The use of Cell Wall Mannoproteins of Candida albicans as immunomodulators in mice vaccinated with Brucella RB51 vaccine

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Abstract

A study were carried out to investigate, the immunomodulatory effect of the Mannoproteins of *Candida albicans* cell wall on the immune response of mice vaccinated with *Brucella RB51* vaccine and considered as biological immunomodulators. The study included two main groups, each group divide into four subgroups, each subgroup involved 20 mice, the first four subgroups were I: treated with distilled water. II: treated with the *Brucella RB51* vaccine only III: treated with the mannoproteins only. IV: treated with *brucella-RB51* strain vaccine and mannoproteins of *Candida albicans*. The V, VI, VII and VIII groups were injected with the immunosuppressive drug prednisolone prior to the forthcoming treatment. All these treatments were carried out on day 1, then the mice were sacrificed on day 8 to estimate serum IFN-γ level and on day I4 for delayed-type hypersensitivity reaction. The doses of the Mannoproteins of Candida albicans cell wall represents 10% of the calculated LD_{50} dose (5714 µg/kg), which were given subcutaneously. The results demonstrated a clear immunomodulatory effects of the mannoproteins of Candida albicans cell wall (improvement of non-specific, and cellular immune response) of the treated mice vaccinated with *BrucellaRB51*. The interferon-γ (IF- γ) showed a significant increased ($P \leq 0.05$) in serum level in Mannoproteins-treated vaccinated mice in comparison with negative and positive subgroups, and group IV showed a highest increase. In delayed-type hypersensitivity reaction, an increased index was significantly increased ($P \leq 0.05$) in Mannoproteins-treated vaccinated mice in comparison with negative and positive subgroups, a best results was observed after 24 hours post-brucelline injection, generally the response after 24 hours was better than 48 hours.

استخدام مانوبروتينز الجدار الخلوي للمبيضات البيضاء كمحفز مناعي للفئران الملقحة بلقاح البروسيلاBrucella RB51

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الخلاصة

درس تأثير بعض المحورات المناعية المتمثلة بمستخلص والمانوبروتينز Mannoproteins المستخلصة من جدار خلايا المبيضات البيضاء *Candida albicans* على الاستجابة المناعية للفئران الملقحة بلقاح البروسيلا العترة *Brucella RB51*. ضمت الدراسة مجموعتين رئيسيتين قسمت كل مجموعة إلى أربعة مجاميع فرعية تحوي المجموعة الواحدة منها على 20 فأر عوملت كالأتي (I) معاملة بالماء المقطر.(II) معاملة باللقاح. (III) معاملة بالمانوبروتينز. (IV) معاملة بالمستخلص مع لقاح البروسيلا. تمت نفس المعاملات في المجاميع الثانوية الأربعة الأخرى مسبوقة بالحقن بمادة البريد نسيلون كمثبط مناعي فقط. أجريت جميع هذه المعاملات في اليوم الأول وقتلت الحيوانات في اليوم 8 لقياس المستوى المصلي للأنترفيرون كاما، في اليوم 14 لقياس (تفاعلات فرط الحساسية المتأخرة). أعطي المستخلص تحت الجلد بجرعة معادلة لـ 10% من نصف

الجرعة المميتة (مانوبروتينز: 5714ميكروغرام/كغم). أظهرت النتائج تأثيرات واضحة للمحورات المناعية المستخدمة في الدراسة وفي المجاميع الممنعة بلقاح البروسيلا ومن خلال تحسن الاستجابة المناعية اللانوعية والمناعة الخلوية مقارنة مع المجاميع غير المعاملة بمستخلص المانوبروتينيز، لوحظ ارتفاع واضح في المستوى المصلي للكاما أنترفيرون في المجاميع الممنعة والمعاملة بمستخلص المانوبروتينيز بالمقارنة بمجاميع السيطرة السالبة والموجبة وسجلت أيضا المجموعة (IV) أعلى مستوى. أما نتائج فحص الحساسية المتأخرة في الجلد فقد أعطت حيوانات المجاميع الممنعة والمعاملة بالمحورات المناعية نتائج أعلى وبفوارق إحصائية معنوية عن مجاميع السيطرة الموجبة والسالبة وأعطت المجموعة (IV) أفضل نتيجة بعد 24 ساعة من حقن، وكانت جميع نتائج فحص الحساسية المتأخرة المسجلة في الحيوانات بعد 24 ساعة أفضل منها بعد 48 ساعة.

Introduction

Materials of fungi have been the interest of different investigators around the globe with their aims to establish the immunomodulator potentials of these materials, and some risks associated with attenuated or killed whole-organism vaccines which can be avoided with vaccines that consist of specific purified macromolecules derived from pathogens (1). *Candida albicans* is one of the fungi species that share the interest of investigators in the field of immune modulation. (2) reported that both *C. albicans*-sensitized and non-sensitized mice were able to mount immediate type and delayed type skin test responses against the cell wall antigens of *C. albicans* but not to the cytoplasmic antigens (2,3). Furthermore (3) have demonstrated that immunization with mannan (a mannoproteins fraction) and mannoproteins derived from digested cell walls of *C. albicans* induced resistance to a systemic candidiasis. Brucellosis is one of the most wide spread infectious disease in the world that causes fetal death as a single agent in human being. This disease is widely distributed in different countries of the world among humans and animals, and there is a positive correlation of infection between animal and human populations (4). Up to now there is no available effective vaccine for protection against brucellosis in humans and animals, although there have been many trails to use a combinations of immunomodulators and vaccines to immunpotentiated the immune mechanism in recipient animals (4,5,6,7,8,9 and10). The immunologists are engaged to design vaccines and vaccine strategies to maximize the responses of the immune system and to avoid the unfavorable complications resulted from *Brucella* vaccines, one of these strategies is the employment of immune modulators to potentiate the immune response and increase the effectiveness of vaccines (2,3,8,10). The present study were designed to evaluate the immunomodulator potentials of *C. albicans* cell wall mannoproteins in mice vaccinated with *Brucella RB51* vaccine. The parameters of evaluation were delayed-type hypersensitivity reaction and interferon-γ serum level.

Materials and Methods

All experiments were carried out on male and female albino mice (Blab-c), which were supplied by the National Centre for Drug Control and Research Baghdad/Iraq. The starting age of mice is rounded (6-8) weeks. They were housed in bio-clean hoods at 20-25ºC with light, dark periods of 14:10 hours. They were fed standard pellets and water, their initial weight was 22 ± 3 grams at the beginning of experiments. Mice were separately caged for a one week preliminary period for acclimatization period. The following culture media were used in carrying out the experiments of the study (agar, Bactodextros, Bacto peptone, Blood agar, Sabourauds dextrose agar, Trypticase Soya agar, Trypticase Soya broth and yeast extract) were the products of Difco Company (U.S.A). Brucelline is a purified ribosomal protein free of lipopolysaccharied, which is prepared from *Brucella* RB51-strains and composed of 50-70% protein and 15-30% polysaccharide (4). Prednisolone is a synthetic glucocorticoid with anti-inflammatory

and It can also be used as an immunosuppressive drug for organ transplantations and in cases of adrenal insufficiency (Addison's disease) (11). Kits were used during the experiments of the study was Mouse IFN-γ ELISA quantitative determination (Bender Med. Systems, Austria). The dried lyophilized seed of *Brucella* RB51 strain was supplied by the Central Veterinarian Laboratory Baghdad/Iraq, the above laboratory receives the strains from the Food and Agriculture Organization (FAO). The Mannoproteins were prepared from the cell wall of a *Candida albicans* isolate as described by (9). The isolate was obtained from the vaginal swab of a healthy woman, were supplied by the Central Health Laboratory Baghdad/Iraq. The LD_{50} was employed to determine the cytotoxic dose of the investigated immune modulators (mannoproteins of *C. albicans* cell wall, in albino mice, according to a method previously described by (8). The brucella vaccine prepared as described by (5). There were eight subgroups in this experiment, which was designed to evaluate the immunomodulator potential of *C. albicans* cell wall mannoproteins in mice vaccinated with *Brucella* RB51 vaccine. The total number of mice were 160 mice (20) mice in each group). All mice were treated on the day (1) subcutaneously:

Group I: A mice were injected subcutaneously with a single dose (0.2ml) of deionized distilled water in day 1. Group II: mice were vaccinated with 0.2 ml of *B*rucella *RB51* vaccine in a similar manner. Group III: mice were injected subcutaneously with a single dose (5714µg/0.2 ml/Kg) of mannoproteins in day 1. Group 1V: The mice were injected subcutaneously with a single dose of mannoproteins (5714µg/0.2 ml/Kg) in day 1, and in day 4, they were vaccinated with 0.2 ml of *B*rucella RB51 vaccine in a similar manner. The V,VI,VII and VIII groups treated were injected subcutaneously with the prednisolone (5 mg/kg) 5 days prior to the treatment regimens that were outlined. All mice were sacrificed on the day 8 to evaluate (serum IFN-γ level), and on the day 14 to evaluate (delayed-type hypersensitivity reaction).

Delayed type hypersensitivity reaction index in a right foot pad was measured to each mouse in all groups, each mouse was injected with 50 ul of brucelline in the right foot pad, then foot Pad swelling was measured twicely (24,48) hours post injection by a digital vernia and given in a unit of millimeter, as suggested by (12). Quantitative Determination of Interferon-γ Serum Level**.** The quantitative determination of IF- γ serum level was carried out by using a mouse IFN- γ ELISA kit (Bender Med Systems, Austria), which is an enzyme-linked immunosorbent assay for quantitative detection of murine interferon-γ (IFN-γ) in murine serum.

Calculations: data results were calculated by interpolation from a standard curve that was performed in the same assay as that for the sample (Fig.1), using a curve fit equation.

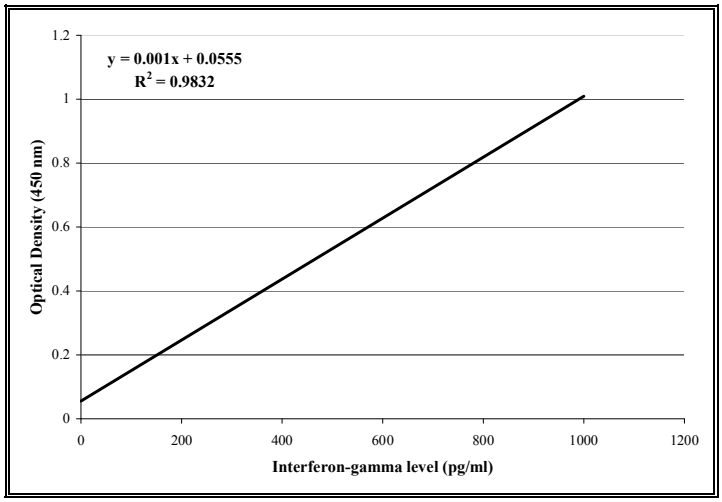

Fig. (1) Standard curve of interferon-γ serum level

- **Statistical Analysis:** Data were analysied for investigated parameters and given in terms of means ± standard errors (S.E.), the means differences betweens were assessed by analysis of variance (ANOVA), least significant difference (LSD) and Duncan multiple rang test, using the computer program SPSS (Statistical Package of Social Sciences) version 7.5. The difference was considered significant when the probability value was equal or less than 0.05.

Results and Discussion

present study came out to add out some understanding about the role of biological material (*Candida albicans* cell wall mannoproteins) in potentiating the immune response (immunomodulators) against brucellosis in mice vaccinated with *Brucella* RB51 vaccine. Experimental design are given in table (1). It was founded that the LD_{50} of *C. albicans* cell wall mannoproteins was (5714µg /kg) (8). Based on these findings, 10% of the LD_{50} of each material (mannoproteins: 5714µg/Kg); was considered as immunomodulator dose in the present study, which was subjected to investigations.

Table (1) Experimental design

Groups	Day I			Day 4		
	D.W	vaccine	Mannoproteins	D.W	vaccine	mannoproteins
I	+	-	-	+	-	-
II	-	-	-	-	+	-
III	-	-	+	-	-	+
IV	-	-	-	-	+	-
V***	+	-	-	+	-	-
VI***	-	-	-	-	+	-
VII***	-	-	+	-	-	+
VIII***	-	-	-	-	+	-

***** injected with prednisolone 5 days prior to mannoproteins treatment**

Table (2) Doses of *C. albicans* cell wall mannoproteins that were used in the assessment of LD_{50}

Immunomodulators	Dose/mouse	Dose/Kg	Number of Animals	Mortality Rate (%)
C. albicans Cell Wall Mannoproteins	20 µg	800 µg	4	0.0
	60 µg	2.4 mg	4	0.0
	120 µg	4.8 mg	4	50.0
	180 µg	7.2 mg	4	50.0
	240 µg	9.6 mg	4	50.0

Table (3) Determination of LD_{50} in mice treated with *C. albicans* cell wall mannoproteins

Type of Immunomodulator	Increase or Decrease in Dose (d value)	Number of Survived Mice after 48 Hours / 4 Animals	Tabulated Value of K	Final Dose (XF value)	LD_{50} Dose
C. albicans Cell Wall Mannoproteins	60 µg/mouse	2	0.381	120 µg/mouse	142.86 µg/mouse

K: Tabulated value; XF: final dose value; d: increase or decrease in dose value

mannoproteins LD_{50} demonstrated a dose of (5714µg/kg), and the 10% of the LD_{50} (14.286 ug/mouse) was also safe and effective in terms of toxicity and produce an immunomodulatory effect. The *C. albicans* cell wall is essential to a approximately for all aspect of the microorganism biology and pathogencity, because it contain materials

that are able to mediate interactions with the host immune response (13). These contents are mainly polysaccharides in addition to proteins and minor amounts of lipids (14). Therefore, it is expected that the isolated mannoproteins are effective immunomodulators. These are in agreement with this conclusion, several researchers suggested the potentianal use of *C. albicans* cell wall mannoproteins in this line of experimental immunology by using different laboratory approaches and animals (3,6,8,9,12,13,14,15, and 17). Results of DTH index after (24, 48) hours are given in table (4) Measuring the DTH after 24 hours revealed that all groups showed an increased index (3.12, 3.9 and 4.97 mm, respectively) and the differences were significant (P≤0.05), as compared to group I (1.98 mm), almost a similar picture was observed after 48 hours, but the index values were lowered.

Table (4) Results of Delayed type hypersensitivity (DTH) index in mice of all groups

Means ±S.D mm.						Probability** ≤	
groups	With prednisolone			Without prednisolone		I	II
	24 Hours	48 Hours	groups	24hours	48 hours		
I	1.98±0.12d	1.90±0.06d	V***	1.69± 0.04e	1.70±0.01e	0.01 N.S	N.S
II	3.9±0.03 a	2.98±0.06b	VI***	3.40±0.1b	2.45±0.04c	0.01	0.05
III	3.12±0.02b	2.23±0.15c	VII***	2.59±0.2c	2.03±0.02c	0.01	N.S
IV	4.97±0.12a	2.87±0.12b	VIII***	4.42±0.04a	2.37±0.04c	0.01	0.01

***Different letters: Significant difference (P≤0.05) between means of the same column.**
****The comparison is between means (I: 24 hours; II: 48 hours) of the two columns (horizontal comparison).**
***** injected with prednisolone 5 days prior to mannoproteins treatment .**
N.S: not significance

Mannoproteins was assessed for their effectiveness on immune cell-mediated immunity (CMI), which can be assessed by determining the level of delayed-type hypersensitivity (DTH) response, and the latter one is an important host defense mechanism against brucellosis (8). With this regard, mice treated with 14.286 µg/mouse of *C. albicans* mannoproteins prior to the time of vaccination induced a significant DTH response against brucelline in comparison with the corresponding control, and the highest thickness was produced after 24 hours of brucelline injection, especially in those immunocomprised mice that were treated with prednisolone. Such findings suggest that the importance of these materials as immunomodulators in a combination with a *Brucella* vaccine program. These results came to confirm the findings of (3, 6, 8, 12, 13, and 16). In this regard, DTH reactions develop when antigen activates sensitized T_{DTH} cells, and these cells generally appear to be a T_H1 subpopulation although T cytotoxic may also be involved. An activation of T_{DTH} cells results in secretion of various cytokines including IL-2, IFN-γ, macrophage-inhibiting factor and tumor necrosis factor (18). The overall effect of these cytokines is to with draw macrophages to the area of injection and activating them, promoting increased phagocytic activity and increases concentrations of lytic enzymes for more potent killing. As lytic enzymes leak out activated macrophages into the surrounding tissue, the localized tissue destruction can be occur. These reactions typically tooks (48-72) hours to develop, which is the time required for initial T_{DTH} cell activation and cytokine secretion to mediate the accumulation of macrophages and the subsequent release of their lytic enzymes (18).

- **Interferon (IFN)-γ Serum Level:** The results of IFN-γ Serum Level are given in table 5. A significant increased serum level of IFN-γ was observed in groups II, III and 1V as compared to group I).

Table (5) Interferon-γ serum level in mice in all groups

Groups	Interferon-γ Serum Level (mean ± S.E.; pg/ml)*			Probability** ≤
	Without Prednisolone Treatment	Groups	With Prednisolone Treatment	
I	43.33 ± 23.40 [c]	V	24.67 ± 4.40[d]	0.01
II	250.00 ± 14.00 [b]	VI	174.67 ± 2.85 [c]	0.01
III	220.67 ± 17.30 [b]	VII	138.33 ± 15.3 [c]	0.01
IV	430.00 ± 22.90 [a]	VIII	191.33 ± 14.7 [bc]	0.01

***Different letters: Significant difference (P≤0.05) between means of the same column.**
****The comparison is between means of the two columns (horizontal comparison).**
***** injected with prednisolone 5 days prior to mannoproteins treatment.**

One of the cytokines is IFN-γ, which was evaluated in this through different experiments in regard to *Brucella* vaccination and immunomodulator (mannoproteins) (6, 8 and 19). Results demonstrated a significant increased (P≤0.01) serum level of IFN-γ in mice treated with the tested immunomodulator. Such findings focus a highlight the importance of mannoproteins of *C. albicans* cell wall as immunomodulator, especially when we consider the immunological importance of IFN-γ in enhancing the cellular immune response, which is important in controlling *Brucella* infection (8, 19) that results reported from this study, it is possible to point out the effectiveness of immunomodulators used on innate immune response and cellular immune response in mice vaccinated with brucella RB51 vaccine (7, 8, 10, 18 and 19). A defining cytokine of the TH1 subset, IFN γ activates macrophages, stimulating these cells to increase microbicidal activity, up-regulate the level of class II MHC, and secrete cytokines such as IL-12, which induces T-H cells to differentiate into the TH1 subset. IFN-γ secretion by TH1 cells also induces antibody-class switching to IgG classes (such as IgG2a in the mouse) that support phagocytosis and fixation of complement. TNF and IFN γ are cytokines that develop inflammation, and it is their secretion that accounts for the association of TH1 cells with inflammatory phenomena such as delayed hypersensitivity. TH1 cells produce IL-2 and IFN- γ cytokines that promote the differentiation of fully cytotoxic TC cells from CD8+ precursors. This pattern of cytokine production makes the TH1 subset particularly suited for response of viral infections and intracellular pathogens. Finally, IFN-γ inhibits the expansion of the TH2 population (20). From the results reported in the present study, it is possible to point out the effectiveness of mannoproteins of *C. albicans* cell wall used on innate immune response and cellular immune response in vaccinated mice. In recent years the understanding and importance of antigen-specific immune responses after vaccination has completely changed. In the past the focus for monitoring a vaccine-specific immune reaction was principally based on the humoral branch of the immune system, and the efficacy of vaccines, as assessed by the induction of protective immunity was mainly correlated with antibodies titers. However, this correlation is often failed and other parts of the immune system have also to be considered; namely the innate immune system and the cellular branch of the antigen-specific immune system. The innate immune system plays its main role in the effective activation of the antigen-specific immune response, in antigen-uptake and antigen-presentation. Furthermore, in order to achieve an effective vaccination, the activation of all T-cell subpopulations is of advantage, but

more important is the generation of antigen-specific memory T and B lymphocytes. From the present results, it is possible to conclude that cell wall mannoproteins of *C. albicans* might be a potential immune adjuvant for inducing active immunity against *brucella* Spp., and may act as immunopotentiators through increasing microsomal proteins. These proteins have a binding activity to antigens, and such binding helps in extending the half-life of the antigen by a gradual release of it over a long period (8, 12, 14, 15 and 19).

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