Degradation of phagocytosed spermatozoa by mouse peritoneal macrophages *in vitro*

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Summary. Mouse peritoneal macrophages were cultured *in vitro* in the presence of autologous spermatozoa. The spermatozoa were found to be phagocytosed and degraded, with accumulation of pigment in the cytoplasm, during the first 5 days.

Phagocytosis of spermatozoa by macrophages is seen in two lesions of the epididymis: sperm granulomata, in which the spermatozoa have escaped from a ruptured ductule (Glassy & Mostofi, 1956), and 'brown patches', in which the phagocytosis occurs in the lumen of an obstructed ductule (Mitchinson, Sherman & Stainer-Smith, 1975). In both conditions the phagocytosed spermatozoa appear to be converted to cytoplasmic pigment granules resembling lipofuscin.

This mechanism might be important in the origin of autoantibodies to spermatozoa. The present study was an attempt to produce sperm phagocytosis *in vitro*, by culturing mouse peritoneal macrophages with autologous spermatozoa, as a first step in the closer examination of the phenomenon.

Eight male mice (strain B10.D2 from the Laboratory Animals Centre, Carshalton, Surrey, U.K.), 3 months of age, were decapitated and injected i.p. with 3 ml sterile Hank's balanced salt solution (HBSS: Flow Laboratories, Irvine, Scotland). After gentle massage, the peritoneum was opened and the fluid was recovered with a syringe, diluted 1:9 with HBSS and dispensed in 1 ml aliquots to each culture dish (35 × 10 mm: Falcon Plastics, Oxnard, California). Ten dishes, 5 'experimental' and 5 'control' for each mouse, were allowed to stand for 20 min while the macrophages adhered to the bottom and were then washed twice with 1 ml HBSS.

The epididymides of each mouse were sliced in 1 ml sterile culture medium so that spermatozoa could be collected without contamination by other cells. The culture medium contained the following constituents in 100 ml: 32.93 ml Eagles modified MEM + Hank's salts + 0.35 g NaHCO₃/l; 32.93 ml Hank's lactalbumin; 32.93 ml heat-inactivated bovine fetal serum; 1.1 ml 200 mM-L-glutamine solution; and 0.11 ml of a solution of 50 mg gentamicin/ml (all materials from Flow Laboratories). 'Control' dishes received 1 ml culture medium and 'experimental' dishes received 0.9 ml culture medium + 0.1 ml sperm suspension from the same mouse.

The dishes were then gassed with 95% air + 5% CO₂ and incubated for up to 5 days at 37°C. At 24-h intervals the medium was replaced with fresh medium without spermatozoa. Every day 2 dishes, one 'experimental' and one 'control' for each mouse, were withdrawn. The cells were fixed with 10% formol saline, stained with haematoxylin and eosin, and cleared and mounted in epon resin. The percentage of cells containing sperm heads and yellowish pigment was then counted, using ×1000 magnification. It was difficult to decide whether or not there was any pigment in some cells; these were counted as positive in 'control' dishes and negative in 'experimental' dishes.

Results and discussion

Some of the sperm suspensions showed immediate head-to-head agglutination, the aggregates ranging from several spermatozoa to about 50. Such agglutination may have been due to auto-spermagglutinins or to non-specific factors such as other cell types, detritus or air bubbles (Phadke & Padukone, 1964; Fjällbrant, 1965).

All the spermatozoa were very motile and were colliding with and adhering to macrophages within seconds. The attachment was usually by the head but a few adhered by the annulus. No spermatozoon was seen to free itself, once adherent, although movements persisted.

Preliminary experiments showed that the spermatozoa were phagocytosed, head first, within a few hours and could be easily seen inside the cell by phase-contrast microscopy, in stained preparations or by electron microscopy (Plate 1). In this study, almost all macrophages contained recognizable sperm heads at 24 h, usually 2–4 in each cell. As the days passed, the proportion of cells containing recognizable spermatozoa decreased progressively (see Table 1), the decline being conspicuous at 48 h in 3 mice and at 72 h in the other 5 animals. The only other difference between these two groups was that the bovine fetal serum was from different batches and the rate of degradation might therefore be dependent on serum factors. The decrease in proportion of cells containing spermatozoa was due to sperm degradation rather than to their dilution by macrophage division, because none of the many cells examined contained a mitotic figure. Similarly low rates of mitosis were observed by Einstein, Scheeberger & Colten (1976) in cultured human monocytes and by Bennett (1966) in mouse peritoneal macrophages. Degradation of spermatozoa in this system takes a surprisingly long time, probably mainly due to the large size of the spermatozoa and their resistant constituents, such as acid-fast lipid (Berg, 1954) and basic keratin-like protein (Henricks & Mayer, 1965).

Table 1. Mean ($\pm$ S.E.M.) percentages of macrophages containing mouse spermatozoa and pigment at 24-h intervals

Culture period (h)	Macrophages + spermatozoa (serum A) (N = 3)	Macrophages + spermatozoa (serum B) (N = 5)	Macrophages + pigment ('control' dishes) (N = 8)	Macrophages + pigment ('exp.' dishes) (N = 8)
24	96.0 $\pm$ 0.0	97.0 $\pm$ 0.8	6.4 $\pm$ 0.6	7.5 $\pm$ 0.5
48	69.7 $\pm$ 1.9	94.8 $\pm$ 1.7	6.6 $\pm$ 0.8	12.5 $\pm$ 0.9*
72	17.3 $\pm$ 3.3	64.2 $\pm$ 3.1	7.1 $\pm$ 0.3	19.1 $\pm$ 1.7**
96	4.7 $\pm$ 1.5	39.2 $\pm$ 3.7	7.3 $\pm$ 0.8	20.9 $\pm$ 0.9**
120	4.3 $\pm$ 1.9	22.8 $\pm$ 2.3	9.5 $\pm$ 0.5	22.9 $\pm$ 1.2**

Values significantly different (paired *t* test) from those in the 'control' dishes, **P* < 0.01, ***P* < 0.001.

In all the preparations, intracytoplasmic yellow-brown pigment resembling lipofuscin gradually accumulated in some of the macrophages (Table 1). A significantly greater proportion of macrophages showed pigment when they had been associated with spermatozoa.

The additional pigment which accumulated in the 'experimental' dishes was probably composed of indestructible remnants of phagocytosed spermatozoa. Dixon (1967) proposed that lipofuscin in cerebral neurones is a remnant of disorientated lipoprotein membranes and spermatozoa contain a large area of membrane in comparison with their volume. The pigment in the 'control' macrophages was presumably remnants of debris phagocytosed by these cells *in vivo* before harvesting.

The significance of the present findings is related to the suggestion that resorption of spermatozoa is a likely starting point for autoantibody production (Rümke & Hellings, 1959; Phadke & Padukone, 1964; Rümke, 1965, 1970; Fjällbrant, 1968; Mitchinson *et al.*, 1975). Although spermophagy has been suggested to be a physiological means of disposal of surplus (Roussel, Stallcup & Austin, 1967), most authors conclude that it is only in pathological states such as seminal obstruction that the phenomenon is significant. The high frequency of sperm autoantibodies in man was therefore puzzling until Mitchinson *et al.* (1975) pointed out that obstructive lesions of the human epididymis are very common.

The postulated train of events in autoantibody production demands that the macrophages carrying spermatozoa reach immunologically competent cells before antigens are destroyed. The present experiments have shown that the phenomenon of spermophagy can be reproduced *in vitro* and the time-course of degradation of the spermatozoa observed. A similar system might be used to

PLATE I

Electron micrograph of a phagocytosed mouse spermatozoon after 24 h in culture. x9000.

measure the duration of persistence of sperm antigen, although there are dangers in drawing close analogies between events *in vitro* and *in vivo*.

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