DEPENDENCE ON PARTICLE SIZE IN THE PHAGOCYTOSIS OF LATEX PARTICLES BY RABBIT ALVEOLAR MACROPHAGES CULTURED IN VITRO

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Abstract……The dependence of the phagocytosis of particulate materials on their size was studied. Rabbit alveolar macrophages (AMs) obtained by lung lavage were cultured in suspensions or monolayers with Latex particles of 1 μm or 2 μm in diameter. After culturing AMs with $10^7-5 \times 10^6$ Latex particles per ml for 15 to 360 minutes, the number of phagocytized particles in each of 100 individual cells was counted by light microscopy. In suspension culture, there was no significant difference in the average number of particles phagocytized per AM between 1 μm and 2 μm particles in the identical conditions as to the particle concentration (particle number/ml) and the incubation time. In monolayer culture, it was difficult to compare the average number of 1 μm particles phagocytized by AM with that of 2 μm ones in the identical condition as to the particle concentration, since the sedimentation velocities at which particles sank to the bottom of culture chamber were different between both particles, resulting in the difference of particle concentration around AMs. The sign of saturation was observed when the average number of phagocytized particles reached approximately 10 particles per AM in either case of suspension culture with 1 μm or 2 μm particles and approximately 45 of 1 μm and 10 of 2 μm particles in monolayer culture.

Key words: Alveolar macrophage, latex particle, particle size, phagocytosis

INTRODUCTION

Alveolar macrophages (AMs) are thought to be an important clearance vector due to their ability to phagocytize, transport and digest inhaled particles. Therefore, the potential toxicologic hazard from particles depositing within the alveolar region of lung...
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is dependent to some extent upon the phagocytic activity of AMs.

It has been well known that the phagocytosis of particles by macrophage in vivo was affected by their physico-chemical state, such as the particle size (Zilversmit et al., 1952), chemical form (Matsuoka et al., 1972) and characteristics of surface (Watanabe et al., 1981). As to AMs obtained by lung lavage and cultured in vitro, Hart and Pittman (1979) have shown that there was an inverse relationship between the rate of beryllium uptake by AMs and the solubility of the beryllium in the incubation medium. Shulman-Satin (1981) have reported that the colloidal carbon was phagocytized more actively than Latex particles and described that the fact probably depended upon different physico-chemical characteristics of these particles. Up to now, however, the influence of particle size on the uptake by AMs still remain obscure (Valberg et al., 1982). In the present study, we examined this point in vitro by using the rabbit alveolar macrophages and Latex particles. Latex particles were used to exclude the influence of additional antigenic or toxic factors.

**MATERIALS AND METHODS**

Male Japanese white rabbits, aged 14-20 weeks old, were used as AM donors. Animals were anesthetized with sodium pentobarbital given intravenously at a dose of 30 mg per kg body weight and exanguished by the cutting of aorta abdominalis. The trachea of each animals was incised and intubated. Lung lavage was performed two times with about 60 ml of chilled Hank’s balanced salt solution (HBSS). The lavage solution recovered was centrifuged at 350 × g for 5 minutes and the resulting cell bottom was washed twice with HBSS and once with Eagle MEM. A total of 10^7 - 2 × 10^7 cells per animal was obtained by above procedure.

AMs were cultured in two different methods, suspension and monolayer culture, with Eagle MEM containing 20% of fetal calf serum (Gibco, USA). After counting the total cell number, the cell suspension was adjusted to be 0.8 × 10^6 and 0.4 × 10^6 cells per ml medium for monolayer and suspension culture, respectively. Thereafter the cell suspension for monolayer culture was placed in tissue culture chambers (Labtek, USA) and incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The cell suspension for suspension culture was poured into 20 ml siliconized test tubes, then placed in a reciprocating-shaker at 80 rpm at 37°C. After 1 hour preincubation, Latex particles with 1 μm or 2 μm in diameter (Dow Chemical Co, USA) were introduced into the culture medium to be at the concentrations of 10^7 - 5 × 10^6 particles per ml and cultured for scheduled time up to 360 minutes.

After culturing for 15, 30, 60, 120 and 360 minutes, the cells cultured in monolayers were washed with HBSS until most of non-phagocytized particles were washed away, being observed under a phase-contrast microscope. The cells cultured in suspensions were centrifuged at 350 × g for 5 minutes and washed three times with HBSS and the resulting cell bottom was smeared onto a glass slide. Adherent cells on the culture chambers and smears in both culture methods were dried with air, fixed in ethanol, and
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stained with May-Grünwald Giemsa stain. For each sample, the number of phagocytized particles in each of 100 individual AMs was counted by light microscopy and calculated the mean.

In counting the number of particles phagocytized by AMs, the particles which were attached to the cell membranes of AMs were included with ones ingested (internalized) for the following reasons. 1. It was difficult to determine under light microscope whether Latex particles were actually ingested or were merely attached to the cell membrane. 2. Gardner et al. (1973) have described that proper xylene treatment of AMs dissolved the extracellular 1 μm Latex particles leaving only the intracellular ones. However, xylene treatment seemed not to discriminate intra and extracellular particles with 2 μm in diameter clearly, based on our preliminary experiment. 3. The concept of phagocytosis in broad sense includes the attachment of particles to the cell membranes of phagocytes (Watanabe et al., 1981).

Experiments were repeated a minimum of 3 times in each combination of particle concentration and incubation time. The mean value which was defined as the average number of phagocytized particles and the standard deviation were presented. Statistical significance was determined by the paired Student's t test.

RESULTS

Relation of incubation time to average number of phagocytized particles in suspension culture: In any combination of particle concentration and incubation time, there were no significant differences in the average number of phagocytized particles between 1 μm and 2 μm particles. The increase in the average number of phagocytized particles was proportional to the incubation time up to 360 minutes at the particle concentration of 5×10⁷ and 10⁸, whereas this proportionality was obscure to some extent after 120 minutes of culture at the concentration of 5×10⁹ (Fig. 1).

Relation of particle concentration to average number of phagocytized particles in suspension culture: The average number of phagocytized particles in each incubation time at the particle concentration of 10⁸ was approximately twice than that in the corresponding incubation time at the particle concentration of 5×10⁷, but such relation, i.e., the proportionality between particle concentration and average number of phagocytized particles, was lost between the particle concentration of 5×10⁷ and 5×10⁹ (Fig. 1).

Relation of incubation time to average number of phagocytized particles in monolayer culture: In monolayer culture, the phagocytic rate, i.e., the number of phagocytized particles per minute, was increased as the time went at early stage of culture. The phagocytic rate increased more rapidly in 2 μm particles than 1 μm ones. On the other hand, the phagocytic rate decreased remarkably in the late stage of culture at high concentration of 2 μm particles, whereas such a phenomenon was not observed distinctly in 1 μm particles. Average number of particles phagocytized by AM cultured with 2 μm particles for 360 minutes at the particle concentration of 5×10⁹ was nearly
equal to those at the particle concentration of $10^9$ (Fig. 2).

*Relation of particle concentration to average number of phagocytized particles in monolayer culture:* The same data shown in figure 2 was represented in a logarithmic graph of particle concentration and average number of phagocytized particles (Fig. 3). The straight line with slope of 45 degrees indicates that the average number of phagocytized particles is proportional to the particle concentration. When the average number of phagocytized particles went over approximately 45 of 1 μm particles per AM and 10 of 2 μm ones, they became not to be increased in proportional to the particle concentration.

Fig. 1. Relation of incubation time and average number of phagocytized particles in suspension culture. Vertical bars indicate the standard deviation.

Fig. 2. Relation of incubation time and average number of phagocytized particles in monolayer culture.
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![Graph showing phagocytosis of latex particles](image)

Fig. 3. Relation of particle concentration and average number of phagocytized particles in monolayer culture. The broken lines indicate straight lines with slope of 45 degrees.

**DISCUSSION**

It had been supported by many investigators that macrophages cultured in monolayers and adhering to the surface of glass had different characteristics from ones in suspension culture. To investigate the dependence of phagocytosis of particulate materials on their size, two culture methods, suspension and monolayer culture, were used in this study. However, there were some disadvantages in each culture method in this study. Since AMs were globular form in suspension culture, particles phagocytized were arranged in three dimensions. Therefore, it was difficult to count more than approximately 30 of particles per cell accurately. This indicates that suspension culture is irrelevant to examine the phenomenon, 'saturation of phagocytosis' observed in phagocytic cells which have phagocytized a large quantity of particles. On the other hand, we were able to count a considerable large number of phagocytized particles in monolayer culture. As shown in figure 2, the phagocytic rate increased at early stage of monolayer culture. This fact may be attributable to the continuous increase in the substantial particle concentration around AMs. Rapider increase in phagocytic rate in 2 μm particles than 1 μm ones might be interpreted by the fact that the sedimentation velocity of 2 μm particles is higher than that of 1 μm ones. Therefore, it was difficult to compare the average number of phagocytized particles between 1 μm and 2 μm particles at identical particle concentrations. In conclusion, we examined the difference in the average number of phagocytized particles between 1 μm and 2 μm particles in suspension culture and the phenomenon, 'saturation of phagocytosis' in monolayer culture.
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In suspension culture, there was no significant difference in the average number of phagocytized particles between 1 μm and 2 μm particles in the same particle concentration. Schroeder (1982) also has shown the effect of particle size on internalization of 0.49 or 0.76 μm Latex particles by LM fibroblasts in the same particle concentration, 2000 particles per cell and demonstrated that equal number of the particles were internalized independent of the particle size.

As shown in figure 3, the proportionality between the average number of phagocytized particles and the particle concentration in monolayer culture was lost when the average number of phagocytized particles increased above approximately 45 of 1 μm particles and 10 of 2 μm ones per cell. This phenomenon may be attributable to the saturation of phagocytosis as reported in numerous investigations in vivo (Normann et al., 1965 and Murray, 1963). The sign of saturation was also recognized in suspension culture when more than 10 of either 1 or 2 μm particles were phagocytized by AM. It is interesting that the sign of saturation was observed when the same number of 2 μm particles were phagocytized both in suspensions and monolayers, but this fact was not the case in 1 μm particles. Two possible mechanisms may be advanced to explain the phenomenon, i.e., the different characteristics of AMs between two culture methods and the difference in the methods used to wash non-phagocytized particles.

In the present study, particles which were attached to the cell membrane of AMs were included in counting of phagocytized particles. But it is necessary to distinguish the true phagocytosis (internalization) from cell attachment by such a method as electron microscopic examination of thin sections.

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REFERENCES


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