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A STUDY ON SOME CELL MEDIATED AND HUMORAL IMMUNE RESPONSE PARAMETERS ASSOCIATED WITH EXPERIMENTAL INFECTION OF MICE WITH *CANDIDA ALBICANS*

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ABSTRCT
The Study was designed to identify some immunological parameters associated with *C. albicans* isolated from infected women with vaginitis in the central health laboratories, the strain were re-identified according to cultural, microscopic characters of yeast and using API candida test to confirm the diagnosis of C. *albicans*. The LD_{50} were $5x10^5$ CFU/ml, dose increased to become $6.25x10^5$ CFU/ml , and only 0.25 ml taken of *C. albicans* suspension and injected intra-peritoneal into BALB/C mice in the different groups according to type of immunological test required, there was good immune response cell mediated and humoral immune response. Cell mediated immune response showed high level using DTH- skin testreaction, there were differences of skin thickness at the injection site of sonicated Ag. MIF test also showed positive in infected group of mice, characterized by complete inhibition of migration of peritoneal cells in tissue culture plates using sonicated Ag with different concentrations. Humoral immune response showed high level of Abs following experimental infection of mice with *C. albicans* using PHA in which mean level of Abs titer reach to 64 and decreased gradually up to 7 at 30 days post infection to sure the high Ab titer we use Elisa which also give high level of Abs against mannoprotein in different groups of infected mice with *C. albicans*. Also other test IFAT used to confirm these results of Abs respone, this test (IFAT) considered the first test used for defection of *C. albicans* Abs in infected mice in Iraq. The result observed that high level of CMI in infected mice with *C. albicans* detected by DTH- skin test and MIF test and high level of humoral immunity in infected mice detected by PHA, ELISA and IFAT.

KEY WORDS: CMI and HI in mice infected with *C. albicans*.

INTRODUCTION

Relatively little is known about the pathogenesis of fungal infections or the mechanism of immunity to fungal diseases, but the infections often arises due to deficiency in host rather than because of any inherent pathogenic properties of the fungus [1]. The bases of resistance of candidiasis is complex and incompletely understood[2], it include the specific and non- specific immune responses[3].The nonspecific immune response include phagocytic cells and complement whereas, the specific immune response include the cell mediated immunity and humoral immunity[4]. Among the phagocytic cells the neutrophils and macrophages which kill the *C. albicans* by secretion of antimicrobial peptides and granulated enzymes and by releasing cellular mediated cytokines and chemokine's in addition to the using of oxidative mechanism with liberation of free oxygen radicals and non oxidative mechanism by releasing lysosomes peptides which has cytotoxic and lyse effect on foreign body [5,6]. Both humoral and cell mediated immunity are important in defense mechanism against Candida infection [7], they found that humoral immunity against the candidiasis mediated through production of IgM. IgA and IgG against mannan a main surface Ag on *C. albicans* similarly, this surface Ag are represented on the T cells and stimulate their proliferation with subsequent cytokines synthesis during the cell mediated immune response[8]. The importance of this zoonotic fungal disease, this study aimed to identify some aspects of cell mediated immunity by DTH – skin test and macrophage migration inhibition test and humoral immunity by passive haemagglutination test , enzyme linked immunosorbent assay and indirect fluorescent antibody technique test.

MATERIALS & METHODS

Candida albicans strain obtained from the central health laboratories, reidentified again to confirm the diagnosis depending on cultural characteristics microscopic examination, chlamydospores formation test, Germ tube test and API candida test (Biomerieux, France). White mice type (BALB/c) used in this experiment 8-10 weeks age, after the determination of LD_{50} of *C. albicans* in the mice which was found according to method (9) $5x10^5$ CFU/ml, we increased the dose to become $6.25x10^5$ CFU/ml , and only 0.25 ml of the dose used for infection and immunization of the mice .

Cell mediated immunitytests

1- Delayed type hypersensitivity (skin test)

This test used in twenty mice infected with *C. albicans* and ten normal mice injected with phosphate buffer saline (PBS), at the 15th days ofter infection and by using different concentrations of sonicated *C. albicans* Ag prepared according to[10] and PBS inoculated at the footpads intradermally in each mice then footpads thickness read by caliper after 24 and 48 hrs post inoculation of Ag or PBS[11]

2- Macrophage migration inhibition test (MIF)

This test was done in twelve mice (six) infected and (six)

non infected control – according to method[12] to detect migration area of intraperitoneal sensitized macrophages against *C. albicans* Ag at 14th day post infection of mice with *C. albicans*, the test done after collection of sensitized peritoneal macrophages washed in RPMI – 1640 and following their counting in neubauer chamber, the cells taken in capillary tubes and put in tissue culture plates containing RPMI – 1640 and different concentration of *C. albicans* Ag. The migration area for sensitized macrophages from infected animals and control were measured according to this following equation.

Migration inhibition index: migration cell area in absence of Ag: migration cell area in presence Ag

Humoral immunity test

1- Passive haemaglutination test

This test was done according to the method[12] to determine anti body titer against *C. albicans* Ag in 40 infected mice with *C. albicans* and 10 normal control at 3 days intervals for 30 days post infection and after making serial dilutions of sensitized sheep RBCS with *C. albicans Ag* against rabbit serum to determine the optimal Ag concentration give good agglutination of sensitized sheep RBCS, then a serial serum dilutions from infected mice and control were used against optimal Ag concentration and incubate at room temperature for 2 hrs. then read antibody titer for each mice .

2- Enzyme linked immunosorbent assay (Elisa) this test was used to detect anti – mannan antibodies in sera of 30 mice infected with *C. albicans* and in 10 non infected control according to company instructions (BioRad, France) Rabbit antimouse conjugate were used , it gives best reading after adding the substrate , colour changes of enzymatic reaction were measured by optical density (OD), by using micro Elisa reader, positive and negative control samples were read and compared with standard control of kit . optical density for positive samples give more than optical density for positive standard value of kit and this indicated that increase in the antibody titers in infected mice with *C. albicans*.

3- Indirect immunofluorescence test

This test used to standardize immunological analysis, it done according to company instructions (Euroimmuns , France) in 20 mice (15 infected with *C. albicans* and 5 non infected (control), at 15th day past infection using BIOCHIP slides and following preparation of BIOCHIP slides, the fluorescence area was read with fluorescent microscope, objective for organ sections 20X . for infected cell 20X, for cell substrate 40X. The results showed presence of antibodies against *Candida albicans* that appear as a fluorescent area clearly in reaction fields, similarly yeast cells clearly identified in the reaction field under fluorescent microscope

RESULTS

Cell mediated immunity tests

1- Delayed type hypersensitivity (skin test) the test was more evident in footpad of mice the swelling was more effective after 24 hrs and then slightly varnished after 48 hrs especially following inoculation the concentrated sonicated Ag in footpad of mice and the swelling was moderate to slight in skin thickess following inoculation of gradual diluted sonicated Ag (Table –1).

TABLE 1: skin reaction in mice immunized with *Candida albicans* and control

No. of animals used for each conc.	Antigen concentration 2mg/ml	(A)Before inoculation (mm)	(B) After 24 hrs of inoculation (mm)	(C) After 48 hrs of inoculation (mm)
4	Concentrated	1.25±0.007	2.78±0.02	2.61±0.02
3	Conc. 1/10	1.40±0.2	2.35±0.22	1.89±0.03
3	Conc. 1/100	1.17±0.01	1.62±0.03	1.38±0.03
10	Control group (PSB)	1.14±0.008	1.24±0.009	1.18±0.009

TABLE 2: Macrophages migration inhibition indices in infected and control groups of mice

Groups	Animal No.	Antigen concentrating µg/ml			PHA
		Concentrated (net)	1/10	1/100	0
Immunized group (6 mice)	1	0.116	0.417	0.583	0
	2	0.100	0.625	0.833	0
	3	0.125	0.442	0.667	0
	4	0.108	0.500	0.750	0
	5	0.142	0.617	0.791	0
	6	0.158	0.541	0.708	0
Mean		0.124	0.524	0.722	
±SE		0.009	0.038	0.04	
Control group (6 mice)	1	0.167	0.991	1.778	0
	2	0.226	1.000	2.254	0
	3	0.191	1.125	1.633	0
	4	0.225	1.108	2.417	0
	5	0.308	1.022	2.742	0
	6	0.375	1.117	2.191	0
Mean		0.248	1.06	2.21	
±SE		0.034	0.027	0.19	

2- Macrophages migrating inhibition test: The inhibition of peritoneal macrophages migration showed invitro at 24hrs using different concentrations of Ag, the concentrated Ag gave complete inhibition of migration whereas, the gradual dilution of Ag concentration gave gradual migration inhibition comparable to complete migration of macrophages in control PBS in vitro(Table 2).

Humoral immunity tests

1- Passive haemagglutination test (PHA) the results of agglutination in bottom of wells were good with optimal Ag concentration 1/10 that diluted with PBS, the mean of antibody titer for different groups of mice that arranged between 2.5 for group 1 and increased to 64 for group 5,6 and decreased to 7 for group 10 (Table 3).

TABLE 3: titration of antibodies in mice infected with *C. albicons* and control

No. animals used in each group	Animal group	Days after inoculation	Final antibody titration (mean)	SE
4	1	3	2.5	±0.5
4	2	6	7	±1.0
4	3	9	16	±5.6
4	4	12	32	±11.3
4	5	15	64	±22.6
4	6	18	64	±22.6
4	7	21	32	±11.3
4	8	24	28	±12
4	9	27	16	±5.6
4	10	30	7	±1.0
10	Control group	0	0	0

2- Enzyme linked immunosorbent assay

It is found that the serum samples with anti – mannan antibodies concentration less than 5 Au/ ml (C <5) are considered negative, and between 5-10 Au/ml (C5-10) are considered equivocal whereas, serum sample containing anti – mannan Abs concentration more than 10 Au/ml considered to be positive (Table – 4) , the results indicated that the group 5 was higher in concentration of anti – mannan antibodies during measuring optical density values in the different groups of infected and control mice.

TABLE 4: maximum, minimum and average of optical density values for ELISA according to the appointed term for each value

Group No.	Days after inoculation	Maximum OD	Minimum OD	Mean	SE
1	3	0.731	0.661	0.696	±0.03
2	6	0.973	0.817	0.877	±0.07
3	9	1.180	1.034	1.107	±0.07
4	12	1.353	1.134	1.241	±0.70
5	15	1.411	1.129	1.401	±0.01
6	18	1.327	1.390	1.266	±0.06
7	21	1.015	1.205	0.978	±0.03
8	24	0.796	0.941	0.734	±0.06
9	27	0.627	0.611	0.619	±0.008
10	30	0.621	0.597	0.609	±0.01

3- Indirect immunofluorescence antibody test (IFAT). All the serum samples collected at the 15th day post infection with *C. albicans* and the results showed presence of Abs against *C. albicans* and yeast appear as a fluorescent areas clearly in reaction field and the results were evaluated as weak , moderate and strong (table -5) and fig 1.

TABLE 5: results of immune florescent antibody titer of 15 sera of infected mice with *C. albicans*

No. of animal used	IgG Antibody Titer	Evaluation
2	10	Weak
4	32	Moderate
9	100	Strong
Total infected mice: 15		
Control animals: 5	0	Negative

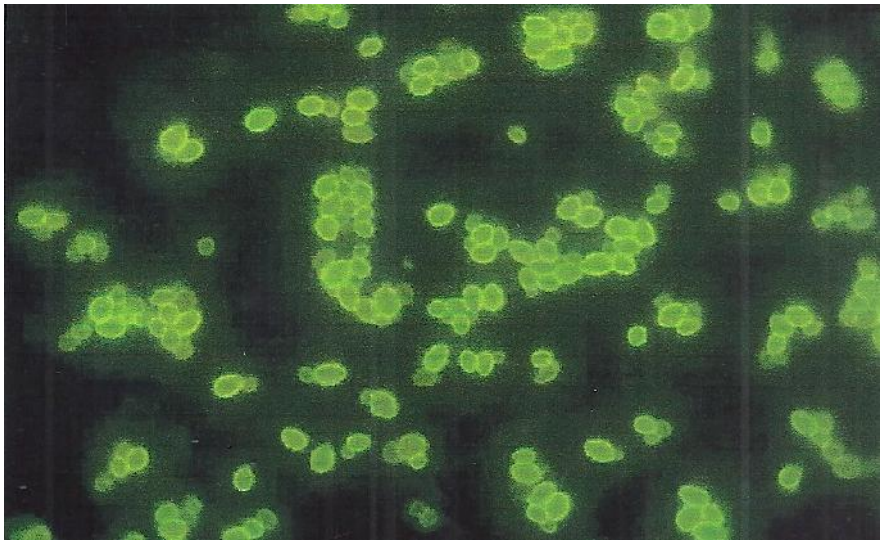

FIGURE 1: positive results of immune fluorescent antibody against *C. Albicans*

DISCUSSION

BALB/C mice used as a good animal model to human candidiasis during the experimental study with *C. albicans* due to its high sensitization for experimental intraperitioneal infection and production high level of cell and humoral mediated immune response which was evident in this study due to the persistence of *C. albicans* Ags in body for long time following intraperitoneal route of infection[13]. DTH skin tests and migration inhibition test used in this study and both test were well detected cell mediated immunity, DTH–skin test depend on lymphokines which lead to infiltration and aggregation of neutrophils, macrophages and lymphocytes in site of inoculation of sonicated Ag in addition to congestion and edema infiltrate the site of inoculation[13] in footpad of mice which is more evident in this study by measuring skin thickess. Lymphocytes showed high sensitivity to MIF test by induction of inhibition of migration of sensitized macrophages when exposed to sonicated Ag invitro in this study in contrast to the complete migration of that occur without presence of the sonicated Ag.

MIF is one of lymphokines produced by sensitized lymphocytes as a response to *C. albicans Ag*, in infected mice which lead to aggregation of macrophages and lymphocytes at the site of injection[14] and inhibit their migration due to MIF secretion which more evident invitro in this study . Also the inhibition of macrophage migration area was higher in infected than in immunized mice[15] which observed in this study and explained that due to viability of *C. albicons* Ags in living organism comparable to killed Ag used for immunization .

Humoralimmune response detected by passive haemagglutination test, ELISA and IFAT test and both of these tests gave an indication for high level of Ab response against *C. albicans* Ag in infected animals in this study. PHA gave a rise in Ab response after 15-18 days post infection and lowdown Ab response after 30 days the variation in Ab response was related to period of post infection and genetic differences in groups of mice and also the difference of immune response in mice within the same group[16] which was observed in this study and this variation in Ab response was related to hereditary factor or due immune insufficiency in the animal within one group .

Enzyme linked immune sorbent assay (Elisa) were used to detect anti– mannan Abs against *C. albicans* which consider one of important serological test used in epidemiological studies due to high sensitivity and specificity compared with other tests, this what was observed in this study and other studies[17,18] Viable *C. albicans* during the infection of mice used in this study give good immune response in mice and is better than killed organism used in other studies because killed organism demand more than single dose to stimulate immune response whereas , viable organism demand one dose to produce long life immunity because it contain all the Ags which some of it lost during killing of organism in addition to continuous multiplication of viable *C. albicans* and production of their toxins inside the body give long life immunity[19] . Indirect fluorescent antibody test used a new sensitive and specific test, it is easy, rapid and their performance indices are similar to Elisa.

This study revealed that *C. albicans* specific Abs detected in sera of infected mice as a fluorescent area clearly in the reaction fields and yeast cells clearly identified in every reaction field under fluorescent microscope , similar results described, by[20,21], in patient sera infected with candidiasis .

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