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Effects of Resistance Exercise Intensity on Cytokine and Chemokine Gene Expression in Atopic Dermatitis Mouse Model

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Abstract

Background and Objective

Although the evidence is unclear, literature indicates that resistance exercise reduces inflammation in colorectal disease. The purpose of this study was to identify the effects of colon tissue on cytokine and chemokine gene expression with changes in resistance exercise intensity.

Material and Methods

We divided male BABL/c mice into 6 groups (each group n=10, total=60) (control group: CON, low resistance exercise group: EX_L, high resistance exercise group: EX_H, atopic dermatitis group: AD, atopic dermatitis+low resistance exercise group: AD+EX_L, atopic dermatitis+high resistance exercise group: AD+EX_H) and subjected them to ladder climbing resistance exercise for 4 weeks. After 24 h of each exercise schedule, a real-time polymerase chain reaction was performed to determine mRNA expression of interleukin-6 (IL-6) and chemokine ligand 20 (CCL20).

Results

The AD group showed significantly higher mRNA expression of IL-6 and CCL20 compared with the CON, EX_L, EX_H, AD+EX_L, and AD+EX_H groups ($p<0.05$).

Conclusion

In conclusion, both high and low resistance exercise effectively decreases the concentration of IL-6 and CCL20 in mice with and without AD.

Atopic dermatitis (AD) is an allergy involving chronic inflammation of the skin.[1] The symptoms include scab formation with itching, erythema, exudation, edema, and boils; the condition requires long-term medical care and is often accompanied by complications such as rhinitis and asthma. It is

assumed that these phenomena are the result of genetic, environmental, and immunologic factors and stress.[2] However, no accurate mechanism of atopy is currently known.

Recently, it has been reported that immunological diseases such as atopy are closely related to kinesitherapy.[3,4] In addition, the risk of disease and development of cancer due to inflammation in the large intestine as a result of decrease in physical activity and dietary habits has been reported.[5] Recent risk factors for disease of the large intestine include decrease in physical activity, inflammatory enteropathy, and stress, which induce inflammatory as well as allergic responses in the large intestine.[6,7] Additionally, physical activity confers a preventive effect on disease of the large intestine in comparison with inactivity.[8] Similarly, it has been reported that exercise has a positive effect on large intestine-related disease;[9] especially, resistance exercise shows positive effects in terms of improvement in muscular strength and organs. Moreover, gene expression patterns differ based on the intensity of the exercise.[10]

A recent study reported that atopic dermatitis (AD) is related to immunological mechanisms, and T lymphocytes play an important role in AD, an autoimmune disease.[11] Atopy results from an imbalance between Th1 and Th2 responses; Th2 also produces interleukin-6 (IL-6).[12] AD in a mouse model is usually induced by a chemoantigen such as 2,4-dinitrochlorobenzene (DNCB) and house dust mites.[13]

IL-6 is closely related to atopy and is a well-known inflammatory cytokine. Macrophages in the mucous membrane of the digestive tract are known to induce inflammation in response to endotoxins and bacteria, as well as to regulate secretion of IL-6.[14] Additionally, IL-6 is part of the structurally homonomous cytokine superfamily, is produced by various cells involved in the inflammatory response, and acts as a chemoattractant to white blood cells around the area of inflammation.[15] Therefore, it would be very interesting to elucidate the role of IL-6 in areas of the body influenced by exercise.[16]

Chemokines are substances that can regulate inflammation-related cellular migration and can be rapidly expressed and secreted. Chemokine ligand 20 (CCL20), an important factor in autoimmune disease, has been recently reported to be involved in atopy.[17] Though previous atopy-related studies have included exercise, there is a lack of studies on the effects in the large intestine. The aim of this study was to investigate the effect of resistance exercise exerted on gene expression within the large intestine during induction of atopy. Therefore, this study details the effects of ladder resistance exercise on protein and mRNA expression of CCL20, a representative chemokine, and IL-6, an inflammatory cytokine, and their relation to large intestine disease in a mouse model of AD.

METHODS

Experimental Animal and Dietary Method

A total of 60 male BABL/c mice (8 weeks old and weighing 20 g each) were randomly assigned into 6 groups of 10 mice each (control group: CON, low resistance exercise group: EX_L, high resistance exercise group: EX_H, atopic dermatitis group: AD, atopic dermatitis+low resistance exercise group: AD+EX_L, atopic dermatitis+high resistance exercise group: AD+EX_H). Commercially available Samtako mouse food (Samtako, Osan, Korea; protein 22.5%, fat 3.5%, low-fibre, ash 9.0%, calcium 0.7%, phosphorus 0.5%) and water were supplied at libitum, with light cycles set to 12 hours, ambient temperature of 22±2°C, and humidity of 70–80%. Animals were housed at a vivarium in the Gwangju Institute of Science and Technology. All study protocols were approved by the Gwangju Institute of Science and Technology.

DNCB and House Dust Mite Dermatitis Induction

Atopy dermatitis was induced by applying and stripping tape (3M tape, 5 times) off the ear lobes of 8-week-old, male BABL/c mice. Additionally, 10 μl DNCB (1%) and house dust mites (10 mg/mL) were administered once a week for 4 weeks. The animals were subjected to ladder resistance exercise as previously described.[1,13]

Experiment Design And Exercise Method

The ladder exercise device used in this study was manufactured as previously described.[18] Firstly, mice performed the ladder climbing exercise without weights applied to the tail to acclimatize them to the exercise for a week, using a 1 m ladder. Ladder climbing exercise was performed 8 times at 2-minute intervals.

Low Intensity Exercise (LIE) included a weight measuring 40–50% of body weight attached to the tail, High-Intensity Exercise (HIE) included a weight measuring 40% to 50% of body weight attached to the tail for a week, and 80% to 100% of body weight was applied from the 2nd week onwards. The frequency of exercise was 3 times a week. The mice were sacrificed after 24 hours to measure the effects of acute exercise after 4 weeks.

Analysis Method

1. RNA isolation. The large intestine tissue (<50 mg) was collected without RNAse in a 1.5 mL Eppendorf tube, using a surgical device to obtain RNA, to which, 1 mL Trizol reagent (JBI, Korea) was added. Tissue was pulverized, chloroform was added, and the mixture was centrifuged at 15,000 rpm at 4°C for 20 to 30 seconds. Next, isopropyl alcohol was added, placed at room temperature for 7 minutes, and centrifuged at 15,000 rpm at 4°C for 20 minutes. The pellet was washed with 75% ethanol (EtOH) in diethyl pyrocarbonate (DEPC) water, centrifuged at 13,000 rpm and 4°C for 5 minutes, and the supernatant was discarded to obtain the pellet suspended in DEPC water. The RNA thus extracted was dissolved in 0.1% DEPC water, and the absorbance was measured at 260 nm/280 nm using an ultraviolet spectrophotometer. The 5 best performing mice that completed the resistance exercise were selected from the 10 in each group. The RNA quality was evaluated, and RNA samples from 3 mice with highest quality were used for real-time polymerase chain reaction (PCR) analysis.

2. Real-time, quantitative reverse-transcriptase (qRT)-PCR. Real-time qRT-PCR was performed using DyNAmo SYBR Green qPCR kit and DNA Engine Opticon (MJ Research, USA). cDNA mold and specific primers were added to 2X PCR master mix, conditions were adjusted, and PCR was conducted. The primers used were as follows: IL-6 sense 5′-CCG GAG AGG AGA CTT CAC AG -3′ antisense 5′-GGA AAT TGG GGT AGG AAG GA-3′, CCL20 sense 5′-CGG AGT CAA CGG ATT TGG TCG TAT -3′ antisense 5′-AGC TTC TCC ATG GTG GTG AAG AC-3′, GAPDH sense 5′-AAT GCA TCC TGC ACC ACC AA-3′ antisense 5′-GTA GCC ATA TTC ATT GTC ATA-3′. PCR product was quantified using GAPDH, and mRNA level of the genes of interest from each group was compared with that of the control group; the extent of increase in gene expression was calculated using the following equation: fold change = 2-ΔΔCT, ΔΔCT= (CT, Target-CT, GAPDH) time x-(CT, Target-CT, GAPDH) time 0, time x = randomized time, time 0 = the moment that expression level of the gene of interest quantified by GAPDH became equal to unit value of the control group without any treatment.

Statistical Analysis

All results are expressed as mean ± standard deviation using SPSS 20.0 (IBM Corp., Armonk, NY, USA). A Kolmogorov–Smirnov test was conducted to confirm normal distribution as the number of subjects was small. One-way analysis of variance was conducted to examine the difference between groups. Post-hoc testing (least significant difference method) was conducted to identify the groups showing differences. Statistical significance was set at $p<0.05$.

RESULTS

Atopy Induction and Changes in the Shape of the Large Intestine with Resistance Exercise

Changes in the shape of the large intestine and that of tissue cells after exercise is shown in Figure 1, stained by haematoxylin-eosin. The results show no specific inflammation or tissue necrosis in any group (×200).

Inflammatory Cytokine, IL-6 mRNA Expression in the Large Intestine

Real-time qRT-PCR results showed significantly higher IL-6 mRNA expression in AD group as compared with CON, EX_L, EX_H, AD+EX_L, and AD+EX_H groups ($p<0.05$) (Table 1 and Figure 2).

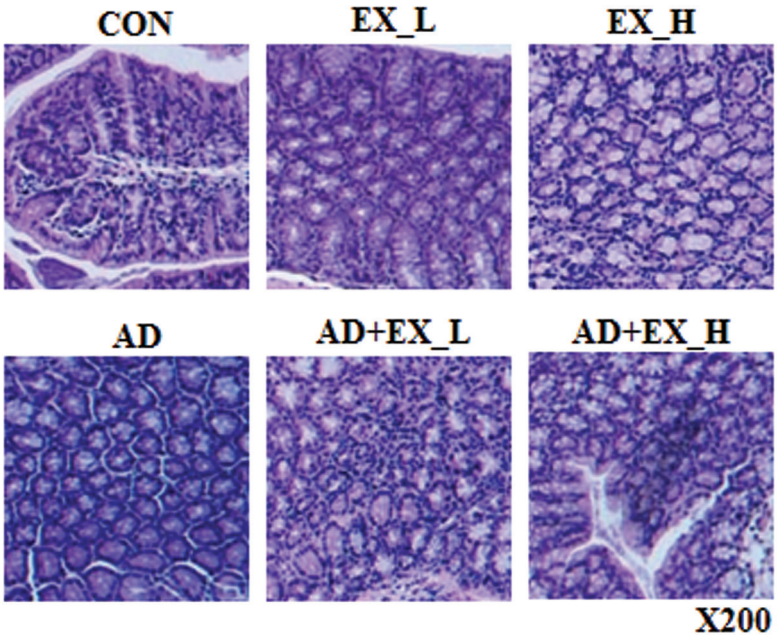
Chemokine CCL20 mRNA Expression in Large Intestine

Real-time qRT-PCR results showed significantly higher CCL20 mRNA expression in AD group compared with CON, EX_L, EX_H, AD+EX_L, and AD+EX_H groups ($p<0.05$) (Table 2 and Figure 3).

DISCUSSION

The aim of this study was to investigate the effect of ladder resistance exercise on mRNA expression of

FIG. 1 Microphotographs of left ear sections collected on Day 28 post-atopic dermatitis and exercise. Sections were stained with haematoxylin and eosin. Original magnification ×200.



CON = control group; EX_L = low resistance exercise group; EX_H high resistance exercise group; AD = atopic dermatitis group; AD+EX_L = atopic dermatitis+low resistance exercise group; AD+EX_H = atopic dermatitis+high resistance exercise group.

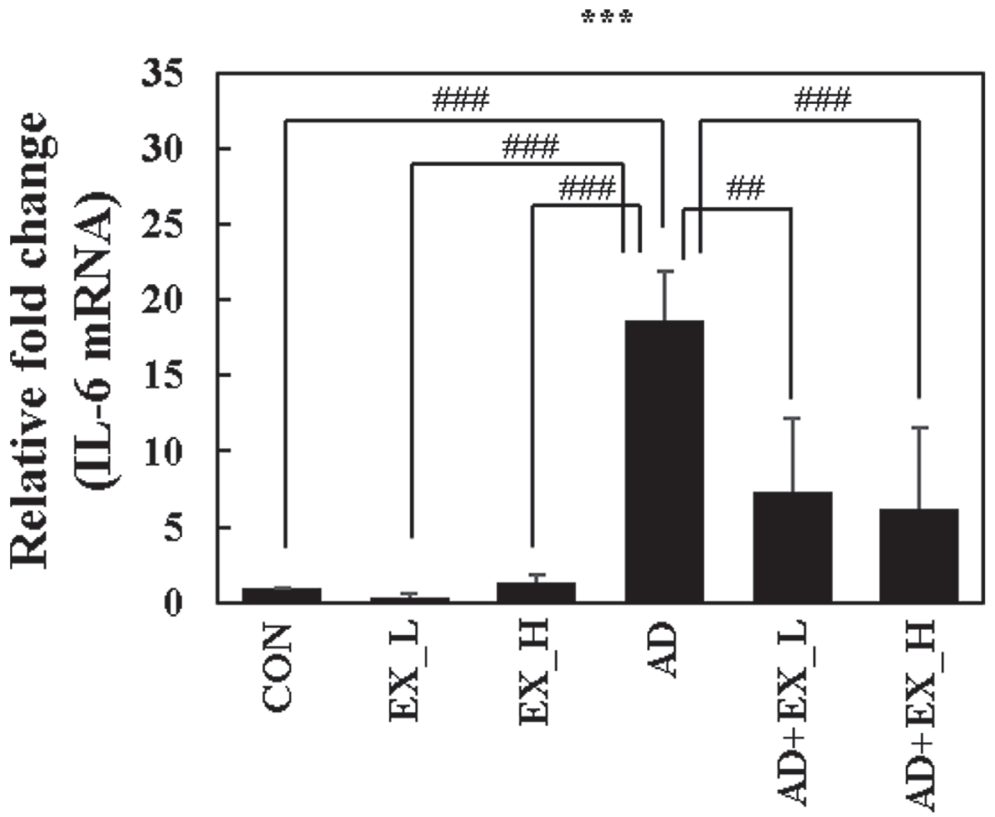
TABLE 1 Change in IL-6 mRNA Gene Expression

Variables	Group	Fold	*F*	*p*
IL-6	Control	1.00 ± 0.00$^{###}$	13.956	<0.001***
	Low resistance exercise group	0.34 ± 0.21$^{###}$		
	High resistance exercise group	1.39 ± 0.48$^{###}$		
	Atopic dermatitis	18.59 ± 3.24		
	Atopic dermatitis group + Low resistance exercise	7.33 ± 4.78$^{##}$		
	Atopic dermatitis group + High resistance exercise	6.25 ± 5.28$^{###}$		

Data are expressed as the mean ± standard deviation
$^{***}p < 0.001$, $^{##}p < 0.01$; $^{###}p < 0.001$ compared with atopic dermatitis group. Tested by one-way analysis of variance with post-hoc (least significant difference method).

IL-6, a representative cytokine in the large intestine and CCL20, a chemokine, in atopic mice. Several studies regarding the relationship between atopy and exercise have been reported,[19] as the condition of atopy is becoming increasingly prevalent.[1] It has been reported that resistance exercise has a positive effect on muscle generation, and it can prevent various diseases.[20] Atopy can induce inflammation

FIG. 2 IL-6 mRNA gene expression relative to exercise intensity and presence of atopic dermatitis for 4 weeks, as determined by real-time quantitative, reverse-transcriptase polymerase chain reaction.



CON = control group; EX_L = low resistance exercise group; EX_H, high resistance exercise group; AD = atopic dermatitis group; AD+EX_L = atopic dermatitis+low resistance exercise group; AD+EX_H = atopic dermatitis+high resistance exercise group.
$^{***}p < 0.001$; tested by one-way analysis of variance.
$^{\#}p < 0.05$; $^{\#\#\#}p < 0.001$ compared with atopic dermatitis group (post-hoc, least significant difference method).

in body tissues,[13] but exercise specifically increases anti-inflammatory and anti-immune cell numbers, preventing inflammatory diseases.[21] Therefore, this study was performed to investigate the relationship between ladder resistance exercise and mRNA expression of IL-6 and CCL20 in atopic mice.

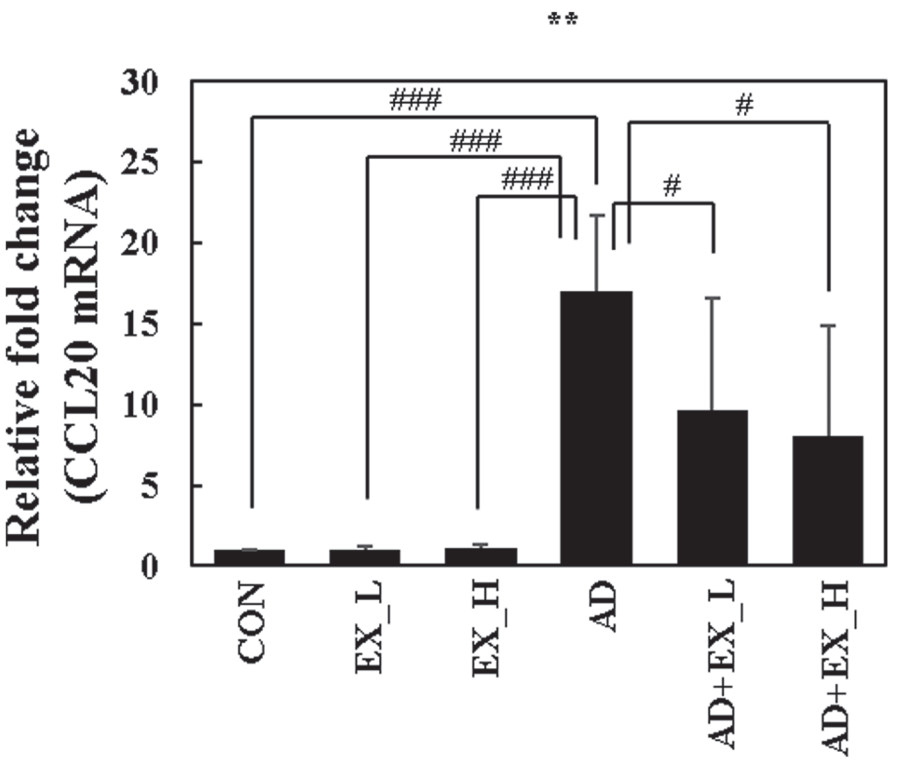
It has been recently reported that HIE increases cytokines in ear tissue in atopic mice.[19] However, there is a lack of studies regarding the relationship between induction of atopy in the large intestine and exercise. The results of the present study show that the AD group exhibited significantly higher IL-6 mRNA expression in tissues of the large intestine in atopic mice compared with CON, EX_L, EX_H, AD+EX_L, and AD+EX_H groups. Levels of IL-6, a representative inflammatory cytokine specifically increase in inflammatory intestine disease and Crohn disease[22]; however, exercise can control the levels of this inflammatory cytokine.[23] This pattern indicates that ladder resistance exercise, based on its intensity, should have a positive effect on the atopic large intestine, suggesting in turn, that exercise itself can induce chemokines. The results of the present study show that mRNA expression level of CCL20, a chemokine in the large intestine, was significantly higher in the AD group compared with CON, EX_L, EX_H, AD+EX_L, and AD+EX_H groups. Thus, exercise either induces or restricts inflammation by controlling the intensity

TABLE 2 Change in CCL20 mRNA Gene Expression

Variables	Group	Fold	*F*	*p*
CCL20	Control	1.00 ± 0.00$^{\#\#\#}$	9.961	0.001**
	Low resistance exercise group	1.05 ± 0.13$^{\#\#\#}$		
	High resistance exercise group	1.11 ± 0.25$^{\#\#\#}$		
	Atopic dermatitis	16.97 ± 4.78		
	Atopic dermatitis group + Low resistance exercise	9.69 ± 6.86$^{\#}$		
	Atopic dermatitis group + High resistance exercise	8.06 ± 2.50$^{\#}$		

Data are expressed as the mean ± standard deviation.
$^{**}p < 0.01$, $^{\#}p < 0.05$; $^{\#\#\#}p < 0.001$ compared with atopic dermatitis group.
Tested by one-way analysis of variance with post-hoc (least significant difference method).

FIG. 3 CCL20 gene expression in relation to exercise intensity and presence of atopic dermatitis for 4 weeks, as determined by real-time quantitative, reverse-transcriptase polymerase chain reaction.



CON = control group; EX_L = low resistance exercise group;
EX_H = high resistance exercise group; AD = atopic dermatitis group; AD+EX_L = atopic dermatitis+low resistance exercise group;
AD+EX_H = atopic dermatitis+high resistance exercise group.
$^{**}p < 0.01$; tested by one-way analysis of variance.
$^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ compared with atopic dermatitis group (post-hoc, least significant difference method).

of the immune response. It is suggested that atopy induction leads to an inflammatory reaction in the large intestine, increasing cytokine and chemokine levels; however, low- or high-intensity ladder resistance exercise can restrict this phenomenon.

The main limitations in this study were its inability to identify various inflammation-related makers, such as tumour necrosis factor alpha and C-reactive protein, and that additional human studies are necessary to determine the applicability of our results in a rat model. Nevertheless, the finding that atopy causes inflammation in all tissues is a clinically significant result. Especially since our study found that while inflammatory cytokines, chemokines, and intestinal disease markers were markedly increased, resistance exercise can significantly decrease their expression. These results suggest that resistance exercise can be effective for alleviating large intestinal inflammation in atopic patients.

CONCLUSION

In conclusion, mRNA expression of IL-6 and CCL20 was increased in atopic mice; however, IL-6 and CCL20 mRNA expression was significantly decreased in the mouse large intestine by low- and high-intensity ladder resistance exercise. This means that IL 6, an inflammatory cytokine that functions as an autoimmune disease marker in the large intestine, also induces inflammation during atopy. However, resistance exercise can inhibit this phenomenon. Thus, the results of this study suggest that resistance exercise may serve as a protection mechanism in the future, to prevent autoimmune disease.

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The authors have no conflicts of interest to declare.

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