Cell Size of Alveolar Macrophages: An Interspecies Comparison

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Alveolar macrophages (AM) play a critical role in the removal of inhaled particles or fibers from the lung. Species differences in AM size may affect the number and size range of particles/fibers that can be actually phagocytized and cleared by AM. The purpose of this study was to compare the cell size of rat, hamster, monkey, and human AM by selective flow cytometric analysis of cell volume. Resident AM from CD rats, Syrian golden hamsters, cynomolgus monkeys, and nonsmoking, healthy human volunteers were harvested by standard bronchoalveolar lavage procedures. Morphometric analysis of AM was performed using a flow cytometer that generates volume signals based on the Coulter-type measurement of electrical resistance. We found that hamster and rat AM had diameters of 13.6 ± 0.4 μm (n = 8) and 13.1 ± 0.2 μm (n = 12), respectively. Comparatively, the AM from monkeys (15.3 ± 0.5 μm, n = 7) and human volunteers (21.2 ± 0.3 μm, n = 10) were larger than those from rats and hamsters. The AM from humans were significantly larger (p < 0.05) than those from all other species studied, corresponding to a 4-fold larger cell volume of human AM (4990 ± 174 μm²) compared to hamster (1328 ± 123 μm²) and rat (1166 ± 42 μm²) AM. In summary, we have found marked species differences in the cell size of AM. We suggest that the number and size range of particles/fibers that can be phagocytized and cleared by AM may differ among species due to inherent or acquired species differences in AM cell size. — Environ Health Perspect 105(Suppl 5):1261–1263 (1997)

Key words: alveolar macrophages, flow cytometry, cell size, cell volume, cell diameter, species differences, rat, hamster, monkey, human

Introduction

Alveolar macrophages (AM) are fundamentally involved in mediating the removal of inhaled particles/fibers from the lung. The phagocytosis of fibers that deposit on the alveolar surface by resident AM is one of the major mechanisms by which alveolar epithelial and interstitial cells are protected against the action of fibers. The actual engulfment of fibers by AM is thought to be dependent on fiber size, in particular fiber length (1). Relatively short fibers are suggested to be completely phagocytized by AM, whereas much longer fibers cannot be totally engulfed. Once relatively insoluble fibers have been phagocytized by AM, their removal is thought to be achieved mainly by the translocation of the fiber-containing phagocytes up the mucociliary apparatus (1). However, proliferation of particle clearance can occur through volumetric overload of AM that is, in turn, determined by the normal AM volume (2,3).

Obviously, AM-associated phenomena such as phagocytosis, AM-mediated clearance, and AM overload should be, at least in part, dependent on AM size. We speculate, therefore, that variations in cell size of resident AM among species may result in species differences in the number and size range of particles or fibers that actually can be phagocytized and cleared by AM. Significant differences in AM morphology and cell size among species have already been reported by Haley et al. (4). As the authors indicated, however, the value of their morphometric data is limited because of spreading and flattening artifacts that occur during cytocentrification. Thus, the purpose of our study was to quantitate and compare the cell size of resident AM from two rodent species, rat and hamster, and two primate species, monkey and humans, using selective Coulter-type flow cytometric analysis of unfixed cells in suspension.

Methods

Alveolar Macrophage Donors

Resident rodent AM were harvested from 12 male CD rats (Crl:CD(SN)BR; 250-350 g) and 8 Syrian golden hamsters (Lak:LVG(SYR)BR; 120–150 g). The animals were obtained from Charles River (Kisslegg, Germany). They were kept on a 12-hr light/dark cycle in a conventional, nonbarrier rodent housing unit in polycarbonate cages. Water and standard rat/hamster laboratory diets supplemented with 18,000 IU/kg vitamin A, 1280 IU/kg vitamin D3, and 120 mg/kg vitamin E (ssniff, Soest, Germany) were supplied ad libitum. Resident monkey AM were obtained from three male and four female adult cynomolgus monkeys (Macaca fascicularis), weighing 3.5 to 7.0 kg, that were bred and raised at local animal facilities. The animals were kept in stainless steel cages, and primate chow diet (Alma, Kempten, Germany), fresh fruit, and tap water were supplied ad libitum. Resident human AM were obtained from 10 nonsmoking male volunteers (mean age 25.6 ± 1.2 years) living in the vicinity of Munich and who were not undergoing therapy at the time of the study and had no recent history of pulmonary disease.

Bronchoalveolar Lavage

Rat and hamster AM were obtained by bronchoalveolar lavage (BAL) as described previously (5). Animals were anesthetized by an ip injection of sodium pentobarbital (rat, 30 mg/kg bw; hamster, 24 mg/kg bw). After cannulation of the trachea, the thorax was opened and the lungs were mobilized. The lungs of hamsters were lavaged with ten 5-ml aliquots, and the lungs of rats with ten 10-ml aliquots of sterile, nonpyrogenic phosphate-buffered saline solution (PBS) (Serva, Heidelberg, Germany). Monkey AM were obtained by fiberoptic bronchoscopy and BAL as described previously (6,7). The animals were anesthetized with 15 mg/kg im ketamine hydrochloride (Ketanest, Parke-Davis, Munich, Germany)
and 2 mg/kg im xylazine (Rompun, Bayer, Leverkusen, Germany). With the animal in supine position, a flexible fiberoptic bronchoscope (BF P10, Olympus, Munich, Germany) was wedged into the intermediate bronchus of the left or the right lung. BAL was performed using five 20-ml aliquots of sterile saline solution that was instilled and withdrawn immediately by hand suction. Human AM were harvested by fiberoptic bronchoscopy with BAL as described previously (8). A flexible fiberoptic bronchoscope was wedged into a subsegmental bronchus of the middle lobe or lingula, and five 20-ml aliquots of sterile saline solution were infused, and aspirated immediately.

### Processing of Bronchoalveolar Lavage Samples

Processing of BAL samples was identical for each species studied. The pooled samples were centrifuged at 300 x g for 10 min; the cell pellet was washed twice and resuspended in phosphate-buffered saline. Total cell counts were assessed with a standard hemacytometer (Coulter Electronics, Krefeld, Germany). Air-dried cyt centrifuge smears (500 rpm x 5 min) served to identify the cellular populations after staining with May-Grünwald-Giemsa. A total of 300 cells was counted to determine the percentages of alveolar macrophages, lymphocytes, neutrophils, eosinophils, and mast cells. Cell viability was determined by trypan blue exclusion and proved to be greater than 90% for rat, hamster, and monkey AM, and greater than 75% for human AM.

### Flow Cytometric Analysis of Alveolar Macrophage Cell Size

All morphometric data were obtained using a FACS Analyzer flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with an argon laser and a nozzle orifice of 75 μm as described previously (9). This flow cytometer generates volume signals based on Coulter-type measurement of electrical resistance (10). These signals are directly proportional to the volume of a particle cell and its electrical resistance, in contrast to the forward scatter signal of other flow cytometers that is proportional to the cross-sectional area of a particle cell and to its refractive signal (11) and is considered an unreliable measure for changes in cell volume (12). We calculated morphometric data, such as cell volume and diameter, from selectively gated cells in suspension.

A minimum of 10^4 events was collected with an acquisition rate of 150 events/sec for each sample. The AM population was selectively analyzed by electronic gating of volume-side scatter dot plots; data were recorded in list mode on a Consort 30 data handling system and stored for later analysis. To convert the arbitrary units of the Coulter-type volume signals into geometric units, we generated a calibration curve by plotting the known diameters of a set of microsphere samples of five different particle sizes (Flow Cytometry Standards, Research Triangle Park, NC) against the corresponding volume signals.

### Statistics

Data were expressed as arithmetic means ± standard error of the mean (SEM). Multiple group comparisons were performed using Kruskal–Wallis analysis of variance on ranks, followed by Dunn’s test for multiple comparisons. A two-tailed p value of less than 0.05 was considered significant.

### Results

The BAL differential cell counts are summarized in Table 1. In all species studied, the cells recovered by BAL were greater than 90% AM. The low numbers of neutrophils (< 4%), eosinophils (< 2%), and lymphocytes (< 5%) strongly indicate that AM were obtained from healthy, non inflamed lungs in each species studied. The relatively high number of mast cells detected in the BAL from cynomolgus monkeys appears to be a species-specific phenomenon, as reported previously (7,14).

Table 2 summarizes the morphometric data obtained by selective Coulter-type flow cytometric analysis of unfixed cells in suspension. The cell sizes of hamster and rat AM were not statistically different from each other. Comparatively, the AM from monkeys and nonsmoking, healthy human volunteers were significantly larger (p < 0.05) than those from rats and hamsters. The AM from human volunteers were significantly larger (p < 0.05) than those from all other species studied, corresponding to a roughly 4-fold larger cell volume of human AM compared to hamster and rat AM.

### Discussion

This study was designed to quantitate and to compare the cell size of resident AM from two rodent species, rat and hamster, and two primate species, monkey and humans. A number of reports on the cell volume and diameter of AM from several species, such as mice (4,13), rats (4,15–20), guinea pigs (21), rabbits (22), dogs (4,23,24), pigs (25), monkeys (4), and humans (4,9,26,27), can be found in the literature. The comparability of the data reported, however, is limited because of variations in the methods applied. Quantitative estimations of cell volume and diameter are strongly dependent on both the methods of cell processing, such as fixation, staining, and cytocentrifugation (4,28), and the morphometric technique used. Based on these considerations, we have decided to perform our studies using resident unstained, unfixed AM in suspension and a flow cytometer that generates volume signals based on the Coulter-type measurement of electrical resistance.

The mean cell volume we have found for rat AM (1160 μm³) is in excellent agreement with those reported by Lum et al. (1128 μm³) (17), Dethloff et al. (998 μm³) (19), and Strom et al. (1100 μm³) (18). The mean diameter of rat AM we have calculated here (13.1 μm) is almost identical to that measured by Lum et al. (12.9 μm) (17). The mean cell volume (1328 μm³) and diameter (13.6 μm) of the second rodent species studied, the hamster, proved to be similar to those measured for rat AM. The AM from the two primate species were significantly larger than those from the two rodent species. The AM from cynomolgus monkeys had a mean diameter

### Table 1. Interspecies comparison of BAL differential cell counts (%)

| Species | AM | Rat, n=12 | Hamster, n=8 | Monkey, n=7 | Human, n=10 |
|---------|----|----------|--------------|-------------|-------------|
| Lymphocytes | 1.6 ± 0.4 | 1.9 ± 0.6 | 4.6 ± 0.8 | 5.0 ± 1.4 |
| Neutrophils | 1.4 ± 0.5 | 3.6 ± 1.1 | 0.9 ± 0.1 | 2.1 ± 0.6 |
| Eosinophils | 0.2 ± 0.1 | 1.1 ± 0.7 | 1.9 ± 0.9 | 0.2 ± 0.2 |
| Mast cells | 0 | 0 | 1.9 ± 0.9 | 0.1 ± 0.1 |

Values are means ± SEM.

### Table 2. Interspecies comparison of alveolar macrophage size

| Species | n | Diameter, μm | Volume, μm³ |
|---------|---|--------------|-------------|
| Rat | 12 | 13.1 ± 0.2 | 1186.9 ± 41.5 |
| Hamster | 8 | 13.0 ± 0.4 | 1237.9 ± 123.4 |
| Monkey | 7 | 15.5 ± 0.5* | 1926.4 ± 193.1* |
| Human | 10 | 21.2 ± 0.3* | 4989.9 ± 174.0* |

Values are means ± SEM. *p < 0.05 vs hamster and rat AM. **p < 0.05 vs monkey AM.
of 15.6 µm and a mean cell volume of 1926 µm³. Human AM had a mean diameter of 21.2 µm and a mean cell volume of 4990 µm³. These values are higher than those reported by Crapo et al. (27) but lower than those reported by Haley et al. (4). The much larger diameters measured by Haley et al. for rat (18 µm), cynomolgus monkey (23 µm), and human AM (26 µm) are probably a result of the cell spreading and flattening that occur during cytocentrifugation (4).

Our data confirm previous studies on differences in AM size among species. Even though the AM from rats and hamsters were similar in size, the mean size of human AM was statistically greater than that for all other species studied, including nonhuman primates. Moreover, we assume that our quantitative data on AM size obtained by Coulter-type measurements of unfixed cells in suspension more accurately reflect the real dimensions of AM than morphometric analyses of cytopsins that result in much larger diameters due to preparation artifacts. The size of AM appears to be one of the limiting factors of AM-associated events such as phagocytosis, AM volumetric overload, dissolution of particles/fibers within AM, and AM-mediated clearance. Therefore, we suggest that species differences exist in the size range of particles/fibers that can be phagocytized, dissolved, and cleared by AM, due to inherent or acquired species differences in AM size.

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