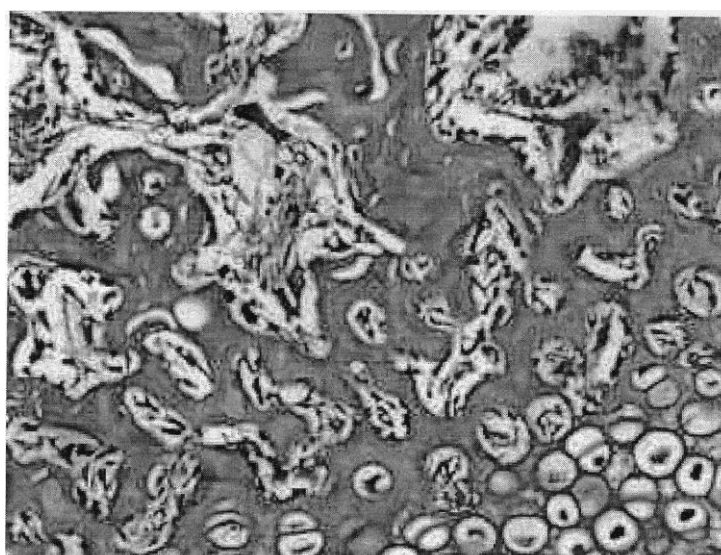


Fig: 11. Histological section in joint of immunized infected animals shows no clear pathological lesions(H&E40X).

Discussion

The results of immunity examination indicated that the WSSAg stimulated both cellular and humoral immune responses and these results are consistent with those reported by Al-Nueimy (18) who reported both cellular and humoral immune response in rabbits immunized with WSSAg. The results of clinical signs and bacterial isolation in non-immunized infected animals indicated that the animals were infected with virulent *Staph.aureus* which evaded the innate immune system of the host body and cause septic arthritis. *S.aureus* produce a large number of potential virulence factors, these factors

enable the bacteria to evade host defenses, and thus to establish infection and may also trigger a cascade of host proinflammatory and immunomodulating molecules(19). Also Gemmel et al.,(20) concluded that simultaneous production of alpha- and gamma-toxin is a virulence factor in *S. aureus* arthritis and alpha-toxin of *S.aureus* might play a major role in the pathogenesis of septic arthritis. Severe bacterial isolation from infected joints at 30 days post-infection, in the present study, may be indicated the persistence of *Staphylococcus aureus* infection in joints. Many attempts have

been made to understand which components of *S.aureus* are of importance for the development and persistence of infection. one mechanisms leading to long-lasting persistence of *S.aureus* in the joint may be due to avascular of the joint cavity(hyaline cartilage) since this area represents a large fraction of the overall surface area of the joint cavity, the extravasation of cells and plasma proteins belonging to the immune system may be delayed and quantitatively low(21). Another factor importance for the regulation of *S.aureus* persistence in the joints is tissue tropism ,which delayed the elimination of microorganisms by immune system, these including collagen adhesions, bone sialoprotein adhesions, and protein A as well as fibrinogen adhesions ,expressed by *S.aureus* (22).Our results explained abscent clinical signs with mild or absent bacterial isolation from joints of immunized-infected animals, these results may be indicated that the WSSAgS elicited sufficient protective immunity that destroyed most *S.aureus* or prevent its adhesion to joint tissues, these results are in agreement with AL-Nueimy (18) who showed that which overcome the local defense and elaborate exotoxin and enzymes that cause articular cartilage damage as well as the infection extend to perichondral area ,as the destructive process continues, Large effusions may be occur in infection of the joint and this lead to, impair the blood supply and result in aseptic necrosis of bone this evidence is consistent with data reported by Nagai et al,1994(30) who investigated that *S.aureus* produce super antigens which produced inflammatory response associated with destruction bone tissues also Guo-min Deng et al.(31),reported that peptidogcan (PGN) exerts a centrol role in cartilage and/or bone destruction triggered by *S.aureus* infection.Superantigen-producing staphylococci stimulated production of IL-4 locally in the joint (33) these cytokine ,in early phase of infection, produced by mast cells in synovia, basophils and eosinophyls and later by 1H2(34),the presence of IL-4 primed

CD4+Tcells develop into TH2 cells(35) and these cytokine which is potent B lymphocyte-differentiating properties, would increase autoantibody production and hence development of immune complex-mediated arthritis(35) .*S.aureus* Map (MHC class TI analog protein) response protein, is a secreted protein that can bind to a variety of ECM components, including fibronectin, fibrinogen, vitronectin, bone sialoprotein, and thrombospondin (36) and it act to shifting T cells response in a Th2 direction and may play a critical role in SA survival since the induction of The (cellular immunity) responses during the course of SA infections have been associated with bacterial clearance in mice (37)it is clear that cellular immunity is critical in orchestrating the clearance of systemic SA infections and in preventing re-infection with the same or similar pathogen (38) According to these evidences and the results of pathological changes in articular joints of non-immunized and immunized animals we postulated that the strain of *S.aureus* used in the present study is highly virulence and WSSAgS stimulated macrophages to produce IL-12 and TNF-alpha that activated production of IFN-gamma by NK cells which favor the differentiation of the IFN-gamma producing TH1 cells which activated macrophages and increased bactericidal ability to destroy *S. aureus* . and these results showed clear correlation with results of cell mediated immunity. These investigations were in agreement with those mentioned by(39) who suggested that IL- 12 is necessary to induce IFN-gamma production by NK cells and differentiation of TH1 cells. And also our results are agreement with investigation that mentioned by Elisabet and Andrej 2006(40) who reported that several vaccine such as Staphylococcal polysaccharides, and enterotoxins WSSAgS provide good immune response against Staphylococcal bone infection, also Kouji et al.,2010(23) ,reported that immunized mice with the clumping factor A(CLFA) protected animals against *S.aureus* infection.CLFA

is a surface protein that binds to fibrinogen and fibrin and promote colonization of *S. aureus* and its evasion of host defense mechanisms. Elisabe et al., (24) demonstrated that immunized mice with CLEA developed less severe arthritis post-challenged with *S. aureus*. According to results given above, we suspected that the WSSAgS used in our study containing all types of *S. aureus* Ags such as cell wall-associated proteins, which stimulated Abs production that prevent bacterial adhesion and enhancing opsonophagocytosis, these idea was supported by level of Abs titer and, result of skin test. The present study showed erosion and necrosis of the articular cartilage with severe inflammatory cells infiltration, these investigation may be due to the particular organism's pathological properties, such as the chondrocyte proteases of *S. aureus*, as well as to the host's polymorphonuclear and mononuclear leukocytes response which are synthesized and secreted cytokines and other inflammatory product, resulting in the hydrolysis of essential collagen and proteoglycans. These evidence supported results of other authors. Greshman et al., (25) explained that *S. aureus* stimulate the host inflammatory response via survival and replication inside PMNs. (26)

demonstration that alpha-toxin promotes the adherence of neutrophils to endothelial cells which is important step in the early inflammatory reaction. It is also known that alpha-toxin causes the release of large amounts of IL-1B, IL-6 and TNF- α , (28). IL-1 is a proinflammatory cytokine that causes joint damage both directly, by activating osteoclasts, and indirectly, by triggering synovial macrophages to produce proinflammatory mediators, e.g., TNF- α . Alpha-toxin and gamma-toxin which are pore-forming toxins, trigger the release of various inflammatory mediators, such as synovial phospholipase A₂, prostaglandin I₂ platelet activating factor, leukotriene B₄, and nitric oxide (29) all these chemical mediators are important in the development and progression of *S. aureus* induced arthritis. (30) our study revealed that intrasynovial *S. aureus* inoculation lead to suppurative inflammation of perichondral area and the inflammation and necrosis of the articular cartilage extended to adjacent subchondral bone that cause osteonecrosis, sequestra and compact bone marrow with inflammatory cells, these results may be due to highly virulent *S. aureus* protects against Staphylococcal infection via devoid of their superantigenic properties and collagen adhesion.

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الخلاصة

من أجل معرفة تأثير المستضد الكلي المتكسر لجرثومة المكورات العنقودية على التهاب المفاصل المتسبب بهذه الجرثومة تم اخذ 15 أرنباً أبيض نيوزلندي ومن كلا الجنسين قسمت عشوائياً إلى ثلاث مجاميع بالتساوي وعوملت كما يلي:

المجموعة الأولى منعت بجرعتين من المستضد الجرثومي (0.5ml, 3.3mg/ml protein concentration) الفرق بينهما 14 يوماً وبعد 28-30 يوماً من إجراء التمنيع تم إجراء فحص المناعة الخلوية والخلطية على التوالي ثم تم إصابة المجموعة الأولى والثانية بجرعة نصف مل من العالق الجرثومي الحاوي على 1×10^{10} cfu/ml في المفصل knee joint بينما جرعة المجموعة الثالثة بجرعة نصف مل من المحلول الملحي المعقم في المفصل واعتبرت سيطرة سالبة . وبعد 30 يوماً من إعطاء جرعة التحدي تم قتل جميع حيوانات التجربة وأخذت نماذج للعزل الجرثومي والفحص المرضي أوضحت النتائج بأن المستضد الكلي المتكسر للمكورات العنقودية حفز استجابة مناعية خلوية وخلطية جيدة صد جرعة التحدي بهذه الجرثومة حيث لوحظ تورم المفصل مع عدم قدرة الحيوان على استخدام قدمه مع عزل جرثومي كثيف من المفاصل في الحيوانات غير الممنعة المصابة بينما لم تسجل أعراض سريرية أو عزل جرثومي في الحيوانات الممنعة المصابة وكذلك سجلت تغيرات مرضية سه شديدة في مفاصل الحيوانات غير الممنعة المصابة تميزت بالتهاب قيحي للمنطقة حول الغضروف مع التهاب وتنخر غضروف المفصل وامتداد هذه التغيرات إلى الحويصلات العظمية ونخاع العظم مكونه التهاب قيحي وتنخر العظم ولم تسجل تغيرات مرضية واضحة في مفاصل الحيوانات الممنعة.