ACTIVE IMMUNISATION OF MICE AGAINST MUSCLE DAMAGE MEDIATED BY *CANDIDA ALBICANS*

DONNA L. OBLACK AND I. A. HOLDER*

Department of Microbiology, University of Cincinnati College of Medicine and

**The Shriners Burns Institute, Cincinnati, Ohio, USA*

USING an experimental murine model to examine early events in the pathogenesis of infection with *Candida albicans*, we described the appearance of a muscle lesion in moribund animals 12 h after intravenous injection of *C. albicans* (Oblack, Schwarz and Holder, 1978). Severe interstitial myocarditis was demonstrated histologically and markedly elevated levels of the muscle enzyme, creatine phosphokinase (CPK), were found in the sera of these animals. The tissue specificity of the CPK enzyme (Hess *et al.*, 1964; Dawson and Fine, 1967) enable us to investigate the effect of *C. albicans* on the host with an enzyme assay as an indicator of in-vivo muscle damage.

The possible significance of muscle damage in the pathogenesis of *Candida* infection was realised when it was discovered that the injection of virulent strains of *C. albicans*, *C. stellatoidea* and *C. tropicalis* produced elevated serum CPK levels, whereas avirulent isolates of seven species of *Candida* were without effect (Oblack, Schwarz and Holder, 1979). In addition, the data suggested that damage to host tissues was restricted to sites where large numbers of *Candida* were present and that metabolic activity on the part of the infecting organism was required (Oblack *et al.*, 1979).

There are few reports on the successful immunisation of animals with *Candida*. Increased survival of animals actively immunised with whole or disrupted cells of *C. albicans* (Mourad and Friedman, 1961; Soles, Lim and Louria, 1966; Giger, Domer and McQuitty, 1978) or *C. albicans* ribosomal preparations (Saunders, Solotorovsky and Tewari, 1975) have been documented. The passive transfer of immune sera has also extended the survival of animals after systemic challenge with *C. albicans* (Mourad and Friedman, 1968; Al-Doory, 1970).

The present paper reports investigations on active immunisation of mice with viable, nonviable, and disrupted cells of *C. albicans* and the effects of these treatments on survival and muscle damage after subsequent intravenous challenge with *C. albicans*.

MATERIALS AND METHODS

Animals. Female, outbred Swiss mice, strain CD-1 (Charles River) weighing 22–24 g were used in all experiments.

Organisms. *Candida albicans* strain CA2 was obtained from the Mycology Laboratory of the Cincinnati General Hospital. The LD₅₀ for this yeast was found to be 3×10^5 colony-forming units (c.f.u.) (Oblack *et al.*, 1979). *Candida albicans* strain CA526 was kindly provided by Dr. H. F. Hasenclever, Hamilton, Montana. Because intravenous (i.v.) injection of mice with 10^7 c.f.u. of this organism produced no mortalities in 21 days (Oblack *et al.* 1979) this strain was considered avirulent.

Active immunisation of mice. Vaccines of viable *C. albicans* strain CA2 or cells disrupted with a Mickle Disintegrator (Brinkmann Instruments, Des Plaines, Illinois) were prepared by the method of Mourad and Friedman (1961). In addition, a third vaccine of heat-killed cells (100°C, 15 min.) was similarly prepared. Animals were immunised with 0.2 ml of the respective vaccines, according to the schedule of Mourad and Friedman (1961) and were challenged on the

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Requests for reprints should be sent to Dr I. A. Holder, The Shriners Burns Institute, 202 Goodman Street, Cincinnati, Ohio 45219, USA.

day after the last immunisation with 1 LD50 *C. albicans* strain CA2. For comparative purposes, a disrupted cell vaccine from the avirulent *C. albicans* strain CA526 was used to immunise animals before challenge with *C. albicans* strain CA2 in one experiment.

Survival of immunised mice. Groups of 14–15 mice immunised with the respective *Candida* vaccines and a control group of animals given injections of saline were examined daily for 28 days after i.v. injection of *C. albicans*, and the number of deaths was recorded.

Enzyme assay. Animals were anaesthetised with methoxyflurane (Metofane, Pitman-Moore, Washinton Crossing, New Jersey) and bled by cardiac puncture. Individual sera were tested within 3 h for CPK activity as previously described (Oblack *et al.*, 1979) or frozen at -20°C and tested within 48 h.

Counts of C. albicans in cardiac tissue. Hearts were aseptically excised from animals 12 h after the i.v. injection of *C. albicans* and the number of c.f.u. was determined by the method of Saymen *et al.* (1972). Data were expressed as the $\log_{10}$ of the number of c.f.u. per g. (wet weight) of tissue.

RESULTS

Muscle damage in immunised mice

Groups of mice were immunised with vaccines of viable, heat-killed or disrupted cells of *C. albicans* before challenge with 1 LD50 of the homologous organism. Previous experiments had established that the serum CPK levels of uninfected mice ranged from 100 to 400 IU/litre (Oblack *et al.*, 1979). Twelve hours after challenge with 1 LD50 *C. albicans*, serum CPK levels of the control mice were over 1400 IU/litre (table I). However, elevated serum CPK levels were not found in animals immunised with *Candida* vaccines of either viable, nonviable, or disrupted cells. It is noteworthy that animals immunised with a vaccine of disrupted cells from an avirulent strain of *C. albicans* were not protected against the muscle-damaging effects of *C. albicans*.

TABLE I

Serum creatine phosphokinase levels of immunised mice 12 h after intravenous challenge with 1 LD50 of Candida albicans

Immunogen	Serum creatine phosphokinase (IU/litre)
<i>C. albicans</i> , disrupted	274 $\pm$ 81*
<i>C. albicans</i> , viable	563 $\pm$ 102
<i>C. albicans</i> , nonviable	378 $\pm$ 66
<i>C. albicans</i> (avirulent), disrupted	1408 $\pm$ 179
Saline	1436 $\pm$ 172

* Data are expressed as the mean $\pm$ the standard error of the mean (SEM) of five animals.

Numbers of C. albicans in cardiac tissue

The amount of the CPK enzyme released into serum after muscle injury was shown to be directly related to the challenge dose of *C. albicans* (Oblack, *et al.*, 1979). To determine whether protection against muscle damage in the immunised mice was related to a reduced number of organisms in the tissue, the c.f.u. of *C. albicans* were enumerated in samples of cardiac tissue collected 12 h after i.v. injection of the challenge dose. These data are presented in table II. Twelve hours after *Candida* challenge, the number of c.f.u. of *C. albicans* found in the cardiac tissue of mice immunised with *Candida* vaccines was similar to the number found in the control animals.

Survival of immunised mice after challenge

The previous data (table I) which indicated that immunisation with the *Candida* vaccines

TABLE II

Numbers of yeast cells recovered from the hearts of immunised mice 12 h after intravenous challenge with 1 LD50 of C. albicans

Immunogen	Number (log ₁₀) of colony-forming units of <i>Candida</i> per g of heart
<i>C. albicans</i> , disrupted	4.19 ± 0.04*
<i>C. albicans</i> , viable	3.96 ± 0.07
<i>C. albicans</i> , nonviable	3.98 ± 0.04
Saline	4.26 ± 0.09

* Data are expressed as the mean ± SEM of five animals.

protected mice against early events during *Candida* infection prompted an examination of the effects of immunisation on survival of mice over an extended interval. After intravenous challenge with 1 LD50 of *C. albicans*, the control mice began dying in the second post-challenge week and only 20% of the animals survived for 4 weeks (table III). However, immunisation with the *Candida* vaccines provided substantial protection as evidenced by the 86–100% survival of immunised mice for 28 days.

TABLE III

Survival of immunised mice after intravenous challenge with 1 LD50 of Candida albicans

Immunogen	Percent* survival at week			
	1	2	3	4
<i>C. albicans</i> , disrupted	100	100	100	100
<i>C. albicans</i> , viable	100	100	93	86
<i>C. albicans</i> , nonviable	100	100	87	87
Saline	100	73	47	20

* Of 14–15 mice in each group.

DISCUSSION

Successful (Mourad and Friedman, 1961; Hasenclever and Mitchell, 1963; Soles *et al.*, 1966; Giger *et al.*, 1978) and unsuccessful (Hurd and Drake, 1953; Winner, 1956) attempts at active immunisation of animals against *C. albicans* infections have been reported. In these investigations, protection was assessed by the survival of immunised animals after varying challenge doses of *C. albicans*. It was our intention to examine the effects of immunisation upon an event that occurs early in the pathogenesis of *Candida* infection, that is, a muscle lesion as evidenced by elevated levels of the CPK enzyme in serum. The results (table I) indicate that animals could be protected against muscle injury by active immunisation with vaccines prepared from viable, nonviable, or disrupted *C. albicans*. It was further shown that, in addition to protection against early tissue injury, immunisation was also effective in extending survival after *Candida* challenge (table III); these data confirm the previous observation made by Mourad and Friedman (1961).

The nature of the immunity that we observed in these experiments remains to be elucidated. The role of humoral immunity in resistance to systemic *C. albicans* infection has been implicated by the transfer of immunity to recipients by immune sera (Mourad and Friedman, 1968; Al-Doory, 1970). In addition, the passive transfer of immune sera also protected mice against a localised thigh lesion (Pearsall, Adams and Bunni, 1978). On the other hand, cellular immunity was not required for defence against systemic candidiasis (Rogers, Balish and Manning, 1976) or localised candidiasis (Pearsall *et al.*, 1978). An increase in humoral immunity associated with opsonising antibodies, and enhanced fungicidal activity of the host defence cells through an increase in cellular immunity would have been reflected in a decrease in the numbers of *Candida*

cells in the tissues. However, protection against muscle injury occurred in the absence of reduced tissue counts of *Candida* (table II). These data imply the possibility of humoral immunity directed against a muscle-damaging factor elaborated by *C. albicans*. While it is still unknown whether immunity occurred through the same mechanism in animals immunised with different *Candida* vaccines, if a common mechanism is subsequently shown to exist, the present data suggest that an effective endogenous immunogen is present in virulent *C. albicans*, is absent from avirulent *C. albicans*, and is heat stable.

SUMMARY

The effect of active immunisation on muscle damage after systemic infection with *Candida albicans* was examined in a murine model. Mice were successfully immunised against acute muscle damage by vaccines of viable, nonviable, or disrupted *C. albicans*. In addition, markedly enhanced survival of immunised mice was demonstrated over a 4-week interval. There was protection against muscle damage in immunised mice without a reduction in the number of organisms present in cardiac tissue. The data indirectly implicate the role of humoral immunity in the protection afforded by immunisation.

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