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Migration Inhibition of Mouse Macrophages by *Brucella* Antigens

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Mice are suitable donors of peritoneal macrophages for the capillary migration inhibition test, provided a period of preincubation with antigen is used

A correlation has been shown between the development of cellular immunity and the presence of delayed-type hypersensitivity (3, 8, 9). A popular in vitro test for delayed-type hypersensitivity is macrophage migration inhibition (2, 4, 6, 7; J. R. David, Fed. Proc. **27**:6-12). This technique has been used effectively in guinea pig and human systems but has had only limited success, except with pooled alveolar macrophages, in the mouse system (1, 10, 11). By employing only peritoneal exudate cells in our capillary migration tests, we have demonstrated delayed-type hypersensitivity in a genetically heterogeneous population of 6- to 8-week old female (Swiss-Webster, code ROR) mice infected with *Brucella abortus*.

Intravenous injections of 10^6 *B. abortus* (strain 19, Weybridge) cells were used to infect mice 20 days before macrophage harvest. The mice received 2.5 ml of sterile, light mineral oil (Lubinol, Purepac, Elizabeth, N.J.) intraperitoneally 4 days before the peritoneal exudates were collected. Infected mice exhibited delayed-type hypersensitivity (Table 1) as measured by the footpad test (8) with a crude sonic extract of *B. abortus* 2308 (5) as the antigen.

The mice were killed by cervical dislocation, the peritoneal walls were aseptically exposed, and their peritoneal cavities were injected with 6 ml of cold, sterile Hanks balanced salt solution. After brief agitation, the exudates were harvested by using disposable syringes. Syringes containing the oil-exudate suspensions were placed vertically in chipped ice, and the oil was allowed to separate from the lower phase containing the suspended cells. The cell suspensions were transferred to precooled siliconized sterile conical glass tubes and centrifuged at 165 × *g* for 10 min at 4 C. The cells were washed by suspending and sedimenting in 10 ml of cold sterile HBSS for 3 to 5 min at 165 × *g*. Each pellet, consisting of approximately 10^6 cells, was suspended in 1 ml of medium (85% NCTC 135, 15% normal guinea pig serum, 100 μg of streptomycin/ml, and 100 units of penicillin/ml) with or without 100 μg of *Brucella* antigen per ml [the crude sonic extract

TABLE 1. *Footpad tests for delayed hypersensitivity in uninfected mice and mice infected with 10^6 cells of Brucella abortus strain 19 Weybridge 20 days prior to testing*[a]

Mice tested	Increase[b] in footpad thickness (mm)	Relative increase in thickness (%)[c]
Uninfected (normal)		
1	0.80	25
2	0.50	18
3	0.70	30
4	0.30	11
Infected		
1	2.20	96
2	1.85	77
3	2.20	88
4	1.75	80
5	1.85	75

[a] Results obtained 24 hr after injection of 0.05 ml of *Brucella* antigens (10 mg/ml) suspended in Hanks balanced salt solution containing 5% bovine serum albumin.

[b] Increase in thickness of injected right rear footpad over noninjected left rear footpad.

[c] Group averages dropped to 11% for uninfected and 56% for infected when concentration of eliciting antigens was reduced to 2 mg/ml. The average increase for the uninfected group was 21%. For the infected group, the average increase was 83%.

described previously (5)]. When antigen was incorporated into the suspension, 0.5 ml was transferred to a sterile plastic tube (Falcon no. 2001) and incubated for 1.5 hr at 37 C in an atmosphere of 5% carbon dioxide and 95% air to allow a period of preincubation with the antigen before the capillary test. The remaining 0.5 ml was stored on ice, i.e., not preincubated with antigen, to insure an adequate supply of macrophages for the migration test. (This step may become unnecessary, but presently it is difficult to obtain complete recovery of adhering macrophages after the preincubation step.) Finally, the 0.5-ml suspensions were combined, centrifuged at 165 × *g* for 5 min, and suspended in a 1:10 (v/v) cell-to-medium ratio. The concentrated cell suspension was distributed evenly between two washed sterile capillary tubes, which subsequently were heat-sealed at one end and centrifuged at 270 × *g* for 3 min. The tubes were cut

TABLE 2. *Effect of preincubation and culture with antigen on the migration of macrophages from noninfected and infected mice*

Noninfected donor mice		Infected donor mice			
Wt of migration area (mg)[a]	Group calculation[b]	Wt of migration area (mg)			Group calculation
17.4		13.2	7.6	14.1	
28.0	$\bar{x}$ = 24.5	2.8	8.4	4.7	$\bar{x}$ = 8.7
16.0	$\hat{\sigma}$ = 8.09	2.4	13.7	11.2	$\hat{\sigma}$ = 5.9
35.7	$\hat{\sigma}_{\bar{x}}$ = 3.6	4.5	14.9	10.8	$\hat{\sigma}_{\bar{x}}$ = 1.4
23.9		5.8	23.7	10.7	
		3.6	0.1	4.2	

[a] Each value represents a different mouse.

[b] Symbols and calculation: $\bar{x}$ = arithmetic mean; $\hat{\sigma}$ = sample variance $\sqrt{\frac{\Sigma x^2}{N-1}}$; $\hat{\sigma}_{\bar{x}}$ = standard error $\frac{\hat{\sigma}}{\sqrt{N}}$ (N = number of donor mice).

at about 0.1 mm below the cell-fluid interface and anchored in plastic tissue culture dishes (Falcon, no. 3001) by sterile silicone stopcock grease. The peritoneal exudate from each mouse was handled separately to avoid pooling. The capillary tubes were incubated for 18 hr at 37 C in 2 ml of medium in an atmosphere of 5% carbon dioxide and 95% air, with or without antigen. Migration areas were magnified ×30 and projected onto paper by using a Unitron microscope (PH-BMIC) with inverted objectives. The migration outlines were traced, cut out, and weighed on an analytical balance.

Macrophages from infected mice, preincubated and cultured in the presence of *B. abortus* antigens, had a migration area only one-half to one-third that of control (uninfected) mice (Table 2). Macrophages from infected mice were not inhibited and migrated as well as controls when the preincubation step was not employed. Exudates from *B. abortus*-infected mice migrated two to three times farther when preincubated and cultured without antigen than when preincubated and cultured with antigen (Table 3).

The reason the preincubation period is required is not completely clear. Experiments are in progress to determine the nature and effects of products released during the early and late phases of this period.

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TABLE 3. *Effect of preincubation and culture with and without Brucella antigens on the migration of macrophages from infected mice*[a]

Culture medium without antigens			Culture medium with antigens				
Wt of migration area (mg)		Group calculation	Wt of migration area (mg)				Group calculation
9.2	24.5	$\bar{x}$ = 16.5	4.0	2.4	7.1	10.8	$\bar{x}$ = 6.94
4.3	16.8	$\hat{\sigma}$ = 8.9	6.9	6.2	1.6	18.8	$\hat{\sigma}$ = 4.2
22.6	11.0	$\hat{\sigma}_{\bar{x}}$ = 3.4	9.6	6.2	7.6	9.2	$\hat{\sigma}_{\bar{x}}$ = 1.1
27.6			3.4	5.0	9.2	3.0	

[a] See footnotes to Table 2 for explanation of figures and symbols.

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