The Effects of Zymosan Upon the Mast Cell Population of the Peritoneal Fluid[1] (36725)

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Mast cells are not generally acknowledged to be phagocytic. It was, therefore, of interest to discover that mast cells would engulf zymosan (yeast cell walls) following the intraperitoneal injection of these particles into rats (1). Electron micrographs substantiated the intracellular location of zymosan particles and demonstrated an increase in the numbers and in the size of microvilli on the mast cell surface closest to the zymosan particles. Since this time, a wide variety of particulates which differ in size, chemical nature, and surface charge also have been shown to be taken up by rat mast cells (2). In order to learn more about this phenomenon, two lines of observation were followed: (a) quantitative studies were made of the absolute numbers of mast cells in the peritoneal fluid of zymosan-injected rats and (b) observations were made on the ability of several species other than rats to phagocytose zymosan.

Materials and Methods. Male rats of the Sprague-Dawley-Holtzman strain (Charles River Breeding Laboratories; North Wilmington, MA) weighing 90 to 110 g were studied. Experimental rats received a single, intraperitoneal injection of 10 mg zymosan in 0.2 ml saline; control rats received an injection of 0.2 ml saline. Zymosan (Lot OB-298; Standard Brands, Inc., New York, NY) was sterilized by boiling for 5 min in a saline solution and then centrifuged and resuspended in sterile, pyrogen-free saline (0.15 *M* NaCl) with the aid of a Cyclo-mixer (Clay-Adams; New York, NY). At predetermined intervals (2 hr, 2, 4, 10, 20, and 40 days) rats were decapitated, and the absolute numbers of free-floating mast cells of the peritoneal fluid were determined hemacytometrically. For this, each rat was injected intraperitoneally with 5 ml 0.9% saline solution which contained 0.1% EDTA (dipotassium ethylenediaminetetraacetic acid). The abdominal area of the animal was massaged gently for 1 min, the cavity was incised, and the lavage fluid containing the suspended cells was collected with an ordinary medicine dropper. The amount of actual peritoneal fluid present at all intervals studied was negligible in comparison to the volume of lavage fluid so that all estimates were based on an initial volume of 5 ml. Wet mounts were made of lavage fluid and were examined using phase contrast microscopy. In addition, brush smears of peritoneal fluid cells were stained with Wright–Giemsa and examined at 2, 4, 8 and 16 hr after zymosan injection and at daily intervals for 3 wk thereafter.

Equal volumes (0.2 ml) of the lavage fluid and of 0.1% methylene blue chloride (C.I. 922) in 100% propylene glycol were mixed. After 5 min at room temperature and remixing, a sample was placed in a Speirs-Levy counting chamber. Two hundred to 1000 mast cells were counted per rat. The remaining cell suspension was centrifuged, following which the sedimented cells were resuspended in several drops of fetal calf serum. This suspension was centrifuged and brush preparations were made from the resulting cell button. Some of these were stained with a Wright–Giemsa preparation buffered at pH 6.6. Others were stained with 0.025% acridine orange and examined by fluorescence microscopy (3).

In the second group of experiments, a single intraperitoneal injection of zymosan was given to rats, mice, hamsters and gerbils. Peritoneal lavage was performed using 5 ml saline in rats, 1 ml in mice and 2 ml in

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hamsters and gerbils. Evidence for ingestion of zymosan by mast cells was sought by examining peritoneal fluid cells stained by acridine orange and examined using fluorescence microscopy (3).

Results. Although macrophages engulfed most of the intraperitoneally injected zymosan, mast cells also rapidly ingested it. Samples of peritoneal fluid examined after 5 min revealed zymosan particles adhering to the surfaces of mast cells but not yet interiorized. After 15 min, examples of zymosan within mast cells were noted. Under fluorescence microscopy, these particles were delineated by a dark rim which may be the perimeter of a vacuole in which they were located. By 2 hr postinjection, 40 to 60% of the mast cells contain at least one particle. A typical distribution of the numbers of zymosan particles in mast cells appears in Table I. Figure 1A depicts the appearance of zymosan particles indenting the surface of a mast cell, and Fig. 1B shows the intracellular location of several zymosan particles.

Within several minutes after the intraperitoneal injection of zymosan, cellular aggregates began to appear in the harvested washings of the peritoneal fluid, some of these being large enough to be detected without the aid of magnification. Microscopic examination of unstained wet mounts using either phase optics or acridine orange-stained smears and fluorescence microscopy revealed these aggregates to be composed primarily of macrophages containing ingested zymosan. Mast cells, some with intracellular zymosan, were also present in these clumps. Masses composed of 50 or more cells were common during the first hour after injection, and after several hours even larger cellular accumulations were found clingling tenuously to omental fringes.

TABLE I. A Typical Distribution of Zymosan Particles in Peritoneal Fluid Mast Cells of a Rat that Received 10 mg Zymosan via Intraperitoneal Injection and Was Sacrificed 2 hr Later.

Mast cells (%)	No. of zymosan particles/cell
38	1
20	2
1	3
1	5
40	0

This rapid clumping of cells corresponded to the precipitous fall in the numbers of free floating mast cells (and macrophages). As shown in Fig. 2, by 2 hr after injection of zymosan the absolute numbers of free mast cells fell to less than 25% of control levels and little sign of recovery was noted during the next 40 days. The remaining mast cells were, in general, smaller than those in the saline-treated controls. In contrast, the absolute numbers of free mast cells in the fluid of the saline-injected control rats increased, more than doubling over the same time period.

Although the majority of free mast cells contained zymosan after 2 hr, by 24 hr only an occasional mast cell contained zymosan. Macrophages displayed cytological evidence which indicated progressive digestion of the intracellular zymosan. Mast cells did not.

The injection of zymosan did not cause a marked immediate disruption of mast cells. Although smears of peritoneal fluid cells always revealed some spilling of mast cell granules, this might have been the result of mechanical trauma. Numerous mast cells in the hemacytometer appeared intact even when containing one or more ingested particles.

The acute response to the injection of zymosan was a short-lived inflammatory reaction. Large numbers of neutrophils appeared in the peritoneal fluid and reached their highest levels between 4 to 8 hr during which time interval they were the predominant cell. They also participated in the phagocytosis of zymosan. Over the next few days their numbers waned rapidly and many neutrophils exhibited karyorrhexis and karyolysis. Concomitantly, the mononuclear cells, predominantly macrophages, whose numbers had fallen initially, began to increase and in subsequent days again became the primary cell of the peritoneal fluid. Macrophages avidly ingested and degraded the engulfed zymosan particles as well as degenerating neutrophils. In so doing, they became hypertrophic and

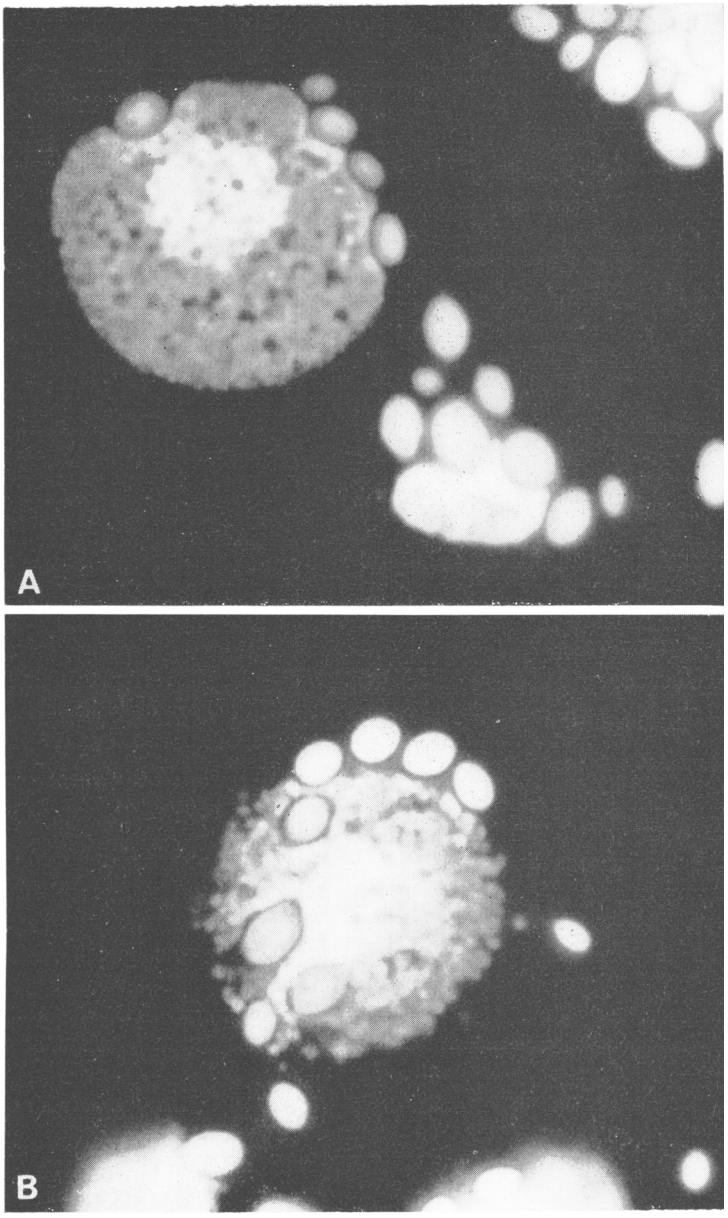

FIG. 1. Rat mast cell–zymosan interaction as seen with fluorescence microscopy after staining with acridine orange. Original, magnification, ca. 1000 (A). Five zymosan particles are indenting the surface of the mast cell. (B) A mast cell with three intracytoplasmic zymosan particles, one zymosan particle apparently in the process of being engulfed, and 4 zymosan particles at the surface. Evidence of slight degranulation at surface.

only gradually did the overall cytological picture return to its preinjection appearance.

Whereas the peritoneal fluid mast cells of rats avidly took up zymosan particles, this was not the case for the peritoneal fluid mast cells of either mice, hamsters or gerbils. Table II summarizes these findings. Even the reported uptake in 2% of gerbil mast cells

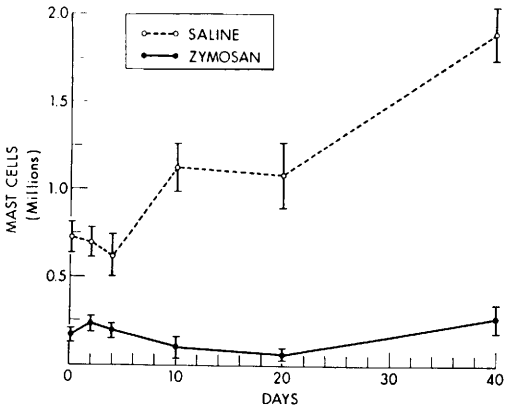

FIG. 2. Absolute numbers of peritoneal fluid mast cells following single intraperitoneal injection of 10 mg zymosan. Each represents the average value for 5 to 7 rats. The vertical lines delineate ± 1 SE.

may be overestimated, because it occasionally proved difficult to determine with certainty whether a particle was intracytoplasmic or merely indenting the surface of the cell. Although mast cells were not phagocytic in these other animals, the local macrophages were.

Discussion. Why should mast cells engulf zymosan? We do not know, but this action does not appear to be part of an intracellular digestive process similar to that carried out by typical macrophages which ingested and degraded this particulate. Electron microscopy has revealed no evidence that mast cells can digest zymosan particles in the same way and to the same extent as macrophages, and mast cell granules in the vicinity of ingested zymosan showed no morphologic alterations (1). The overwhelming superiority of the macrophages to ingest zymosan avidly was also seen in the present studies. Macrophages competed successfully with mast cells for zymosan particles and it was necessary to use high enough dosages to "saturate" the macrophages before significant uptake by mast cells was noted.

On the other hand, the occurrence of limited degradative phenomena cannot be excluded completely, because rat mast cells have been shown to contain several hydrolytic enzymes possessing acid pH optima (4) plus a recently discovered glucosaminidase which is found predominantly in their granules (5), and it has been stated that the presence of these enzymes "indicates a capability of the mast cell for the breakdown of the connective tissue ground substance."

On the other hand, the interiorization of particulates by mast cells may be related to the processing of immune complexes. Mast cells, like lymphocytes, can bind certain immunoglobulins to their surface membranes, and these may become coupled to antigens. Lymphocytes have been shown to endocytose complexes of anti-Ig with membrane-bound Ig (6). It remains to be determined whether mast cells share this ability as has been suggested (7). Whereas the function of phagocytosis by mast cells remains speculative, their ability to engage in this activity appears to be incontrovertible.

Recent work (2) has offered ample proof

TABLE II. *In Vivo* Phagocytosis of Zymosan by Peritoneal Fluid Mast Cells in Several Species of Animals.

Animal	Strain	Sex	Wt (g)	No.	Zymosan	Mast cells (%) containing zymosan[a]
Rat	Sprague-Dawley	Male	90–110	6	10.0	50
Mouse	CF #1	Male	20–30	6	0.3	<1
		Male	20–30	6	0.5	<1
		Female	20–30	6	0.5	<1
		Male	20–30	6	1.0	<1
Hamster	Unknown	Male	35–45	4	1.0	<1
Gerbil	Unknown	Male	65–68	4	1.0	1–2

[a] At least 300 mast cells were examined in each animal for evidence of zymosan uptake.

that rat mast cells not only ingest a wide variety of particulates including thorotrast, ferritin, microspheres and poxvirus but are also able to incorporate some of these materials into their specific granules for extended periods of time.

If mast cells do have a long-term function for storing certain materials, this does not appear to depend upon maintaining large numbers of these cells in the peritoneal fluid. Following a single intraperitoneal injection of zymosan in rats, the absolute numbers of free-floating mast cells fell precipitously and remained at low levels for at least 40 days while the absolute numbers of mast cells in the control group rose. This concurs with observations made several decades ago that mast cells are susceptible to a variety of foreign substances which cause their rapid disappearance from tissues (8). The slow rate of recovery also is in accord with more recent reports concerning the slow turnover of these cells in the rat (9, 10). Furthermore, the rise in the absolute numbers of mast cells in the controls confirms earlier investigations of this phenomenon in normal, growing rats (10, 11).

Summary. A single intraperitoneal injection of zymosan into rats resulted in the engulfment of these particles by mast cells as well as by macrophages. There was a rapid fall in the numbers of free-floating mast cells which appeared to be a result of cellular clumping, and the numbers of peritoneal fluid mast cells remained depressed for at least 40 days. Unlike rats, neither mice, hamsters, nor gerbils displayed large scale engulfment of zymosan by peritoneal fluid mast cells.

1. Padawer, J., and Fruhman, G. J., Experientia **24**, 471 (1968).

2. Padawer, J., Lab. Invest. **25**, 320 (1971).

3. Riva, H. L., and Turner, T. R., Obstet. Gynecol. **20**, 451 (1962).

4. Lagunoff, D., and Benditt, E. P., Ann. N.Y. Acad. Sci. **103**, 185 (1963).

5. Lagunoff, D., Pritzl, P., and Mueller, L., Exp. Cell Res. **61**, 129 (1970).

6. Unanue, E. R., Perkins, W. D., and Karnovsky, M. J., J. Immunol. **108**, 569 (1972).

7. Mota, I., *in* "International Review of Cytology" (G. H. Bourne and J. F. Danielli, eds.), Vol. 15, p. 363. Academic Press, New York (1963).

8. Webb, R. L., Amer. J. Anat. **49**, 283 (1931).

9. Allen, A. M., J. Nat. Cancer Inst. **28**, 1125 (1962).

10. Blenkinsopp, W. K., J. Cell Sci. **2**, 33 (1967).

11. Padawer, J., and Gordon, A. S., J. Gerontol. **11**, 268 (1956).

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