villi are found after 24 h of culture; here, the serosal side of the explants is not covered with the epithelium even after 72 h of culture, and oedema is absent. With FBS supplemented medium, short and irregular villi are formed only after 48 h of culture. In a few explants, the epithelium has reached the serosal side after 72 h.

At the fine structural level, endocrine and mucous cells seem to differentiate properly with all the media used. However, absorptive cells remain poorly differentiated.

Like serum, amniotic fluid is a very complex mixture. It is known to contain cortisol[9], somatomedin[10], dopamine[11], disaccharidases and lysosomal enzyme activities[12], glucose and potassium[13], fibronectin[14], oxytocin[15], diiodothyronines[16], epidermal growth factor[17], etc. For the moment it is not known if ingested amniotic fluid has any effect in vivo. However, in vitro, mouse amniotic fluid is able to promote cell growth in duodenal explants; furthermore, MAF also sustains the early stages in the formation of intestinal villi.


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## Differences in phagocytic activity of methacrylate copolymer particles in normal and stimulated macrophages


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*Summary.* Methacrylate copolymer particles were found to be optimal for a study of phagocytosis in normal and stimulated macrophages, and, after their simple coating with antiserum, for a specific cell labelling with minimal spontaneous adhesion to the cell surface.


Phagocytosis of particles represents one of the several functional parameters used for characterization of macrophages. Besides bacteria and erythrocytes particles commonly classified as 'inert' have been employed. This group includes, e.g., colloid carbon, starch, silica, carbonyl iron or nickel, latex, etc. However, certain types of cells avidly form bonds with such particles exhibiting no specificity or ability for subsequent phagocytosis. It is beyond the possibilities of optical microscopy to differentiate between phagocytized particles, particles specifically bound to the surface of a phagocyting cell, and particles adhering spontaneously to cells of various types. A complete removal of the nonspecifically bound particles requires additional treatment (e.g., a trypsin-versene procedure in the case of the latex spheres[3]) which consequently limits the possibilities of a surface-marker study. These difficulties seem to be partly removed by the use of the methacrylate copolymer particles prepared in this laboratory. The nonspecific adherence of these particles to the cell surface is considerably reduced, while the binding of an antiserum or other ligands to these particles could be achieved using a simple procedure, thus opening up new possibilities for specific cell labelling.

*Material and methods.* Cells. Peritoneal exudate cells were obtained from peritonea of untreated, or thioglycollate-treated (TG), or proteose peptone-treated (PP) 3–4-month-old female mice of the A/J strain (breeding colony of the institute). The stimulation procedure consisted of an i.p. injection of 3 ml TG-medium (Institute of Microbiology, Prague) or 1.5 ml 10% PP (Difco) 3 days prior the cell harvest.

Particles. Methacrylate copolymer particles (mean particle diameter 0.5 μm) were prepared according to Rembaum and his coworkers[4,5] by gamma irradiation-activated ($Co^{60}$; 8 kGy) copolymerization of a nitrogen-bubbled 2% aqueous solution of a mixture consisting of 2-hydroxyethyl-

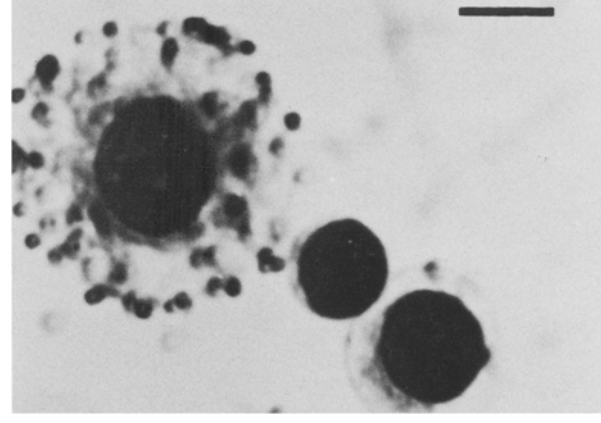

Fig. 1. Peritoneal exudate macrophage (stimulated with thioglycollate medium) with methacrylate copolymer particles as seen in the light microscope. 2 lymphocytes are negative (bar: 10 μm).

methacrylate, methacrylic acid, and methylene-bis-acrylamide (Serva; w/w/w ratio = 39:10:1). Thorough washing with phosphate buffered saline (PBS) followed the irradiation in order to remove all detectable monomers.

Experiments. The phagocytic activity was tested by incubating peritoneal exudate cells with methacrylate copolymer particles (1:50) at 37 °C in PBS under permanent agitation for 30 min. Neither serum nor other phagocytosis-enhancing substances were added. In some experiments the particles were coated with a rabbit anti-mouse macrophage serum (AMS). The suspension volume of 0.1 ml containing $10^8$ particles was incubated with the same volume of diluted (1:10) AMS and permanently agitated at 37 °C for 1 h. The AMS-coated particles were then washed 5 times in PBS and resuspended in the same medium. Preparation of AMS is described by Unanue[6]. Screening of the inhibition of phagocytosis was performed by adding 20 mM sodium azide (Avondale Laboratories) to the incubation medium. Each experiment was carried out at least in quadruplicate.

Electron microscopy. Specimens for electron microscopy

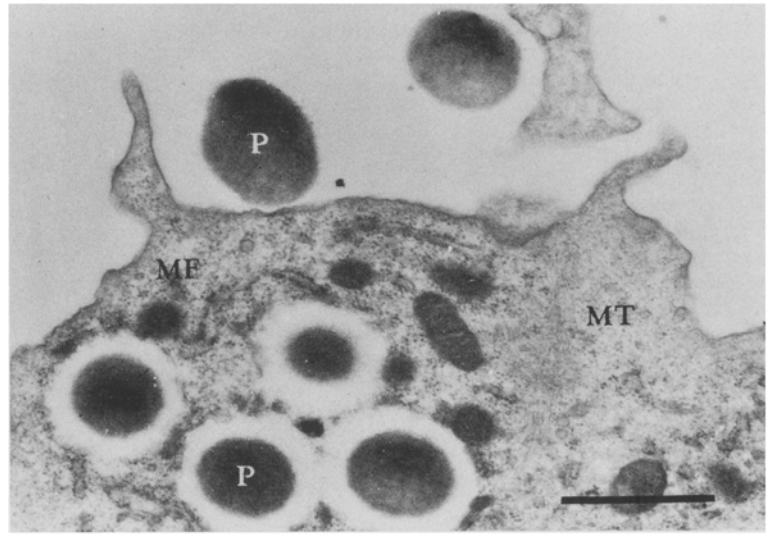


Fig. 2. Electron micrograph of a macrophage from the mouse peritoneal cavity on day 3 after stimulation with proteose peptone. The cytoplasm is filled with microfilaments (MF) and microtubules (MT). Adherence state and final phagocytosis of methacrylate copolymer particles (P) may be observed (bar: 1 µm).

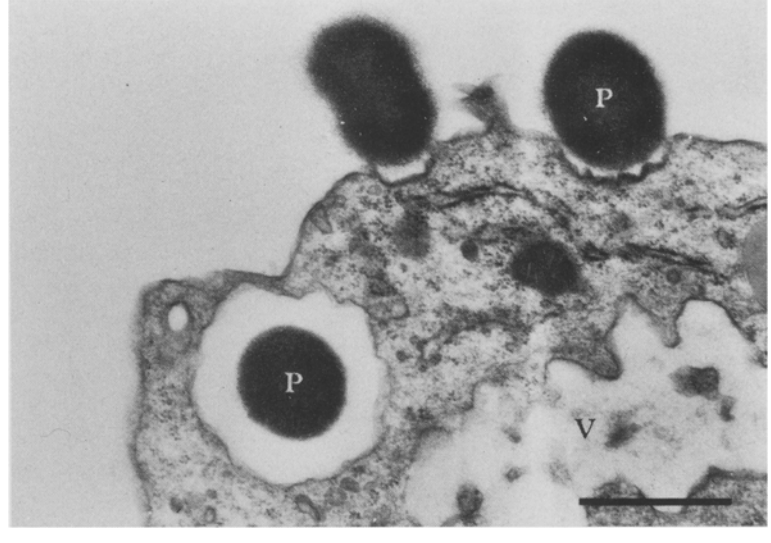


Fig. 3. Mouse peritoneal macrophage on day 3 after stimulation with thioglycollate medium. The cell surface is smooth, cytoplasm is full of vesicles (V) of irregular shapes containing phagocytized stimulating medium. Simple phases of methacrylate copolymer particle (P) ingestion can be seen (bar: 1 µm).

Phagocytosis of methacrylate copolymer particles by normal (untreated) and stimulated mouse peritoneal exudate cells

| Cell type | Percentage of macrophages[a] in peritoneal exudate | Percentage of phagocytosis-positive macrophages[b] | Percentage of adherence- and/or phagocytosis-positive lymphoid[c] and mast[c] cells |
|---|---|---|---|
| Normal | 38.5 ± 4.9 | 2.4 ± 0.8 | < 0.1 |
| Normal[d] | 38.5 ± 4.9 | 73.7 ± 6.0 | < 0.1 |
| Thioglycollate-stimulated | 92.0 ± 6.4 | 97.3 ± 2.2 | < 0.1 |
| Thioglycollate-stimulated[e] | 92.0 ± 6.4 | 0.3 ± 0.2 | < 0.1 |
| Proteose peptone-stimulated | 63.4 ± 8.3 | 87.8 ± 7.1 | < 0.1 |

Values are mean ± SD; [a] as determined from morphologic characteristics; [b] with 7 and more particles; [c] with 3 and more particles; [d] particles coated with anti-macrophage serum; [e] 20 mM sodium azide in medium.

were fixed with glutaraldehyde and post-fixed with osmium tetroxide. After dehydratation in alcohol the specimens were cleared in propylene oxide and embedded in Durcupan ACM (Fluka). Phagocytic activity was determined in series of thin sections contrasted with uranyl acetate and lead citrate in a Tesla BS 500 electron microscope with the accelerating voltage of 60 kV. Macrophages with 7 or more particles were considered as phagocytic positive cells.

*Results and discussion.* Macrophages have at least 3 functional types of receptors for attachment and subsequent ingestion of particles: the Fc receptors, the complement receptors and the nonspecific receptors. Phagocytosis via the last type has been studied using 'inert' particles, for example latex or latex-like particles. Under normal conditions, methacrylate copolymer particles adhere neither to the lymphoid cells nor to the peritoneal exudate macrophages obtained from unstimulated donors. After the stimulation with PP or TG-medium the peritoneal macrophages acquire the ability to engulf these particles (table). This result supports the observations[7,8] showing an increased phagocytic activity in stimulated mouse peritoneal macrophages, and is in contradiction with some data on a decreased phagocytic ability[9,10] (especially after the TG-stimulation), although no detailed comparison of the degree of phagocytosis of different types of particles has been done[11]. The addition of sodium azide prevents phagocytosis of these particles. No significant increase of phagocytosis occurs when mouse or calf serum is added to the incubation medium. The experimental data indicate that the stimulation is due either to an increased adhesion and the ingestive power of mouse peritoneal macrophages, or to an increased number of nonspecific surface receptors for this type of particle. The changes in the uptake of particles therefore seem to be accompanying the functional alterations during the stimulation processes and reflecting modifications in the properties of the cell membrane. This suggestion is supported by the numbers of spontaneous rosettes of macrophages with sheep erythrocytes and by changes in intercellular adhesivity[12].

The electron microscopic observations show a smoothened cell surface and a rearrangement of the cortical regions of the cytoplasm of the cells phagocytosing the methacrylate copolymer particles. Pictures show a random distribution of particles over the cell surface and inside the cells (figure 1), often near vacuoles containing stimulating medium (figures 2 and 3). The partial shrinking of the particles during preparation of the specimes for electron microscopy does not seem to be of great importance for our purposes.

Methacrylate copolymer particles could be useful in studies of the phagocytosis of macrophages and, after coating with specific ligands, in specific labelling of cells. In other words, these particles may serve as markers for specific identification of cells by optical as well as by electron microscopy.

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## The influence of sodium-8-chlorotheophyllinate (S8CT) on immune processes[1]

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*Summary.* S8CT injected at the time of immunization significantly enhances specific IgM-production but has no effect on IgG-formation. Mitogen (PHA-P) induced macrophage migration inhibition of cells of S8CT pretreated animals is reduced. The same effect is observed, when normal cells are tested in the presence of S8CT in vitro. Blast transformation of B-lymphocytes but not of thymocytes is significantly stimulated by S8CT. Acid phosphatase activity is also stimulated in B-cells and - to a lesser degree - in cortisone-resistant T-lymphocytes whereas the activity of the total thymocyte population is reduced. No effect was seen on phagocytosis and intracellular bactericidal activity. A stimulatory effect of S8CT for B-cells is postulated.

Cyclic 3′, 5′ adenosine monophosphate (cAMP) and substances enhancing intracellular cAMP-level such as catecholamines or theophylline were described earlier as modulating the immune response[2-5]. Results obtained with theophylline, however, seem not to be homogeneous: no effect on antibody formation or inhibition were found using doses above 10 mg/kg[6-8] when theophylline was applied in vivo. With 1 exception[9], an enhancement of antibody formation[3], depending on the time of contact[10,11], was shown when lymphocytes were antigenically stimulated and treated with this substance in vitro. We report here the results of studies of how different immune processes (antibody formation, mitogen induced macrophage migration inhibition and lymphocyte transformation) are modulated by S8CT. Moreover, the influence of S8CT on the lysosomal acid phosphatase activity of different lymphocyte classes was examined.

*Material and methods.* S8CT (mol.wt 236.62) was synthesized as described previously[12]. The number of direct (DPFC) and indirect (IPFC) plaque forming (IgM and IgG producing) spleen cells was evaluated on day 3 and 7 after immunization ($2 \times 10^8$ SRBC in 0.5 ml saline i.p.)[13]. S8CT