Ingestion of Colloidal Gold by Mast Cells (33455)

JACQUES PADAWER*

Department of Anatomy, Albert Einstein College of Medicine, New York, New York 10461

The ability of rat peritoneal fluid mast cells to phagocytize zymosan (1, 2) and Thorotrast (3) particles has been demonstrated recently. The present report demonstrates that another particulate, namely colloidal gold, is also readily ingested by mast cells.

Materials and Methods. Male rats of the Sprague-Dawley strain (CD-COBS, Charles River Breeding Laboratories, Wilmington, Mass.) were used. Body weights ranged from 126 to 132 g; all rats were 39 days old when injected i.p. with colloidal gold. The colloidal gold was prepared by concentration, to about 9-fold, of a commercial preparation (Colloidal Gold Solution, Lange, Magar Chemicals, Inc., Cornwall-on-Hudson, N.Y.) by heating in an open vessel under reduced pressure. Animals were killed 2 hr or 3 days after injection and free-floating peritoneal mast cells were prepared for electron microscopy as described elsewhere (2).

Results. Colloidal gold is taken up by rat mast cells. At 2 hr postinjection, light microscopic observations of peritoneal mast cells revealed blue deposits of varying size within their cytoplasm. Electron microscopy revealed these to consist of large vesicles in which aggregates of colloidal gold spherules were contained (Fig. 1). At this time, occasional individual gold spherules were also seen in contact with some of the mast cell specific cytoplasmic granules.

Animals killed 3 days postinjection still displayed mast cells with blue deposits, as seen in the light microscope. Electron microscopy showed gold spherules associated solely with granules at this time. Some spherules occurred individually—one to several per mast cell granule (Fig. 2). In other instances, groups of aggregated spherules were seen, generally at the periphery of the granule substance, but always within the membranous investment of the granule (Fig. 3).

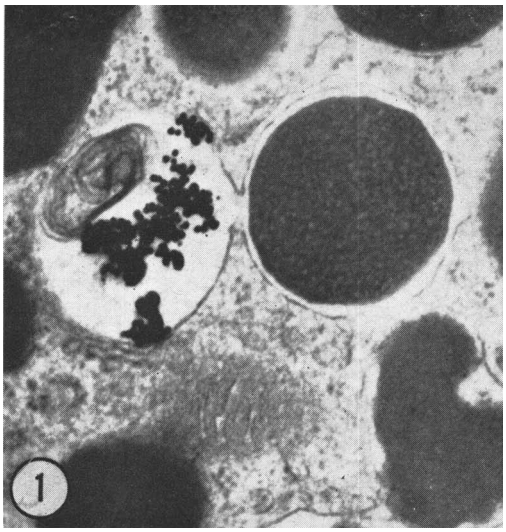

FIG. 1. Peritoneal fluid mast cell 2 hr after intraperitoneal colloidal gold injection. A vesicle containing aggregated gold spherules appears to be connected directly to the space that surrounds one of the specific cytoplasmic granules. The latter is almost fully mature. Uranium acetate–lead citrate strain, ×41,600.

In all cases, only a very small proportion of the cell's granules contained gold colloid. Gold-containing mast cells were common. Gold spherules were not associated with any other cellular organelle than the vesicles or the specific granules mentioned above. Many macrophages and some eosinophilic leukocytes are normal constituents of rat peritoneal fluid. A substantial influx of neutrophilic leukocytes occurred after injection of the gold suspension. Whereas many macrophages were found to contain ingested gold particulate confined within phagocytic vacuoles, no gold spherules were seen within either eosinophilic or neutrophilic granulocytes.

Discussion. Although mast cells are not generally held to be phagocytic (4, 5), it appears that this notion should be seriously challenged. Indeed, the demonstration that

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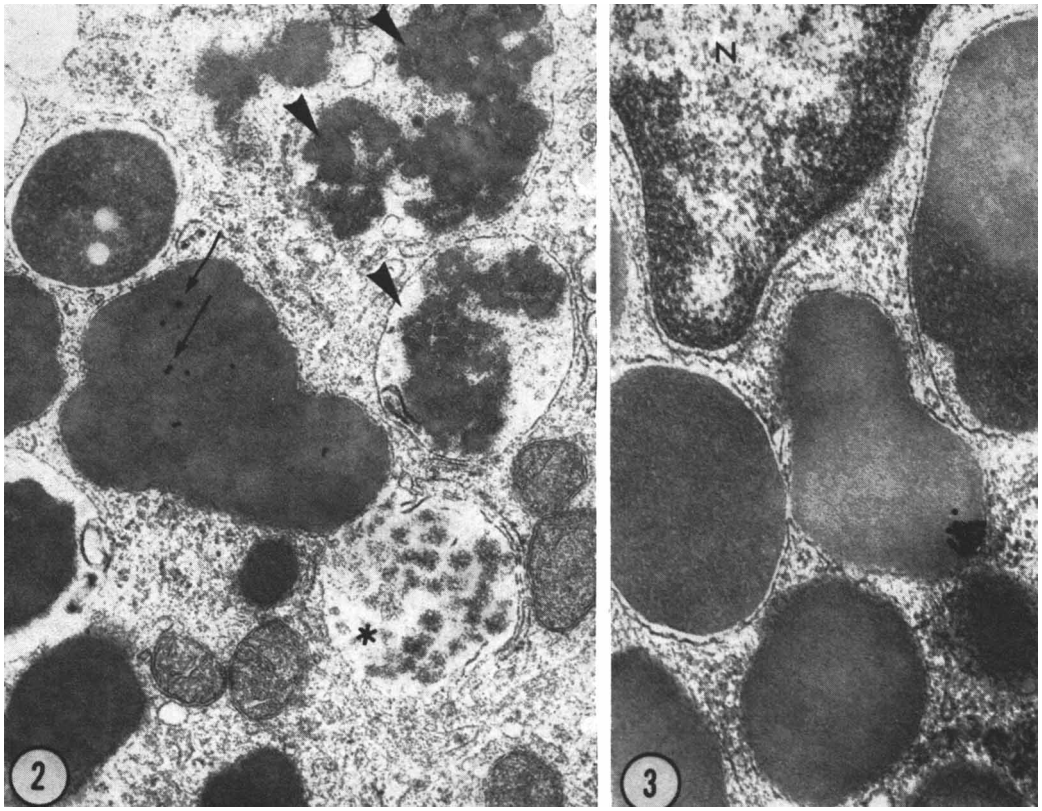

FIG. 2. Mast cell, 3 days after intraperitoneal injection of colloidal gold. Several gold spherules (arrows) are located within the matrix of a developing granule which is in an advanced stage of compaction. A vesicle containing several progranules is located nearby (*), as are several developing granules in early stages of compaction (arrow heads). Uranium acetate–lead citrate stain, × 31,200.

FIG. 3. Mast cell, 3 days after intraperitoneal injection of colloidal gold. A group of aggregated gold spherules is nested against a mature granule. Uranium acetate–lead citrate stain, ×41,600; N = nucleus.

mast cells can ingest colloidal gold brings to three the number of particulates definitely shown to be taken up—at least by the rigorous methods of electron microscopy. The earlier literature of mast cells includes reports of phagocytosis of colloidal dyes (6), cocci (6), and erythrocytes (7). It also includes denials that colloidal dyes are taken up (7, 8). However, it is the author's experience that light microscopic methods may be misleading in this respect and, furthermore, that there may be a systematic technological bias that reduces selectively the occurrence of phagocytically-active mast cells from the sample obtained for study. This might explain the numerous negative reports in the literature as well as the unconvincing nature of positive reports antedating the successful handling of mast cells for electron microscopic observations.

It is of interest that intimate contact of ingested gold particulate with mast cell granules did not lead to disruption of the latter. This is in agreement with previous findings in which neither zymosan (2) nor colloidal thorium dioxide led to granule disruption.

Summary. Rat peritoneal fluid mast cells can phagocytize colloidal gold. This foreign particulate rapidly becomes localized within the specific cytoplasmic granules of the cell. With the previously demonstrated uptake of zymosan and of Thorotrast particles by mast

cells, the demonstrated phagocytosis of a third particulate substance by mast cells suggests that uptake of substances from the tissue environment may be a process with broad significance with respect to mast cell function.

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Assay of Spleen Cells Forming Antibodies to O-Antigens of Salmonellae (33456)

A. L. OLITZKI[1] (Introduced by W. Braun)

Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey 08903

Quantitative assays of antibody production by single spleen cells in agar have been carried out with red blood cells (1), red blood cells coated with bacterial antigens (2), and with gram-negative bacteria (3) as antigens. The latter are lysed in the presence of antibody and complement (4–6) and sterile plaques are produced around bacteriolysin-producing spleen cells. The experiments described here were carried out to examine (i) whether a direct assay without the use of antigen-coated red blood cells can also be used for testing the immune response to typhoid and paratyphoid vaccines, (ii) whether there exist differences between the response to living or heat-killed microorganisms, and (iii) whether antibody production is primarily limited to species-specific antigens of the microorganisms or comprises, from the beginning of the immune response, species-specific *and* genus-specific antigens.

Materials and Methods. Salmonella typhosa, strain 0901 with the antigenic formula IX, XII and *Salmonella paratyphi A* with the antigenic formula I, II, XII, were kindly provided by Dr. L. S. Baron, Department of Bacterial Immunology, WRAIR, Washington, D. C. Bacteria grown 20 hr at 37° on the surface of nutrient agar plates were used as antigens for immunization and for the assays as well. Forty mice were divided in 4 groups, each of which was inoculated intraperitoneally on the same day with 2×10^8 microorganisms as follows: (i) *S. typhosa,* living, (ii) *S. typhosa,* heat-killed at 65° for 1 hr, (iii) *S. paratyphi A,* living, and (iv) *S. paratyphi A,* heat-killed at 65° for 1 hr.

Assays for the detection of the number of spleen cells producing O-antibodies were carried out as follows: 2 ml of melted 1.4% Noble's agar (Difco) were mixed in small test tubes in a water bath at 50° with 2 ml of sterile, double-strength Eagle's minimal essential medium (MEM), and 0.2 ml of a 0.5% solution of diethylaminoethyl-dextran. The spleens were removed under sterile conditions at 4°, a suspension of single cells was prepared and cells from individual spleens were suspended in 2 ml of Hank's balanced salt solution. If the presence of large numbers of antibody-producing cells was suspect-

[1] Home address: Department of Bacteriology, The Hebrew University-Hadassah Medical School, P.O.B. 1172, Jerusalem, Israel. This work was supported by USPHS Grant AM-08742 to Dr. W. Braun.