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Immunologic Study in Mice Immunized with Whole Cryptococcus Neoformans Fungusorganism

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Abstract A comparative study was conducted to focus on immune response level in different groups assist by some immunologic parameters in mice immunized with the whole cell killed yeast *Cryptococcus neoformans* vaccine. All experiments were carried out on a total number of120 male and female albino mice (Blab-c), mice were divided into equal three groups, the first group received distilled water as (control group), the second group was immunized with *killed yeast Cryptococcus neoformans* antigen and the third group was immunized with *a life yeast Cryptococcus neoformans* antigen. Vaccine efficiency was evaluated according to phagocytic activity percentage, delayed type hypersensitivity reaction test, anti-*C.neoformans* antibodies titer level and gamma globulin fraction percentage in immunized mice serum. All treatments were carried out on day one. Then the mice were scarified and tested at different periods: day 10, phagocytic activity percentage by Nitro Blue Tetrazolium test (NBT) was evaluated, at days 14 tests for delayed hypersensitivity type reaction of skin, and at day 21 and 28 performed the test for anti-*anti-C.neoformans antibodies titer* level in mice serum by indirect immunoflourescent assay and gamma globulin fraction percentage by Gel electrophoresis. Results revealed that third group recorded significantly higher values in their peripheral blood phagocytic activity by nitro blue tetrazolium test measured by ELISA reader and anti- *C.neoformans* antibodies titer level in mice serum at 21 and 28 days by indirect immunoflourescent assay and gamma globulin fraction as compared with control group. Second group results revealed significantly increased in phagocytic activity index of peripheral blood by nitro blue tetrazolium test and the anti- *C.neoformans* antibodies titer level assessed by indirect immunoflourescent test and gamma globulin fraction in mice serum at 21 and 28 days. In delayed-type hypersensitivity reaction, the index was significantly increased in group III vaccinated mice and in group II as comparison with control group, the best results was observed after 24 hours post-*C.neoformans* protein injection. Result concluded that a life C.neoformans antigen produce the best immune response in mice.

Keywords *a life and* heat killed *C.neoformans* immunization in mice

1. Introduction

Cryptococcus neoformans is an opportunistic encapsulated fungal pathogen that causes significant morbidity and mortality in immunocompromised individuals, including patients with AIDS and other immune defects [1]. *Cryptococcus neoformans* is a yeast-like fungus which causes life-threatening meningoencephalitis in 5 to 10% of patients with AIDS. Cryptococcosis is still a significant problem in Asia and Africa [1]. The infectious particle enters the host through the respiratory tract and reaches the lung, where the primary infection is contained in immunocompetent hosts. In contrast, the ability to control infection is severely hampered in immuno-suppressed subjects, resulting in dissemination of fungal cells to cause life threatening meningitis [2]. However, cryptococcosis is an emerging problem in other immunocompromised patient populations and remains a major cause of meningoencephalitis in the developing world [1, 3]. Therapeutic optionsare inadequate to eradicate the fungus. It is widely recognized that a complex interplay between cell-mediated and humoral immune response plays a pivotal role in the control of *C. neoformans* infection [4]. Despite the availability of antifungalagents that are active against *C. neoformans*, cryptococcosis is largely incurable in individuals with immune impairment because the organism cannot be completely eradicated [5]. Hence, modalities that enhance or provide components of the protective immune response to *C. neoformans* represent a rational approach to the management of cryptococcosis [6, 7]. As such, immune-based adjunctive antibody-based therapiesare promising modalities because of their ability to augment host defense mechanisms against *C. neoformans* [6, 8]. In animal models of infection, there is convincing evidence that administration of preformed antibody to the polysaccharide

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capsule can prolong survival and reduce organ tissue fungal burden [6]. The efficacy of some antibodies against *C. neoformans* has led to the development of a highly immunogenic polysaccharide-protein conjugate vaccine for the prevention of cryptococcal infection [9]. Cell-mediated immunity has been extensively implicated as an important defense mechanism against *C. neoformans* infection [1].

2. Material and Method

ACryptococcus neoformans isolate from poultry worker suffering from respiratory infection. Two strains of *C. neoformans* var. *neoformans* were used: a serotype A thinly encapsulated strain (CBS 6995 5 NIH 37; National Institutes of Health, Bethesda, Md.) and a capsular mutant (CBS 7698 5 NIH.

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 eight weeks. They were housed in bio-clean hoods at 20-25℃ with light, dark periods of 14:10 hours. They were fed standard pellets and water, their initial weight was 25 ± 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 (Blood agar, Sabourauds dextrose agar, Trypticase Soya agar, Trypticase Soya broth and yeast extract) was the products of Difco Company (U.S.A). The life yeast Cryptococcus neoformansvaccine prepared as described by [1] *and killed yeast vaccine prepared as described by* [1]. The NBT kit used in the study was the product of Sigma company (U.S.A). There were three groups in this experiment, which was designed to evaluate the immune response in mice vaccinated with Cryptococcus neoformans vaccine. 120 mice were a total number, 40mice 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 cervical region atday 1. **Group II:** mice were vaccinated with 0.2 ml of *killed yeast Cryptococcus neoformans* vaccine in a similar manner. **Group III: mice** were vaccinated with 0.2 ml of *a life Yeast Cryptococcus neoformans* vaccine in a similar manner; *all above treatment were done* at day 1. All mice were sacrificed on the day 8 to evaluated (phagocytic activity by NBT index measured by Elisa reader as suggested by [10], on the day 14 to evaluate (delayed-type hypersensitivity reaction) as suggested by [3] and in day 21 and 28 to assess anti-Cryptococcal neoformans antibody titer by indirect Fluorescent test in the sera of mice that were immunized with *a life and killed Cryptococcus neoformans* vaccine, the procedure of WHO 1997 [11] was adopted to determine such titer. Serum electrophoresis was carried out using a commercially available kit (Hellabio, Spain) for evaluated of gamma globulin fraction in immunized mice. The Hellabio Agarose Gels for protein electrophoresis are intended to be used for *in vitro* diagnosis, and they enable quantitative and qualitative estimation of proteins in serum and other biological materials.

3. Result and Dissection

The results of NBT index were given in table 1. Mice in groups II and III showed different significant increases in the NBT index test which represented the phagocytic activity% (65.99% and 77.45% respectively) as compared to group I (0%), which was injected with deionized distilled water (control group). The best NBT index was recorded in group III.

Table 1. Nitro blue tetrazolium (NBT) index in treated mice

Group	Mean ± SE	Phagocytic activity %
I	0.82 ± 0.30^{b}	0.00
II	2.53 ± 0.20^{a}	65.90
III	2.7 ± 0.14^{a}	77.45

*The different letters denoted that significant differences among the groups p ≤0.01

A life C.neoformans as a vaccine antigen. First, it is a T-cell-dependent type 1 antigen that does induce affinity maturation, class switching, or memory T cells.

Macrophage inflammatory protein-1a (MIP-1a) is a CC chemokine (CCL3) [14] that are required for T1 CMI to *C. neoformans*. MIP-1a is required for optimal recruitment of leukocytes in response to Cryptococci or cryptococcal Ags [15, 16]. We have shown that, in mice infected intratracheally with *C. neoformans*, administration of neutralizing anti-MIP-1a Abs on days 7–13 of infection decreases the recruitment of mononuclear phagocytes and neutrophils into the lung and ablates clearance of the pathogen [13]. Thus, MIP-1a plays an important role in the efferent (effector) phase CMI to *C. neoformans*, but its role in the afferent phase (development/polarization) of CMI. The early expression of MIP-1a could play a role in the development of Th1 immunity to *C. neoformans*. MIP-1a is induced during the early (innate) phase of the immune response to *C. neoformans* infection [12, 15]. In other systems, MIP-1a was shown topromote chemotaxis of Th1 but not Th2 cell lines in vitro [18]. MIP-1a can also drive TCR transgenic Th0 cells to differentiate toTh1 cells in vitro [17] and can decrease IL-4 production from cultured. Th2-type lymphocytes stimulated with schistosomal egg Ag [5]. Anti-MIP-1a Abs can inhibit the development of T1-mediated experimental autoimmune encephalitis [17] whereasMIP-1a knockout mice are protected from Coxsackie virus-inducedmyocarditis and influenza virus-induced pneumonitis [12]. Thus, MIP-1a has the potential to modulate the development of CMI. It remains to be determined whether MIP-1a can modulatethe development of Th1 vs Th2 CMI in vivo, during infections clearedin a T cell-dependent fashion, such as *C. neoformans*. [19, 21]. The sera of treated mice showed some variations.

Groups I showed no anti-*C. neoformans* antibodies at the start titer 1:16 after 21 days, while the other groups II and III showed a higher positive immunoflourescent reaction at the titer 1:128 and 1:256 respectively. These results are given in (Table 2). The sera of mice in groups I showed no anti-*C.Neoformans* antibodies at the start titer 1:16 after 28 days, the groups II and III showed a higher positive immunoflourescent anti-*C. Neoformans* reaction at the titer at the start titer 1:64 and 1:128 in groups after 28 days (Table 3). The results of gamma globulin fraction were given in table 4, in mice immunized with *a life and yeast Cryptococcus neoformans* vaccine, a significant increase in the percentage of gamma globulin fraction was observed as compared to control group, the best level was recorded in groups III as compared to the other two groups.

Classical mechanisms of antibody action include direct effects, such as toxin and viral neutralization and cooperative effects, primarily mediated through effector cells, such as enhancement of phagocytosis by opsonization, complement activation and fixation and antibody dependent cellular cytotoxicity (ADCC) [3]. In recent years additional mechanisms of antibody action against fungi have been revealed, including growth inhibition [14], inhibition of biofilm formation, inhibition of adherence, inhibition of germination and direct antifungal effects [12, 19]. For antibodies to *C. albicans* mannoproteins, *P. carinii* surface antigen and *C.neoformans* GXM, the Fc region and/or complement were essential for antibody efficacy [13, 15], whereas the activity of antibodies to other *C. albicans* mannoproteins (MP65) and HSP 90 is mediated by antibody fragments (Fabs) and does not require Fc regions [16].

An optimal protective response could be acquired by an early administration of preformed specific IgG1 that may potentiate Th1 generation by optimizing the antigen presentation process via phagocytosis enhancement, Fc_ R perturbation, and activation of the complement cascade [20 and 2] would facilitate the scavenging of *C. neoformans* and glucuronoxylomannan (GXM) from the host [13]. Although collaboration between the cellular and humoral responses is certainly determinant for the outcome of *C. neoformans* infection, we believe that a strong predictive value for the efficacy of the interconnection of the two branches of immune response is provided by the cytokine milieu and costimulatory molecule interaction. Opportunistic fungal pathogen *Cryptococcus neoformans* is widely accepted [6, 8, and]; however, the role of B lymphocytes remains controversial. Administration of polyclonal immune sera to mice has been reported to be either protective [16, 17] or ineffective [18, 19] against experimental infection with *C. neoformans*.

Table 2. Anti- Cryptococcal neoformans antibodies by indirect immunoflourescnt test in treated mice after 21 days

Groups	anti- Cryptococcal neoformans antibodies titer after 21 days					
	16	32	64	128	256	512
I	Negative	Negative	Negative	negative	Negative	Negative
II	Positive	Positive	positive	positive	Negative	negative
III	Positive	Positive	Positive	positive	Positive	Negative

Table 3. Anti- Cryptococcal neoformans antibodies by indirect immunoflourescnt test in treated mice after 28 days

GrG Groups	anti- Cryptococcal neoformans antibodies titer after 28 days				
	16	32	64	128	256
I	Negative	Negative	Negative	Negative	Negative
II	Positive	Positive	Positive	Negative	Negative
III	Positive	Positive	Positive	Positive	Negative

Table 4. Gamma Globulin Serum Fraction in immunized mice

Groups	After 21 days	After 28 days	P-value
I	12.15 ± 0.26^{e}	10.14 ± 0.24^{d}	**N. Non-significant**
II	25.03 ± 0.42^{b}	18.11 ± 0.16^{c}	**0.05**
III	33.23 ± 0.12^{a}	28.08 ± 0.14^{b}	**0.01**

Means with different letters in the same column differ significantly ($P < 0.01$)
P-value refers to differences between means in the same row.

Table 5. Skin test Index in treated mice

Groups	Zero time	After 24 hours	After 48 hours
I	1.77 ± 0.04^{c}	2.41 ± 0.04^{b}	2.05 ± 0.04^{c}
II	1.8 ± 0.03^{c}	3.46 ± 0.05^{a}	2.79 ± 0.05^{b}
III	1.79 ± 0.01^{c}	3.69 ± 0.06^{a}	2.94 ± 0.02^{b}

The results of skin test of treated mice after 24 and 48 hours were given (in table 5). The highest treatment efficiency after 24 hours was recorded in group III as compared to control group. The results obtained indicate the role of cellular immune response against the C. neoformans infection. The encapsulated yeast *Cryptococcus neoformans* is acquired via the respiratory tract, and the development of T cell-mediated immunity (CMI) is one of the crucial elements required for clearance of this pathogen [1, 2]. Both CD41 and CD81 T cells play an important role in this process [1-18]. Either a type 1 (Th1) or type 2 (Th2) response can develop following *C. neoformans* infection. A Th1 response promotes clearance of *C.neoformans*, and molecules important in Th1 development include IFN-g, IL-12, and the CCR2 [6]. In contrast, a Th2 response results in IL-4 and IL-5 production, chronic infection with lung eosinophilia, brain dissemination, and death of mice infected with *C. neoformans.* Thus, Th1 CMI in the lungs during *C.neoformans* infection is protective, whereas Th2 CMI isno protective [10-13]. B cell deficient by administration of antimouse Ig as newborns were indistinguishable from controls with respect to organ burdens of yeast, development of delayed-type hypersensitivity, and mortality after experimental intravenous yeast infection [5]. Experimental studies at this laboratory have attempted to model more closely the immunologic events attending a primary pulmonary exposure to *C. neoformans* that is followed by dissemination of yeast to the brain. Mice are vaccinated with a sub lethal pulmonary infection and allowed to develop specific Anticryptococcal resistance. Six to eight weeks after vaccination, they are challenged intravenously with yeast. Vaccinated mice express an acquired resistance to *C. neoformans*. Moreover, transfer of T cells from vaccinated donors into lymph deficient SCID mice protects the latter against intravenous infection with *C. neoformans* [1, 7]. A Th1-type response has been shown to be important in containing *C. neoformans* infection at lung level. It is known that the generation of a Th1 or Th2response depends on many factors, including the cytokine milieu, the antigen concentration and the type of antigen-presenting cells (APC). In addition, Th1 and Th2 responses may coexist in the host until one or the other emerges. A switch from a Th1 to a Th2 response has been demonstrated, while a switch from Th2 to Th1 appears improbable. Th1 generation has been associated with high levels of interferon (IFN and IgG2a whereas a Th2 response correlates with interleukin4 (IL-4), IL-10 and IgG1 production [13].

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