

# Effect of aging on cytoskeleton system of Kupffer cell and its phagocytic capacity \*

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**Subject headings** Aging; Kupffer cell; cytoskeleton; phagocytosis

## Abstract

**AIM** To investigate the age-related alterations of cytoskeleton system in liver Kupffer cell and their relation to the changed phagocytic function.

**METHODS** The phagocytic function of Kupffer cells from rats of various ages (6 mo, 12 mo, 18 mo and 24 mo) were quantitatively evaluated by phagocytosis of polystyrene beads. The actin distribution and measurement of Kupffer cell were determined by a phalloidin-TRITC method; and the myosin and vimentin distribution and measurement with indirect immunohistochemical staining.

**RESULTS** Aging resulted in significant alterations of actin, myosin and vimentin distributions and reductions in Kupffer cell; the 3 cytoskeleton components of 24-month-old Kupffer cell were significantly decreased to 68.0%, 84.9% and 75.5%, respectively of these of 6-month-old Kupffer cell ( $P < 0.01, 0.01$  and  $0.01$ ). And these decreases had significant positive relations with the damaged phagocytosis of the aged Kupffer cell.  $\gamma$  values were  $0.96 (P < 0.05)$ ,  $0.99 (P < 0.01)$  and  $0.95 (P < 0.05)$  respectively.

**CONCLUSION** The cytoskeleton system of the aged Kupffer cell presents an evident state of senescence, which may be an important mechanism of decreased phagocytosis of the aged Kupffer cell.

## INTRODUCTION

Kupffer cells account for about 30% of nonparenchymal liver cells and constitute the largest pool of resident macrophages in the body. They play an essential role in the elimination of foreign substance derived from the systemic circulation mainly through phagocytosis. The age-related alterations in Kupffer cell function are considered to be related to the susceptibility to sepsis after trauma or infection or to tumor of the old people<sup>[1]</sup>. In this study, the distribution and contents of actin, myosin and vimentin in Kupffer cells of various ages, and a quantitative evaluation of phagocytosis of polystyrene beads by primary cultured Kupffer cells of various ages will be described.

## MATERIALS AND METHODS

### *Animals and groups*

Twenty-four Wistar rats of different ages obtained from the Chinese Herb Research Institute of Sichuan Province were rendered into 4 groups (6, 12, 18 and 24 months of age). Each group had 6 rats.

### *Kupffer cell isolation and culture*

Liver nonparenchymal cells were isolated by a collagenase-perfusion method as reported previously<sup>[2]</sup>. Briefly, after anesthesia (30 mg of barbital/kg body wt., intraperitoneally), the liver was perfused *in situ* with  $Ca^{++}$ -free Hanks balanced salt solution at 37°C for 3min. Then 0.05% collagenase (Sigma, Type IV) was added and the liver was perfused for a further 4min with Hanks balanced salt solution. After gentle shaking, the suspension was filtered and hepatocytes were sedimented at  $50 \times g$  for 3min. Non-parenchymal cells from the treatment were collected and sedimented at  $300 \times g$  for 10min. The pellet of nonparenchymal cells was resuspended and cultured with RPMI 1640 medium (containing 15 mmol/L HEPES, 0.05 U/ml insulin, 15 mmol/L L-glutamine, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin) supplemented with 10% newborn calf serum. After 30min, the non-adherent cells were deleted. The viability of the KC was greater than 90% as determined by trypan blue exclusion.

### *Distributions and content measurements of actin, myosin and vimentin of Kupffer cell*

Kupffer cells were fixed on a plastic dish and the phalloidin-TRITC (Sigma) method was used for

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staining actin by a previously reported method<sup>[3]</sup>. The samples were observed under fluorescence microscope (Olympus VANOX) and the actin contents were determined using a fluorometer (Hitachi MPF-4). Indirect immunochemical staining was performed for myosin and vimentin using anti-myosin antibody and anti-vimentin antibody(Sigma)<sup>[4]</sup>. The samples were observed by using a 40-fold objective (Olympus VANOX). The staining images were collected by means of a Panasonic CL320 videotape camera and sent into an image analyzing computer system (CMIAS007) for quantitative assessment of the gray scale.

#### Quantitative analysis of phagocytic activity

The phagocytic function of Kupffer cell was quantitatively measured by a method previously reported<sup>[4,5]</sup>. Cultured Kupffer cells were incubated with RPMI 1640 medium supplemented with 10% fetal calf serum at 37°C for 6 h. After the incubation,  $1.7 \times 10^8$  polystyrene beads/dish (diameter 1.1  $\mu\text{m}$ , Sigma) were added to the cultures, which were maintained for a further 60min at 37°C. The cultures were washed three times with RPMI 1640 medium and the numbers of beads in Kupffer cells were counted under an inverted phase contrast microscope (XSJ-D), Chongqing Optical Electric Appliances Plant). One hundred Kupffer cells randomly selected from each of 5 different cell preparations were used for the phagocytosis study.

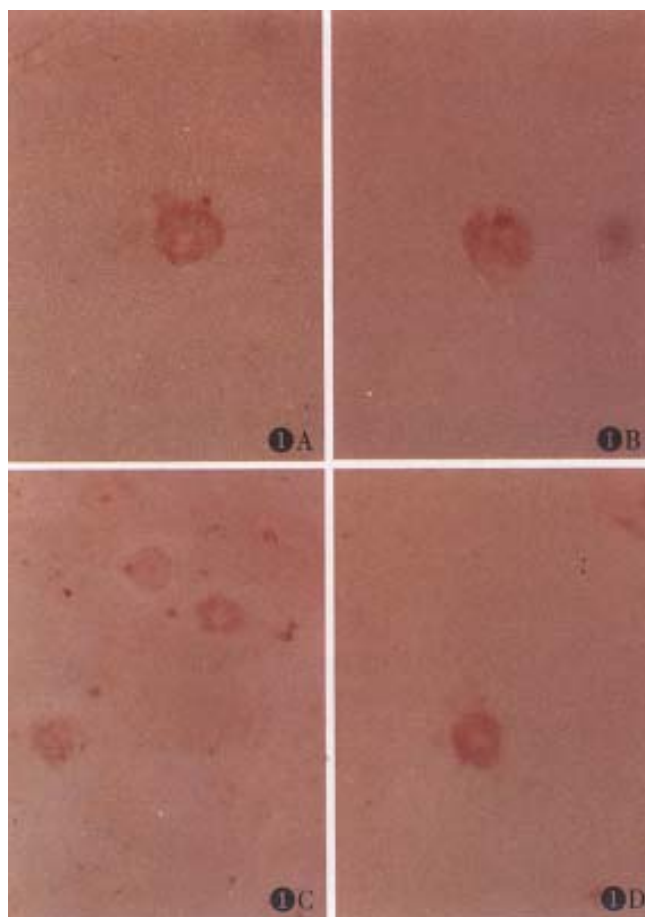
#### Statistical analyses

The results were expressed as  $\bar{x} \pm s$  and statistical analyses were made with one-way analysis of variance and Student's *t* test.

## RESULTS

### Distribution of actin, myosin and vimentin in rat Kupffer cells at various ages

Aging caused morphological changes in cytoskeleton system of Kupffer cells are shown in Figure 1. Kupffer cells of 6-mo-old rat appeared flat and there were several pseudopodia on the surface. The myosin was shown in the pseudopodia. In fluorescent staining for actin, intense specific fluorescence was observed in both the cytoplasm and the peripheral region along pseudopodia. The distribution of actin was similar to that of myosin. Vimentin positive fibres extended throughout the whole cytoplasm and a perinuclear accumulation was also present in 6-month-old Kupffer cells. Kupffer cell of 24-month-old rat appeared small and circular and the number of pseudopodia decreased. Weak and diffuse fluorescence and staining could be observed around the pseudopodia and the nuclei of the aged cells in actin and myosin staining respectively. Weak staining could also be observed throughout the cytoplasm of the aged Kupffer cells in vimentin staining.



**Figure 1** Distribution of myosin and vimentin of 6-month-old (a,b) and 24-mo-old(c,d) Kupffer cells (ABC $\times$ 400). a and c: myosin;b and d: vimentin.

**Table 1** The actin, myosin and vimentin contents of Kupffer cells of each age group ( $\bar{x} \pm s$ )

Group	Actin (n = 6) (ng phalloidin/ $\mu\text{g}$ prot.)	Myosin (n = 5) (gray scale)	Vimentin (n = 5) (gray scale)
6 mo	8.63 $\pm$ 0.60	134.88 $\pm$ 8.14	137.50 $\pm$ 5.71
12 mo	8.24 $\pm$ 0.53	141.46 $\pm$ 5.18	129.91 $\pm$ 7.01
18 mo	6.20 $\pm$ 0.71 <sup>b</sup>	121.89 $\pm$ 7.52 <sup>a</sup>	118.13 $\pm$ 13.54 <sup>a</sup>
24 mo	5.87 $\pm$ 0.49 <sup>b</sup>	114.45 $\pm$ 5.09 <sup>b</sup>	103.08 $\pm$ 8.41 <sup>b</sup>

<sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.01, vs 6 mo.

### Quantitative measurement of the 3 cytoskeleton components of Kupffer cells at various ages

Aging resulted in significant reductions of the 3 cytoskeleton components mentioned above, i. e., the contents of actin, myosin and vimentin in 24-mo-old KC were significantly decreased to 68.0%, 84.9% and 75.5% respectively of those in 6-mo-old KC (*P*<0.01,0.01 and 0.01), (Table 1).

### Phagocytic activities of Kupffer cells from each group

In the 6-mo group,  $19.6 \pm 2.1$  beads were taken up by cultured Kupffer cells during the 60min observation period. In 12-mo, 18-mo and 24-mo

groups,  $20.8 \pm 2.1$ ,  $12.7 \pm 1.5$ ,  $8.6 \pm 2.3$  beads were taken up into Kupffer cells respectively. The difference between 6-month-old group and 12-month-old group was not significant ( $P > 0.05$ ). The difference between 6-month-old group and 18-month-old group and that between 6-month-old group and 24-month-old group were significant ( $P < 0.05$ ).

### Analysis of correlation

Significant positive correlation was found between the changes of actin, myosin and vimentin contents in Kupffer cell aging and the damaged phagocytosis.  $\gamma$  values were 0.96 ( $P < 0.05$ ), 0.99 ( $P < 0.01$ ) and 0.95 ( $P < 0.05$ ), respectively.

## DISCUSSION

Kupffer cell is the main component of the host monocyte-macrophage system. It is crucially important for the host to fight against infection or sepsis<sup>[6]</sup>. The previous study showed that the decreased phagocytosis of the aged Kupffer cell was responsible for the increased severity of pathophysiological changes after endotoxemia<sup>[7]</sup>. So it is of significance to study the mechanism of decreased phagocytosis in Kupffer cell aging.

A 24-month-old rat was used in the present experiment as the aging model, which was comparable to the age of 65 - 75 in the human being, an age period consistent with the standard of old people of our country. So 24-month-old rat may serve as a qualified model for the study of Kupffer cell aging<sup>[2]</sup>.

The mechanism of phagocytosis by Kupffer cells is still not completely understood. The ruffling of cell membrane and formation of pseudopodia play an important role in the phagocytosis of Kupffer cells and this is believed to be accomplished by the cytoskeleton. In the cytoskeleton, actin-myosin interaction through the calcium-calmodulin systems plays a major role in this activity<sup>[8]</sup>. In this system, intracellular  $Ca^{2+}$  combined with calmodulin to form the active calcium-calmodulin complex, which activates an enzyme, myosin light chain kinase, for phosphorylating the light chain of

myosin. Phosphorylated myosin, but not unphosphorylated myosin, can interact with actin to induce the activity of cell membrane and pseudopodia and then phagocytosis. The process of this system is reversible, in that a phosphatase can catalyze dephosphorylation of myosin, restoring it to a form that can not be activated by actin.

Vimentin is another important cellular cytoskeleton component. It is in radial arrangement in cytoplasm, forming a frame to support the actin-myosin system and other organelles. It can prevent the cell from being injured by changing its tension, thus keeping the cellular shape.

In the current study, the distribution and determination of actin, myosin and vimentin in Kupffer cells at various ages were studied and our observations revealed that the distributions of the 3 cytoskeletons dramatically changed and their contents significantly decreased in aged Kupffer cells. The actin fluorescence and myosin staining in the area of the pseudopodia became weak in the aging Kupffer cells. The vimentin staining throughout the whole cytoplasm became weak too. These results indicate that the age-related damage of the cytoskeleton system in Kupffer cell aging is one of the important mechanism responsible for a decrease in phagocytosis.

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