Change in Performance of BALB/c Mouse Pulmonary Macrophage Surface Receptor after Exercise and its Influence on Phagocytic Activity

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Abstract: *Objective: To study the effect of exercise on phagocytosis by pulmonary bronchoalveolar macrophages (BAMs). Methods: A total of 120 seven- to nine-week-old male BALB/c mice were randomly assigned into the following groups based on exercise intensity on a treadmill: control exercise (CE) group, acute moderate exercise (ME) group, and strenuous exercise group. Lung lavage was conducted to collect BAMs from the mice. Phagocytic behavior and surface receptor expression on BALB/c mouse BAMs were analyzed through fluorescence microscopy and flow cytometry. Results: In the SE group, expression levels of macrophage scavenger receptors (surface receptor [SR-A] type I/II and macrophage receptor [MARCO], complement receptor3 (CR3), and intercellular adhesion molecule 1 (ICAM-1) were upregulated; by contrast, expression level of extensive G-type immune globulin receptor (Fc Rs) was not upregulated. The promoting percentage of phagocytosis in the CE group was 100%; the highest promoting percentage of phagocytosis was 161% observed in MARCO, followed by 116% detected in CR3; the promoting percentage of phagocytosis found in SR-A type I/II and ICAM-1 increased by approximately 65%. Indeed, these scavenger receptors were involved in phagocytosis induced by macrophages. MARCO was also necessary to elicit a stimulatory effect on macrophage phagocytic activity. Conclusions: The phagocytosis of unopsonized particles was possibly mediated by MARCO expression.*

Keywords: *Exercise, Pulmonary bronchoalveolar macrophage, BALB/c mouse, Phagocytosis, Scavenger receptor.*

Introduction

Exercise is an important bodily activity. Moderate and regular low-intensity exercise can improve cardiopulmonary function and immunocompetence. Clinical exercise intervention is often recommended for diseases, such as diabetes; exercise intervention also impedes cancer and HIV development [11]. Hoffman-Goetz et al. [8] indicated that exercise can alleviate pulmonary tumor growth. Davis et al. [4, 5] showed that strenuous exercise can inhibit cancer cell proliferation and expansion; by contrast, moderate exercise fails to elicit similar effects.

Studies on the relation of exercise to immunity have originated from the combination of exercise physiology and hematology. Researchers indicated that concentrations and types of leukocytes present in blood under different exercise states vary from other leukocytes when the body is at rest. Epidemiologic studies have suggested that regular and moderate exercise can improve immunity; however, strenuous exercise can reduce immunity because individuals may be susceptible to infection as a consequence of weakened immunity several hours after strenuous exercise [1]. Other researchers showed that exercise can reduce death rates of cancer patients. Therefore, exercise can alter pulmonary immunity and affect pulmonary macrophage activity.

Pulmonary macrophages develop through monocyte differentiation; these cells are widely distributed in pulmonary interstitium. Phagocytosis, immunity, and secretion of pulmonary macrophages occur to eliminate foreign particles, participate in metabolism of substances on the pulmonary surface, and activate immune system when individuals become infected. However, the effect of exercise on macrophages has been rarely investigated; likewise, pulmonary macrophages have also been rarely studied. Fehr et al. [6] discovered that peritoneal macrophage metabolism, lysosomal enzymatic activity, and latex particle digestion occur and reach maximum levels after mice undergo strenuous exercise; these activities yield average levels after mice experience moderate exercise, and these activities are the weakest when mice are not subjected to exercise [6]. Ortega et al. [12] used different exercise methods and animal experiments to investigate peritoneal macrophage activity and reported results similar to those of Fehr et al. [6]. Therefore, exercise can influence macrophage activity, but specific mechanisms remain unclear. Further studies on the effect of exercise on pulmonary macrophages should be conducted.

This research particularly focuses on the changes in expression of macrophage surface receptors and their effects on the phagocytic activity of pulmonary macrophages after acute moderate exercise and after strenuous exercise. This research aimed to provide a basis to further investigate the relation of exercise to the expression of pulmonary macrophage surface receptors. This research also aimed to determine the underlying regulatory mechanism.

Materials and methods

Materials

A total of 120 seven- to nine-week-old pure bred male BALB/c mice with weights ranging from 20 g to 25 g were selected randomly and assigned to the following groups based on exercise intensity on a treadmill: control exercise (CE) group, moderate exercise (ME) group, and strenuous exercise (SE) group. Each group was composed of 40 mice. The SE group was further divided into the following subgroups according to SE duration: 1 h, 4 h, and 16 h: post-SE 0 h; post-SE 1 h; and post-SE 16 h. Pure bred BALB/c mice were purchased from the Institute of Laboratory Animal Sciences, CAMS & PUMC. None of the mice manifested any disease symptoms.

Methods

Running test procedure of the mice

A classified running test procedure [2] was performed in this experiment. Speed and inclination of a ZH-PT experimental animal transmission treadmill were adjusted, and exercise load was gradually increased with time until the mice satisfied the experimental requirements. A gas analyzer was used to measure maximal oxygen consumption ($V \cdot O_2max$). Based on the standard curve described by Fernando et al., exercises with 20%, 50%, or 80% of $V \cdot O_2max$ are respectively defined as low-intensity, moderate-intensity, and strenuous exercise [7]. The CE group was kept stationary for the entire period. ME and SE groups started exercise at 9 m/min and accelerated at an interval of three minutes. The intensity of the ME group was set at 17 m/min. The SE group was subjected to exercise on the treadmill until the mice became exhausted.

Reagents

Chemical reagents, antibodies, protein products, and plastic products were used in the study. Plastic fluorescent granules, fetal calf serum, bovine hemoglobin, purified mouse immunoglobulin type G (IgG), anticoagulant, and cell lysis buffer for the macrophages were purchased from American Sigma Company. Cell culture materials were purchased from

British GIBCO Company. Other chemicals were purchased from Shanghai Shenggong Bioengineering Technology Service Co., Ltd.

Instruments

The following instruments and equipment were used: centrifugal tubes and pipettes (Eppendorf China Co., Ltd.), transmission treadmill (American Diagnostic & Research, Instruments Company), confocal microscope (German Leica Company), centrifuge machine (American Sigma Company), flow cytometer (American Bacton Company), nitrite and nitrite colorimetric detection kit (American MD Company), and ultra-pure water instrument (American Millipore Company).

Macrophage collection

Bronchopulmonary lavage was conducted several times to collect the pulmonary macrophages from the mice. Several tubes containing 1 ml of sterile saline solution were warmed for several hours at 37 °C in a cultivation container before lung lavage. Approximately 0.1 ml to 0.15 ml of Nembutal was injected into the abdomen of the mice. The mice were anesthetized at a supine position and fixed on a dissection disc. Ethyl alcohol (70%) was used to disinfect the abdomen. An incision was made with the shape of "工" from the neck to the diaphragm cortex. The carotid artery was cut to eliminate blood supply, and lungs were exposed carefully to avoid damage to cardiopulmonary structures. A suture needle was used to make a small incision on the trachea after all blood supply was drained. Care was taken not to cut the trachea completely. A cannula was inserted into the trachea and secured with surrounding sutures. A slipknot was added to facilitate pulmonary lavage.

Warm 1 ml of saline solution was injected through the cannula to fill the entire lung carefully to avoid bursting. The solution was aspirated back into the cylinder. The process was repeated five times by using fresh saline solution in each lavage. Six tubes of 1 ml of lavage liquid were collected and placed on ice to retard cell metabolism. The pulmonary macrophages were collected through centrifugation at 800×*g* for 8 min. The supernatant obtained from the tube containing liquid from the first lavage was marked "BAL1S"; the remaining cellular sediments were divided and placed in six separate tubes according to the purpose of the experiment. Cell concentrations were adjusted using saline solution or RPMI 1640 cultivation liquid to reach 1×10^6/ml to 2×10^6/ml. The samples were then analyzed in terms of phagocytosis and cell cultivation.

Phagocytosis analysis

Bovine serum albumin (BSA) and immune globulin were used to coat the plastic particles tainted with fluorescence to analyze the phagocytosis of maladjustment and adjustment particles. The macrophage sample (2×10^6/ml) and the coated fluorescent plastic particles (3×10^8/ml) were mixed at a ratio of 1:40 and then incubated and allowed to react at 37 °C. The mixture was patted gently several times at an interval of 10 min to ensure that particles were uniformly distributed. After 1 h, phosphate buffer solution (PBS) was added to terminate the reaction, and bound cells were separated from unbound plastic particles through centrifugation at 800×*g* for 10 min. The isolated cells bound to the coated particles were fixed in stationary liquid (4% formaldehyde) at 4 °C for 2 h. Fluorescence microscopy and flow cytometry were performed to determine macrophage phagocytic ability. The measured parameters were as follows: percentage of phagocyte (%P) = (number of cells containing phagocytosed particles/total number of cells tested) ×100%; the average phagocytosed particles (PI) = total number of cytophagy particles/total number of cells tested.

Cellular immunofluorescence staining

The fixed macrophage sample was suspended in a centrifuge tube, mixed with a primary antibody (such as MARCO), and stored in a refrigerator at 4 °C overnight. The cells were washed with PBS twice and resuspended in 60 ml of PBS; afterward, a secondary antibody (such as IgG) was added to a fluorophore. The resulting mixture was washed again twice after this mixture was allowed to react for 1 h at room temperature in the dark. The fluorescence intensity of each macrophage with immunofluorescent staining was measured using a flow cytometer. The relative molecular density and distribution of particles with conjugated focal planes were determined.

Data analysis

Data were expressed as mean ± SEM. Differences at $p < 0.05$ were considered significant and those at $p < 0.01$ were considered highly significant. A two-tail test was employed to compare two groups, and one-way ANOVA was utilized to compare groups of more than two. If a significant difference was detected, q-test was further employed to compare two sets of data at a time. Results were processed using SAS 9.2.

Results and discussion

Results

Pulmonary macrophage surface receptors are found in several forms: two types of scavenger receptors (SR-A type I/II and MARCO), first type cell adhesion molecule (ICAM-1), third complement receptor (CR3), and extensive G-type immune globulin receptor (Fc Rs). The results on the expression of macrophage surface receptors after SE are shown in Table 1. After SE, the expression levels of SR-A type I/II, MARCO, ICAM-1 and CR3 increased; by contrast, the expression level of Fc Rs remained unchanged. If the CE group yielded 100%, the percentage of increase in expression of MARCO was found to be the highest at 161%, followed by that of CR3, which was 116%. The percentage of increase in SR-A type I/II and ICAM-1 was approximately 65%.

Table 1. Influence of strenuous exercise on the expression percentage of pulmonary macrophage surface receptors

Receptor	Antibody	MFI of BAM receptor		SE-induced increase, (%)
		Control	Post-SE 0 h	
SR-A type VII	2F8	49.3 ± 7.4	62.6 ± 5.6*	65.4 ± 5.3
MARCO	ED31	9.6 ± 1.4	24.6 ± 7.1*	161.2 ± 8.4
SR-A&MARCO	2F8&ED31	45.9 ± 12.5	71.3 ± 10.7*	68.1 ± 13.9
ICAM-1	Polyclonal	32.2 ± 13.0	57.0 ± 3.9*	64.2 ± 14.0
CR3	M1/70	25.7 ± 2.4	56.2 ± 3.9*	116.6 ± 2.3
Fc Rs	Murine IgG	47.3 ± 11.2	53.6 ± 2.8	14.9 ± 12.8

Note: * comparison with the corresponding CE group and the presence of statistically significant difference at $p < 0.05$ ($n = 10$).

The effect of the increase in expression of macrophage surface receptors on BAM phagocytic ability was analyzed on the basis of the blocking effect of antibodies. BAM phagocytic activity was inhibited by the single plant antibody ED31, and the inhibitory effects were 15% and 55% in the CE group and Post-SE 0 h, respectively (Fig. 1). The inhibitory effects of 2F8 antibody were approximately 5% and 25%, respectively, for both groups. These findings suggest that MARCO and SR-A I/II receptor participate in the phagocytic activity of

pulmonary macrophages; both types of receptors elicit stimulatory effects, and the greatest influence was observed in MARCO.

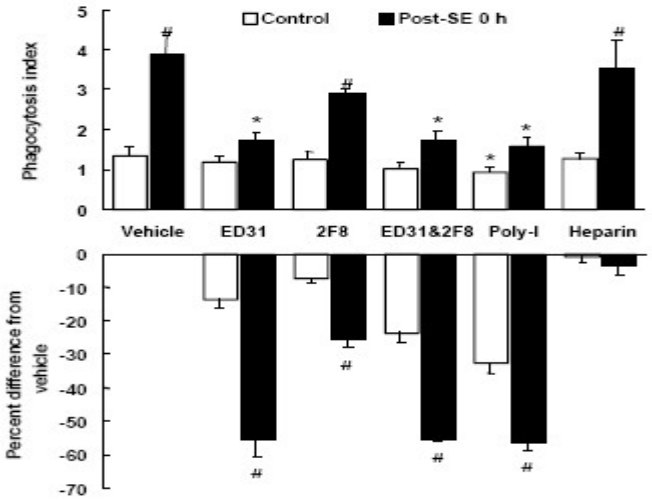

Fig. 1 Effect of surface receptor antibody and inhibitor on BAM phagocytic activity
Note: * significant difference ($p < 0.05$) between the compared phagocytosis adjustment particle values in the same group;
significant difference ($p < 0.05$) compared with the CE group.

ICAM-1 is a multi-plant antibody eliciting a neutralization effect; this antibody was used to determine the effect of the increased expression level of ICAM-1 on BAM phagocytic activity and maladjustment particles (Fig. 2). The increased expression level of ICAM-1 caused a dose-dependent inhibitory effect on CE and Post-SE 0 h groups; by contrast, this increased expression level did not affect G-type immunoglobulin. At the same antibody concentrations, the inhibitory intensity of the Post-SE 0 h group was slightly higher than that of the CE group. The maximum inhibitory percentage exceeded that of the Post-SE 0 h group by approximately 30% and that of the CE group by approximately 20%. This result suggested that the degree of participation of ICAM-1 in maladjusted particle phagocytosis increased after SE; thus, ICAM-1 likely affected macrophage phagocytic activity.

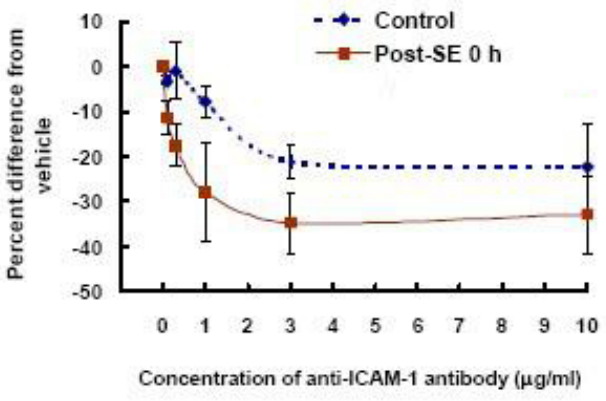

Fig. 2 Effect of the neutralized antibody in ICAM-1 on BAM phagocytic activity

After ICAM-1CR3 combination was achieved, cell adhesive forces increased. The combined effects of the single-plant antibody and the antibody that could bind CR3 were determined. No neutralization effect was found. CR3 was crosslinked and simulatedCR3 was combined by ICAM-1. Fig. 3 shows the results of the effect of increased expression of CR3 on maladjusted

particles phagocytosed by BAM. After CR3 was crosslinked, phagocytic ability increased by >30%. The effect was adequate to reverse the inhibitory effect caused by the ICAM-1 antibody. This result also indicated that the combination of ICAM-1 and CR3 stimulated BAM phagocytic activity.

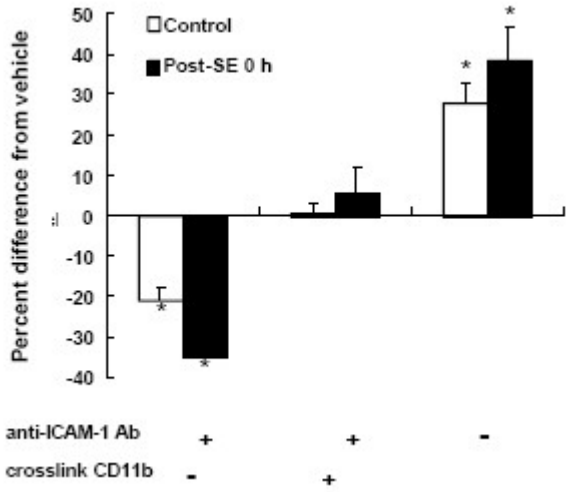

Fig. 3 Effect of ICAM-1 and CR3 crosslinking on BAM phagocytic activity
Note: * significant difference ($p < 0.05$) between the comparison of the adjustment particle phagocytosis values in the same group.

Discussion

Pulmonary macrophage activation induced by exercise is strongly observed in the phagocytosis of maladjusted particles. Scavenger receptors are involved in cell phagocytosis of maladjusted particles. This study showed that SR-A type I/II and MARCO expression levels in the pulmonary macrophages of the Post-SE 0 h group were increased by approximately 65% and 161%, respectively. After a reaction with single-plant antibodies 2F8 and ED31 occurred, BAM phagocytic activity in the Post-SE 0 h group was inhibited. In particular, antagonism by MARCO antibody inhibited phagocytosis; by contrast, SE promoted phagocytosis. Although the expression level of MARCO was lower than that of SA-A type I/II, the effect of MARCO on the phagocytosis of maladjusted cells was considered more important than that of SR-A type I/II. Among surface receptors, SR-A type I/II and MARCO involved in congenital immunity have been extensively investigated [1]. Without adjustment of antibody or complement, binding may exist between host cells and fluorescent particles, environmental particles, or some bacteria. Removal of apoptotic thymic cells by macrophages through SR-A upregulation has been previously shown. The macrophage surface receptors are important in signal transduction and activation of cell phagocytosis of foreign antigens. For instance, the phagocytosis and pathogenesis of mycobacterium tuberculosis are both promoted by SR-A type I/II [13]. This research first demonstrated the activation of expression of the two pulmonary macrophage surface receptors after SE. Only a few studies explored the adjustment of SR-A type I/II and MARCO expression. The hyperplasia-stimulating factors of the macrophage could promote the expression of SR-A type I/II. After thallus or LPS was intravenously injected, MARCO expression increased. In some tissues with normal macrophage levels, no effect on MARCO expression was observed. This result showed the importance of MARCO in immune regulation during bacterial infection [14].

In addition, the expression of CR3, ICAM-1, Fc Rs and other surface receptors was analyzed. Results show that the expression of CR3 and ICAM-1 in the Post-SE 0 h group was both upregulated compared with those in the CE group. In contrast, no significant change was seen in Fc Rs expression. Hashimoto et al. [9] demonstrated that exhausting exercise, such as swimming, can increase the expression of the granular leucocyte CR3 in the blood but has no influence on ICAM-1. Jordan et al. [10] pointed out that marathon can increase the expression of granular leukocyte CR3 in the blood, but moderate running causes no change. The CR3 molecule is involved in absorption, phagocytosis, and cell activation. This research showed that BAM phagocytosis of the IgG/C particles in the Post-SE 0 h group was greater than the phagocytosis of maladjusted particles; therefore, the increase in CR3 expression caused by exercise did not contribute significantly to the phagocytosis of maladjusted particles. This finding might be explained by a remarkable increase in cytophagy of maladjusted particles in the Post-SE 0 h group; thus, the function of an antibody or a competent receptor to promote space activity was impeded. Furthermore, this research found that the neutralized multiple-plant antibody in ICAM-1 can inhibit the phagocytic ability of pulmonary macrophages. The inhibitory effect disappears after crosslinking with CR3 occurred. The single crosslinking of CR3 can also stimulate cytophagy; this result suggested that CR3 and ICAM-1 of the pulmonary macrophage regulate phagocytic activity. The binding activity of the carboxyl terminal structure of CR3 has been investigated, revealing that this substance can combine with β-glucan [13]. This combination exposes the structural domain of CR3, which promotes further interaction with iC3b to activate respiratory burst. ICAM-1 can also stimulate phagocytosis and activity of cytohormone. This function can be achieved through the combination of b2 with molecules belonging to the same protein family. Moreover, signal transmitted via CR3 can facilitate the activity of other receptors or cytohormones. Synergistic effect activates macrophages [3].

In summary, SE can increase the phagocytic ability of pulmonary macrophages in BALB/c mice. Stimulatory effect is achieved through the increased expression level of surface receptors. For example, the expression level of MARCO is increased. Approximately 30 min of exercise is sufficient to increase MARCO levels. Newly synthesized MARCO receptors are initially stored intracellularly and subsequently transported to the cytomembrane when MARCO receptors are stimulated by appropriate factors; these receptors are then expressed on the cell surface. In conclusion, exercise induces increased expression of MARCO, thereby increasing pulmonary macrophage phagocytic activity and accelerating the elimination of foreign particles from pulmonary tissues. These events lessen tissue damage and promote pulmonary immunity. Further studies on the effect of exercise on ME, SE1, and SE16 groups will be conducted.

Conclusions

In this paper, the influence of exercise on the performance of pulmonary macrophage surface receptor was described to further understand the mechanism of pulmonary immune response adjustment though exercises. The experimental results indicate the following: 1. Acute exercises elicit a promoting effect on the phagocytic activity of BALB/c mouse pulmonary macrophage; 2. The change is observed by increasing the performance of the surface receptor; 3. For the players, the expression of the pulmonary macrophage surface receptor MARCO can rapidly clear most of the foreign matters in the pulmonary tissue; as a result, tissue injury is reduced and pulmonary immune ability is enhanced.

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