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IMMUNOLOGIC IMPAIRMENT IN MICE TREATED INTRAVENOUSLY WITH KILLED COCCIDIOIDES IMMITIS SPHERULES: SUPPRESSED RESPONSE TO INTRAMUSCULAR DOSES[1]

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In earlier studies (1, 2), formalin-killed spherule vaccines of *Coccidioides immitis* were found to be very efficacious in mice and monkeys when administered by the subcutaneous and intramuscular (i.m.) routes. We recently investigated intravenous (i.v.) vaccination because Larson *et al.* (3) observed that it was strikingly effective with particulate tubercular antigens (in oil) and also because it had been used on occasion to test other fungal vaccines.

In the present study with *C. immitis*, i.v. administered spherules not only immunized mice poorly, but prevented the development of strong immunity from i.m. doses given up to 35 days earlier. Animals so treated succumbed to challenge with approximately 5 LD_{50}; in contrast, those vaccinated by the i.m. route alone survived up to 200 LD_{50}.

The attributes of this deficient response were of concern for several reasons: with the possible exception of *Cryptococcus* (4), the reaction had not generally been reported for fungal antigens and, inasmuch as it was demonstrable primarily by i.v. injection, the possibility was raised that a similar reaction might have been a factor in poor immunity to numerous other fungi following i.v. vaccination and/or challenge (5). Finally, impaired immunity was markedly pronounced in our model which differed from others demonstrating unresponsiveness in that the challenge was administered intranasally, producing primarily pulmonary disease (6), and that the particulate vaccines producing impaired immunity were comprised of structures degraded very slowly *in vivo* (7).

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Impaired immunity to *Coccidioides* was mediated by immunospecific substances, but heat-killed preparations of reduced immunogenicity were still effective. With formalin-killed preparations, there was no adverse effect on existing immunity when the i.v. dose followed the i.m. dose by 1½ to 3 months; i.v. treatment at 3 months actually potentiated immunity. Although the mechanisms of the deficient response are obscure, its major features are similar to those described for immunologic unresponsiveness induced by soluble bacterial and other antigens (8, 9).

METHODS

The Silveira strain of *C. immitis* was used throughout. Mice were vaccinated with spherules or endospores cultured and formalin-killed as described earlier (1, 10). The animals were later challenged intranasally (11) with the arthrospore phase of the fungus. The vaccine and challenge doses, and the intervals between them, were varied and are detailed in conjunction with individual experiments. Census of fungal numbers in the lungs of mice was made as described earlier (6).

Spherule walls were isolated and purified from mechanically disrupted, formalin-killed spherules as described by Kong *et al.* (12) with the modification that a Braun Model MSK homogenizer (Brownwill Scientific, Rochester, N. Y.) was used; the soluble protoplasmic material was separated, filtered (12) and investigated for its influence on immunity and suppression of immunity. Similarly, the effect of i.v. injection of intact *Cryptococcus neoformans* or *Saccharomyces cerevisiae* on both responses was studied with formalin-killed organisms. *Cryptococcus* was grown in heart infusion broth (Difco) for 48 hr with shaking at 35°C and commercial baker's yeast cake was the source of *Saccharomyces*. In one experiment non-formalinized, autoclaved (121°C, 18 hr) spherules

TABLE I

Mortality in mice challenged intranasally with Coccidioides immitis after vaccination by different routes with formalin-killed spherules

Challenging Dose (No. Arthrospores)	Dead/Total (30 Days Postchallenge)					
	Vaccinated[a]					
	Control	2.4 mg i.m.[b]	1.2 mg i.m.	2.4 mg i.v.[b]	1.2 mg i.v.	1.2 mg i.m. + 1.2 mg i.v.
40	2/9					
60	7/10					
110	7/10					
275	10/10	0/10	0/10	1/3	6/10	3/10
480		0/10	0/10	2/5	9/10	8/10
2040		1/10	1/10	5/5	10/10	7/9
4200		2/9	1/10	—[c]	10/10	6/10

[a] With ⅓ indicated dosage on days 0, 7 and 14; challenged on day 42.

[b] I.m. = intramuscularly; i.v. = intravenously.

[c] No data.

were also studied for the effects mentioned above.

All preparations for i.v. use were sedimented repeatedly by centrifugation and resuspended in pyrogen-free saline (Cutter Laboratories, Berkeley, Calif.); the final suspensions were adjusted to permit later specified doses to be given in a volume of 0.5 ml. When the suspensions were to be used over a period of several days or weeks, 1:10,000 Merthiolate (final concentration) was added.

Female NAMRU albino mice, penbred in our laboratory, were employed in these experiments. The animals were 6 to 12 weeks old at the inception of different experiments, but in any one experiment their age varied no more than 2 weeks.

RESULTS

The weak immunologic response of mice vaccinated i.v. with killed spherules and the markedly impaired response in mice given vaccine by both the i.v. and i.m. routes are shown in Table I.

TABLE II

The influence of spherule dose given intravenously (i.v.) on immunity to Coccidioides immitis induced by intramuscular (i.m.) injection of vaccine

Experiment	Mg Spherules per Dose[a] and Route (s)	% Mortality[b] (30 Days Postchallenge)								
		Challenging dose (no. arthrospores):								
		24	43	89	250	350	465	740	1150–1220	2400–3050
1	None					100		100	100	100
	0.4 i.v.					94		94	89	100
	0.4 i.m.					10		0	22	50
	0.8 i.m.					11		0	0	50
	0.4 i.m. + 0.04 i.v.					39		45	70	47
	0.4 i.m. + 0.2 i.v.					61		70	65	79
	0.4 i.m. + 0.4 i.v.					44		61	83	82
	0.4 i.m. + 0.6 i.v.					38		31	50	66
2	None	44	22	78	90					
	0.4 i.v.				89		100		100	100
	0.4 i.m.				0		0		50	30
	0.8 i.m.				0		22		0	14
	0.4 i.m. + 0.012 i.v.				8		25		9	80
	0.4 i.m. + 0.025 i.v.				8		8		55	40
	0.4 i.m. + 0.05 i.v.				8		8		45	67
	0.4 i.m. + 0.1 i.v.				33		42		42	33
	0.4 i.m. + 0.2 i.v.				33		83		75	80
	0.4 i.m. + 0.4 i.v.				33		67		58	75

[a] Indicated doses given on days 0, 7 and 14; mice challenged intranasally on day 49 (Exp. 1) or on day 46 (Exp. 2).

[b] From 7 to 20 mice per group; mice, treated by both i.m. + i.v. routes, were composed of 10 to 20 per group.

Whereas 60 or more arthrospores administered intranasally were lethal to 70 to 100% of nonvaccinated mice, animals that had been immunized i.m. with three doses of spherules, totaling 1.2 or 2.4 mg, were well protected against challenge doses of up to at least 4200 arthrospores. Neither vaccine dosage was very effective when given i.v.; immunity so induced was sufficient only to reduce mortality rates in mice challenged with 480 or fewer arthrospores. This was also the case in mice vaccinated i.m. with 1.2 mg of vaccine if they received concurrently an additional 1.2 mg i.v., notwithstanding the efficacy of 1.2 or 2.4 mg when given by the i.m. route alone.

The impaired response of i.m. vaccinated mice also treated i.v. with the antigenic preparation is again evident in Table II; those given i.v. as little as 120 to 150 μg of intact spherules were poorly immunized. These dosages contained insufficient antigen to confer immunity by the i.m. route (10). Nor was immunity to 150 to 630 arthrospores induced by any i.v. dose of spherules between 0.01 μg and 1.0 mg (Table III). Conversely, suppressed immunity did not follow i.m. injection of 20 mg of spherules or 4.5 mg of spherule walls (Table IV), the major locus of immunogens (12). The immunogen content of 4.5 mg of walls corresponded approximately to that of 18 to 22 mg of intact spherules.

Spherule walls, given by different routes, induced the same responses in mice as intact organisms: They were highly immunogenic by the i.m, route, poorly immunogenic by the i.v. route and when administered i.v., impaired the response to i.m. doses (Table V). We were therefore interested in determining if the soluble protoplasmic moiety of spherules, which is poorly immunogenic except

TABLE III

Influence of spherule vaccine given intravenously on mortality after challenge with Coccidioides immitis

Spherule Dose	% Mortality[a] (30 Days Postchallenge)		
	Challenging dose (no. arthrospores):		
	60	150	630
mg			
0.0	50	80	
0.00001		60	90
0.0001		95	100
0.001		100	70
0.01		63	90
0.1		100	90
1.0		92	100

[a] From 10 to 15 mice per group; challenged intranasally 40 days after vaccination.

TABLE IV

Influence on immunity to Coccidioides immitis of dose of spherule walls and intact spherules given intramuscularly (i.m.)

Preparation	Dose	% Mortality[a] (30 Days Postchallenge)									
		Challenging dose (no. arthrospores):									
		30	40–50	75–100	175	455	800–1,000	4,800	8,000–10,000	15,000	55,000
	mg										
Spherule walls	0	30	90	70							
	0.0045			70		100	100		100		100
	0.045			10		30	11		50		60
	0.45			10		30	30		20		50
	4.5			0		0	0		0		30
Intact spherules	0		40	100	70		90				
	2.0						0	10	20	40	
	6.0						0	30	20	29	
	10.0						0	20	20	40	
	20.0						0	60	70	20	

[a] From 7 to 11 mice per group. Vaccinated with ⅓ indicated dosage on days 0, 7 and 14 (spherule walls) or ¼ indicated dosage on days 0, 7, 14 and 20 (intact organisms); challenged intranasally 22 or 29 days respectively later.

TABLE V

Influence on immunity of Coccidioides immitis spherule fractions given intramuscularly (i.m.) and/or intravenously (i.v.)

Challenge Dose (No. Arthrospores)	Dead/Total (33 Days Postchallenge)							
	Treatment:[a]							
	None	Freund's adjuvant[b] i.m.	0.3 mg walls i.m.	0.5 mg soluble protoplasmic fraction i.m.	0.5 mg soluble protoplasmic fraction + Freund's adjuvant i.m.	0.3 mg walls i.v.	0.3 mg walls i.m. + 0.3 mg walls i.v.	0.3 mg walls i.m. plus 0.5 mg soluble protoplasmic fraction i.v.
20	1/10	3/10						
40	5/10	5/10		2/10	0/10			
80	7/11	7/10		4/10	0/10	6/10	0/10	0/10
130			0/10	6/10	0/10	9/10	5/9	0/10
475			1/10			7/8	6/8	3/9
1150			3/10					

[a] With ⅓ indicated dosage on days 0, 7, 14; challenged intranasally on day 44.
[b] Freund's complete adjuvant, 1:1 with either saline or soluble protoplasmic fraction.

TABLE VI

Influence on immunity to Coccidioides immitis of autoclaved spherules given intravenously (i.v.) to mice vaccinated intramuscularly (i.m.)

Treatment[a]	Dead/Total (30 Days Postchallenge)		
	Challenging dose (no. arthrospores):		
	60	120	1200
None	3/10	8/10	
1.0 mg spherules i.m.		3/10	5/10
2.0 mg spherules i.m.		0/10	5/10
1.0 mg autoclaved spherules i.m.		7/10	8/9
2.0 mg autoclaved spherules i.m.		4/10	10/10
1.0 mg autoclaved spherules i.v.		4/5	6/6
1.0 mg spherules i.m. plus 1.0 mg spherules i.v.		2/8	8/8
1.0 mg spherules i.m. plus 1.0 mg autoclaved spherules i.v.		2/6	6/6

[a] One dose as shown on day 0; mice challenged intranasally on day 30. Spherules were killed by formalin, except those designated "autoclaved" which were killed by heating at 121°C for 18 hr.

when emulsified with Freund's complete adjuvant (12), similarly impaired the response of mice. The findings in Table V show that the weakly immunogenic soluble fraction, given i.v., suppressed only slightly the development of i.m. induced immunity. The relationship between the capacities of a preparation to confer immunity by the i.m. route and to suppress it by the i.v. route was not, however, an uncomplicated function of immunogenicity. The i.v. injection of autoclaved spherules of markedly reduced immunogenicity still suppressed immunity development in mice vaccinated i.m. with formalinized spherules (Table VI).

Formalinized endospores, somewhat less immunogenic than formalinized spherules (10, 13) but more immunogenic than heated spherules (Table VI), also interfered with immunity development when given i.v. (Table VII, Exp. 1) to either spherule- or endospore-vaccinated (i.m.) mice. Correspondingly, i.v. administered spherules had a similar effect on endospore-vaccinated mice. Both morphologic phases showed suppressive activity by the intraperitoneal route (Table VII, Exp. 2), but this was less pronounced than that induced i.v. Since the spherules ranged from 15 to 20 μ in diameter and endospores from 1 to 3 μ, suppressed immunity induced by either route apparently was not attributable to the large size of injected spherules. This consideration is noted

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