THE INFLUENCE OF WHOLE SONICATE BRUCELLA ABORTUS ANTIGEN ON THE CANDIDA ALBICANS INFECTION IN MICE

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ABSTRACT

The aim of this study is to evaluate the effect of immunization with whole sonicate Brucella abortus antigen on the mice infected with Candida albicans, for that, Twenty one white mice , both sexes, average weight 20-24g, were divided randomly into (3) equal groups.

First group immunized with (0.5)ml of sonicated brucella antigen, two doses, 10 days interval, skin test was done 27 days post-vaccination, 2nd group and 3rd groups served as positive and negative control group respectively. (30) days post-vaccination, 1st and 2nd groups were challenged with (0.5)ml of inoculums (1X10yeast/ml), C. albicans I/P. 3rd group was injected with (0.5)ml of sterile normal saline I/p.

The results showed that the immunized animals revealed cellular immune response, negative fungal isolates and minor pathological changes in compared with positive control. Positive control animals show sever fungal isolates from internal organs with sever pathological changes characterized mainly by polymorphonuclear cells infiltration and multiple foci of granulomatous lesion.

INTRODUCTION

Candidiasis is the infection caused by species of Candida, mostly C.albicans which is the most frequently isolated fungal pathogen in humans and animals (1). C.albicans is a normal commensal of the gastrointestinal and genitourinary tracts of human and of various warm-blood animals (2). In immuno-compromised hosts, the transition of C. albicans into an opportunistic pathogen is not uncommon,and disseminated candidiasis of endogenous origin may occur (3). C. albicans pathogenicity depends on a complex array of microorganisms related putative virulence factors, these include the yeast to mycelium transition, antigentic variably, phenotype switching, adheres to host and tissue, cell surface hydrophobicity, molecular mimicry, immunomodulation of host defence mechanisms and production of extracellular enzymes (4). The three most significant hydrolytic enzymes produced by C.albicans are secreted aspartic proteinases, phospholipase and lipase. Their hydrolytic activity has a number of possible function in addition to simple role of digesting molecules for nutrition,these enzymes used to host tissue invasion by digesting or destroying cell membrane and by degrading host surface molecules and all these enzyme are able to attack cells and molecules of the host immune system to avoid or resist antimicrobial activity (5)

Candida antigen may stimulates specific cell mediated and humoral immune response .Most invasive fungal infection occur in patient with defective cellular immunity but the role of antibodies in resistance to candidiasis is poorly understood (6)The integration of innate and adaptive immune responses is required for efficient control of C.albicans (7).

Resistance to C.albicans infection is determined by phagocytic effector mechanisms enhanced by Th-1type CD4 cell cytokines (8). Neutrophils and mononuclear phagocytic cells play importance role in preventing candidal dissemination (9). Th-1 cytokines play essential role in the
activation of mononuclear phagocytic cells, Th-1 differentiation requires the combined effects of different cytokines, including interleukin-12 (10), this cytokine was both required and prognostic for the development of protective Th1 responses to C. albicans (11) and acted as an adjuvant in response to a Candida vaccine (12).

C. albicans can switch from a unicellular yeast form into various filamentous forms all of which can be found in infected tissues (13), the ability to reversibly switch between these forms is thought to be important for Candida virulence. (14) Filamentous growth form is required to evade the cells of the immune system, whereas the yeast form may be the mode of proliferation in infected tissues (14). Phagocytic cells such as neutrophils, macrophages and dendritic cells rapidly and efficiently phagocyted both yeast and hyphae of the fungus. In vitro, ingestion of yeasts activated phagocytic cells for IL-12 production and priming of T helper type 1 cells whereas ingestion of hyphae inhibited IL-12 and Th1 priming, and induced IL-10 production (15) IL-10 differentiated naive CD4 T cell to Th2 type cells which produced IL-4 and IL-5 and mostly associated with pathology and disease progression (16).

Safety vaccine against C. albicans infection in human and animals was not recognized, the aim of this study is to evaluate the effect of immunization with whole sonicated brucella abortus antigen in mice against C. albicans infection.

MATERIALS AND METHODS

Organisms: organisms used in this study were obtain from the followings:

1- The pathogenic C. albicans was taken from the stock culture collection of Mycology Division, Collage of Al-nahran Medicine. Yeast cells were grown on Sabouraud glucose agar at 28C, Yeast cells were harvested after 48hrs of culture, centrifuged at 1000 rpm, washed twice in sterile normal saline, counted and diluted to desired concentration. Feature of this strain exhibited according to Lodder (17).The number of viable cells was counted by a haemacytometer.

2- Brucella abortus 99 was obtained from unit of zoonotic disease unit, College of Vet, Medicine, Baghdad. Sonicated antigen was prepared according to the method which was described by Hiallibartion and Blazkovec (17) and used Sonicater MSE UK. to destruct the bacteria for 15 minutes with ice.

Experimental design

A total number of 28 white mice, both sexes, were used in this investigation. Their age range between 60-90 days, their weights ranged between 20-34 grams. They were divided into 3 groups:

1- The first group was immunized with 0.5 ml of sonicated antigen (protein concentration 17mg/ml) S/C, two doses with 10 days interval, skin test examination was done at 27 days post-immunization. At 30 days post-immunization, all animals were infected intraperitoneally with a volume of 0.5 ml of inoculums (1X10 yeast/ml).

2- The second group was infected with viable C. albicans at the same dose and route of first group.

3- The third group was injected S/C with 0.5ml of normal sterile saline

All animals were sacrificed at day 20 post-infection, samples were taken from some internal organs for culture and other samples were fixed in 10% formaldehyed solution for histopathological examination according to Luna (18).

RESULTS

Skin test results:

Table:1 showed mean of difference thickness of palm legs of animals at 27 days post-immunization against Brucellen
All the immunized animals remained alive and in good condition. Cultures failed to detect the fungal growth from their internal organs. Two animals died from non-immunized group and all animals gave heavy growth of yeast from kidney, lung, spleen, liver and heart (table: 2).

Pathological examination:
1- Gross examination showed no pathological lesion in the examined organs of immunized animals. Gross lesions of non-immunized animals were mostly confined to the kidney and liver, the lesions characterized by congestion of these organs with white pin-point areas distributed over their surfaces.

2- Microscopic examination:

Non-immunized animals
- Kidney:
  Microscopic section revealed congested blood vessels between renal tubules with inflammatory cells mainly neutrophils in their lumens. Bowman’s capsules loss its normal histological architecture, atrophy of glomerular tufts and dilated of bowman space, acute cellular degeneration of epithelial lining cells of renal tubules also reported as polymorphonuclear cell infiltration in the interstitial tissue of the cortex and around the blood vessels (fig:1).
- Liver:
  The lesions in the liver distributed throughout the liver parenchyma, characterized by area of necrosis and disappearance of hepatic cells which replaced by RBCs (fig: 2), in addition to the presence of multiple foci of granulomatous lesions consisted from neutrophils, macrophages scatter in the liver parenchyma (fig: 3). In other section, abscess which consists from dead and live of neutrophils and encapsulated by fibrous connective tissues was seen in the liver capsule and it adhere to the stomach and esophages layer (fig:4,5).
- Lung:
  Histopathological findings of the lung consisted from hyperplasia of epithelial lining of mucosa of bronchioles with inflammatory cells infiltration mainly neutrophils and macrophages in the subepithelial layer and in the lung parenchyma (fig: 6)
- Spleen:
  polymorphonuclear cells infiltration, hemorrhage and also congestion of blood vessels which contained inflammatory cells in their lumens were seen in the spleen (fig7).

Immunized animals
Histopathological examination of the internal organs showed severe hyperplasia of white pulp of the spleen together with mononuclear cells proliferation around splenic sinuses which appear as a cord-like (fig: 8). No signs of inflammatory lesions observed the lung (fig: 9) lymphocytes aggregate around the central veins (Fig: 10) and blood vessels in the portal area.

DISCUSSION
The present work aimed to determine the effects of sonicated Brucella abortus antigen in immune responses against infection by C. albicans in mice.
The results revealed that Brucella antigen stimulated cell mediated immunity in immunized animals which showed negative fungal isolates from their internal organs compared with non-immunized animals in which heavy fungal isolates were recovered from their organs.
Cell mediated immunity is essential for host protection against virtually all fungal pathogens (19).
Brucella antigens stimulated cellular immune response in laboratory animal’s model measured by lymphocyte blastogenesis and produce protective cytokines (20, 21)
Phagocytosis of *C. albicans* by macrophages must be accompanied by killing; otherwise, macrophages could promote the dissemination of the pathogen and help the fungus to avoid the immune attack (22). Killing of *C. albicans* can be upregulated in macrophages by cytokines such as interferon gamma (INF-γ) or tumor necrotic factor alpha (TNF-alpha), chemokines, or neuroendocrine products (23). Phagocytosis of either form of candida by macrophages and dendritic cells produced sets of cytokines with opposing activities in the developing immune response (24). Macrophage synthesis of IL-12 and TNF-alpha in early infection results in a synergistic stimulation of natural killer cells to synthesize IFN-γ (25). This IFN-γ activates resident macrophages to become bactericidal, particularly through the production of nitric oxide and also induces Th cells to undergo differentiation to CD4+Th1 cells. These Th1 cells synthesize additional IFN-γ, thus positively amplifying the host response, the activated macrophages now have an increased ability to present bacterial antigens to these T cells, resulting in their differentiation into armed effectors cells and the sterile eradication of the bacteria (26).

Histopathological results agreed with results of skin test and fungal isolates. No significant lesions in the internal organs of immunized animals compared with no-immunized group. This results suggest that the Brucella antigens may be induced specific or non specific protective immunity against *C. albicans* in mice. Phagocytosis of the brucella antigen activated the macrophages and dendritic cells to secrete proinflammatory cytokines such as IL-1, TNF alpha and IL-12. (27). These cytokines participate both in the control of the fungus and in the induction of protective Th1 immunity (16). TNF alpha is one of the major secretory products of stimulated macrophage with important role in host defense against disseminated candidiasis (28).

IL-12 allows the CD4+ T cells to differentiate into Th1 cells resulting in phagocytic-dependent immunity clearly represents an important mechanism of anticandidal resistance (29).

The pathological changes in the internal organs of non-immunized animals indicate that a virulence strains of *C. albicans* were used in the present study. Phagocytic cells engulfed Candida by a zipper-type phagocytosis (30). Once internalized, yeast were detected in phagolysosomes where different stages of progressive degradation were seen and may result in an efficient release of fungal peptide for class II-restricted antigen presentation whereas hyphae surviving free in the cytosol may eventually intersect the class I-restricted antigen presentation pathway (31). The activation of CD8+ T cells occurred in response to the filamentous forms of the fungus and activated CD8+ T cells secreted cytokines which induced granulomatous reaction (32), this evidence was in agreement with the present study.

Neutrophils infiltration and necrosis which are seen in some organs of non-immunized animals may be due to virulence factor of *Candida albicans* This fungus secretes hydrolytic enzymes such as phospholipase C that increases penetration into host cell tissues (33). It also play role in perforation of macrophage phagosomes, destruction of the infected cells and spread the Candida to adjacent cells.
Fig 1: histological section of kidney in animal infected with *C. albicans* showed congestion of blood vessels with polymorphnuclear cells infiltration in the interstitial tissue. (H&EX40).

Fig 2: histological section in the liver of animals infected with *C. albicans* revealed necrosis and disappearance of hepatic cells which replaced by RBCs. (H&EX40).
Fig 3: histological section in the liver of animals infected with *C. albicans* explain multiple granulomatous lesion in liver parenchyma & blood vessels (H&EX40).

Fig 4: histological section in the liver of animals infected with *C. albicans* showed abscess adhered with stomach (H&EX20).
Fig 5: histological section in the liver of animals infected with *C. albicans* revealed abscess encapsulated by thick fibrous connective tissue adhered to esophagus (H&EX20).

Fig 6: Histological section of the lung of animals infected with *C. albicans* showed hyperplasia of the epithelial lining of mucosa of the bronchioles with inflammatory cells infiltration in the subepithelial layer (H&EX40).
Fig 7: Histological section of the spleen of animals infected with C. albicans revealed inflammatory cell infiltration with hemorrhage in the red pulp (H&EX40).

Fig 8: Histological section in the spleen of immunized animals at 20 days post-challenge showed hyperplasia of white pulp (H&EX40).
Fig 9: histological section in the lung of immunized animals at 20 days post-challenge showed no pathological lesions (H&EX20).

Fig 10: histological section in the liver of immunized animals at 20 days post-challenge revealed aggregation of mononuclear cells around the central vein (H&EX40).
تأثير التثبيط بالمستضد المتكسر لجرثومة البروسيلا المجففة على الإصابة التجريبية للفئران

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الخلاصة

تهدف هذه الدراسة لتقسيم فعالية التثبيط باستخدام المستضد الكلي الناتج من تكسر جرثومة البروسيلا المجففة على الفئران المصابة تجريبيا بفطر (المبيضات البيضاء) حيث تم استخدام 21 من الفئران البيضاء من كلا الجنسين تتراوح أوزانها بين 20-24جم قسمت عشوائيا إلى ثلاثة مجاميع وتم أضعاف المجموعة الأولى المستضد المتكسر لجرثومة البروسيلا المجففة 0.5 مليلتر (1 ملغ بروتين) وقطر وضع بين كل واحدة وأخرى 10 أيام. وأجري الفحص الجلدي بعد 27 يوما من التثبيط بالجرعة الأولي.اما المجموعة الثانية والثالثة فأعتبرت مجموعتي سيطرة موجهة وسالبية بالثنائي. تم بعد 30 يوما من التثبيط بالجرعة الأولي للمستضد أضعاف جرعة تثبيط تجريبي تحدي فطر (المبيضات البيضاء) مقدارها 0.5 مليلتر 

من محلول تركيزه 1x10^{-6} مللتر/ملغ عن طريق الحقن داخل التجويف البريتيوني (IP). بينما أعطيت المجموعة الثالثة 0.5 مليلتر من محلول الفسليجي المتعادل للمعقم داخل التجويف البريتيوني. 

أظهرت النتائج أن الحيوانات المثبتة أظهرت أعراض متابعة منخفضة ولم يتم عزل الفطر من الحيوانات في هذه المجموعة مع وجود تغييرات مرتبة طفيفة مقارنة مع ما أظهرته مجموعة السيطرة الموجهة (المجموعة الثانية) والتي أظهرت أفات مرتبة شديدة تمثلت بارتفاع الخلايا المتعددة الآتية وبور متعددة من الأورام الحبيبية مع عزل أعداد كثيرة من الفطر من الأعضاء الداخلية لحيوانات هذه المجموعة.

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