

Decrease of T-lymphocyte proliferation in exercise-induced asthma

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The present study was designed to examine the effect of physical exercise on T-lymphocyte proliferation in patients with exercise-induced asthma (EIA). Indeed, a decrease in different immune functions is described in normal man after exercise. Thirty subjects (10 normal and 20 asthmatic subjects with or without EIA) underwent a submaximal exercise test on an electrically driven treadmill. Before and after this test, ventilatory variables were measured, and venous blood was taken to study plasma histamine (RIA) and spontaneous and phytohemagglutinin (PHA)-pulsed T-lymphocyte proliferation (mononuclear cells isolated on Ficoll-Hypaque; tritiated thymidine incorporation). Ten minutes after the end of the exercise, there was a significant FEV₁ decrease only in asthmatic subjects with EIA (mean: $24 \pm 5\%$). In the same group, the mean plasma histamine level was 0.31 ng/ml^{-1} (± 0.06) before the challenge. It rose to 0.62 ng/ml^{-1} (± 0.14) 10 min after the end of the exercise ($P < 0.05$), and returned to normal limits 20 min after the test. In this group, there was also a significant decrease (by about 35%) of spontaneous and PHA-pulsed T-lymphocyte proliferation 2 and 4 h after the exercise. By contrast, exercise challenge had no effect on either plasma histamine level or T-lymphocyte proliferation in the normal group. Our results show a rapid and transient increase in plasma histamine in EIA. This was followed 2 and 4 h later by a significant decrease of T-lymphocyte proliferation. A possible relationship between these two phenomena is discussed.

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The immunology of exercise is presently an active area of research. This matter is important because of the growing number of reports implicating mild infectious disease in reduced performance of the athlete (18, 19, 33). A major subject has been the investigation of circulating leukocyte and lymphocyte subpopulations before and after exercise (8, 17, 20). Of the lymphocyte subpopulations, the number of natural killer cells changes most, and while cytotoxicity increases at the end, it decreases after endurance exercise (6, 10). It has also been shown that during moderate exercise, the lymphocyte proliferation responses to mitogens and antigens may be decreased (22, 32). The mechanisms behind the suppressed lymphocyte function are unknown. However, all these studies have been done in normal man. Exercise of a subject with asthma does, in many cases, result in an early asthmatic reaction. The mechanism by which this reaction is brought about by exercise is still enigmatic. Therefore, the demonstration of mediator release such as histamine in plasma, in association with the bronchial reaction

to exercise, is an important but controversial observation (3, 5, 25, 27). To the best of our knowledge, the effect of exercise on lymphocyte functions has never been studied in exercise-induced asthma (EIA). The purpose of the present study was to compare the effect of exercise on T-lymphocyte proliferation in normal subjects and asthmatic subjects with EIA.

Material and methods

At 0800, healthy and atopic asthmatic volunteers underwent a submaximal exercise test on an electrically driven treadmill and were followed for 24 h. Before the test and at different time intervals after the challenge, ventilatory variables were measured, and venous blood was taken to study plasma histamine, spontaneous and phytohemagglutinin (PHA)-pulsed T-lymphocyte proliferation, and leukocytosis. All the subjects gave their written, informed consent, and the protocol of this study was approved by the hospital ethics committee.

Subjects

Ten healthy men (18–26 years old) were studied (group 1). They exhibited normal pulmonary function and no allergic background, as verified by history, negative skin tests, and RAST for a battery of the most common inhalent allergens.

Ten asthmatic subjects (19–27 years old) with a documented history of EIA (group 2) were also studied and compared with 10 asthmatic subjects without EIA (group 3). All these asthmatic subjects were sensitive to house dust, *Dermatophagoides* mites, and other allergen sources on the basis of history, skin tests, and RAST. However, they were asymptomatic and received no treatment at the time of the study. Before starting the experiment, all subjects had a $FEV_1 \geq 80\%$ of the predicted value (35). FEV_1 was measured with a Godart spirometer before and 5, 15, and 45 min after the challenge.

Exercise challenge

All subjects rested for at least 20 min after arrival in the laboratory. All exercise challenges were performed in the morning. The ambient temperatures varied between 17° and 22°C, and the relative humidity ranged from 40 to 52%.

Exercise challenge was performed on an electrically driven treadmill at a 10% incline at a mean speed of 10 km/h. Exercise was performed for 8 min, during which a heart rate of 90% of the predicted maximum was reached (2, 12).

T-lymphocyte proliferation

Heparinized venous blood (20 ml) was taken before and 2, 4, and 24 h after the challenge. Mononuclear cells were obtained by density gradient centrifugation of blood on Lymphoprep (Nyegaard, Oslo, Norway). Cells were washed three times in Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} (Gibco, UK) and resuspended to 1.10^6 cells/ml in RPMI 1640 containing 5% heat-inactivated fetal serum (Gibco). Triplicates (200 µl) of washed cells were incubated for 72 h (37°C, 5% CO_2) in plasma microwells (Nunc, Denmark), in the presence or absence of purified PHA (Wellcome, Dartford, UK) to a final concentration of 1 µl/ml. Seventeen hours before completion of the culture, 20 µl of tritiated thymidine (methyl ^3H -labeled thymidine; 50 µCi/ml, Amersham, UK) was added to the wells. The cells were harvested. The incorporation of the labeled thymidine in the lymphocytes (isotope uptake) was estimated at the end of cultures, by standard toluene-based scintillation counting methods and was expressed as the mean counts per minute (cpm) of each set of three replicates. At present, in our labo-

ratory, normal values of spontaneous or PHA-pulsed lymphocyte proliferation in healthy volunteers ($n = 80$) were, respectively, $1550 \text{ cpm} \pm 820$ and $41\,200 \text{ cpm} \pm 16\,000$. There was no significant difference from our asthmatic population ($n = 50$).

Plasma histamine determinations

Venous blood (5 ml) was collected in a cold tube containing ethylenediaminetetraacetic acid (EDTA) before and 10 and 30 min after the challenge. Blood was immediately centrifuged at 4°C, and the upper part of the plasma supernatant was carefully collected. Histamine was measured by a specific and sensitive radioimmunoassay (Immunotech, Marseille, France). Its sensitivity was 0.1 ng/ml with an intra- or interassay variation coefficient of $< 8\%$ (29, 30).

Leukocytosis

For determination of leukocytosis and leukocyte subpopulations, venous blood (3 ml) was collected in an EDTA tube before and at the end of exercise, and 5 min, 30 min, 1 h, and 2 h after the exercise. The samples were measured in a coulter (Technicon H1) (9).

Statistical analysis

Student's paired *t*-test was used for statistical comparison; $P < 0.05$ was considered to be statistically significant. Results were presented as mean $\pm$ SD.

Results

Clinical tolerance

Base-line FEV_1 (percentage of the predicted values) measurements were similar in our three groups. Exercise challenge was performed to an average of 91% of maximum predicted heart rate. All asthmatic patients from group 2 experienced a bronchospasm with a fall in FEV_1 of $24 \pm 5\%$ from base line (range 19–33%) after this challenge. This bronchospasm was associated with cough, dyspnea, and, generally, wheezing. However, in these subjects, the symptoms spontaneously resolved in about 15 min. Other subjects (groups 1 and 3) showed no respiratory symptoms and no spirographic modification after the challenge.

Histamine plasma levels

The mean resting plasma histamine level in normal subjects was 0.23 ± 0.04 ng/ml. The asthmatic subjects had significantly higher mean resting plasma

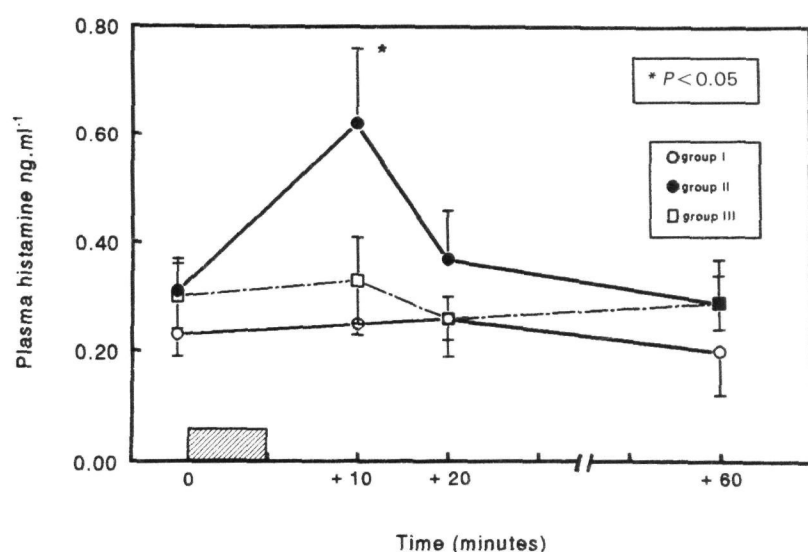


Fig. 1. Changes in plasma histamine after exercise in normal and asthmatic subjects. Each point represents mean $\pm$ SD for 10 subjects.

values: 0.31 ± 0.06 ng/ml ($P < 0.05$). After exercise, plasma histamine levels in subjects from groups 1 and 3 did not change significantly from base line. In contrast, we observed after exercise an early (+10 min) increase in plasma histamine (0.62 ± 0.14 ng/ml) in asthmatic subjects with EIA (Fig. 1). This rise was significant ($P < 0.05$) 10 min after the end of the challenge, the level returning to normal limits after 20 min.

Leukocyte subpopulations

The overall number of granulocytes, especially neutrophils, increased slightly at the end of exercise and 30 min later. It returned to normal limits within 2 h of ceasing exercise (Table 1). Any significant differences were observed among the three groups of subjects.

T-lymphocyte proliferation

Before exercise, all subjects (normal and asthmatic) showed basal spontaneous and PHA-pulsed T-lymphocyte proliferation within normal limits.

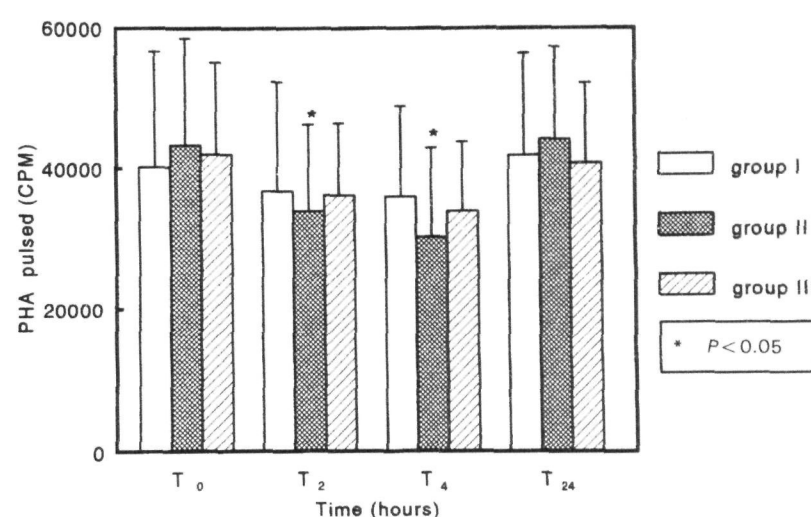


Fig. 2. Effect of exercise on PHA-pulsed T-lymphocyte proliferation in normal and asthmatic subjects. Each bar represents mean $\pm$ SD for 10 subjects.

There was no significant difference among the three groups.

Fig. 2 shows the effect of exercise challenge on PHA-pulsed T-lymphocyte proliferation in our three groups. After the challenge, we observed in normal subjects a slight decrease of PHA-pulsed T-lymphocyte proliferation 2 and 4 h later. The decrease in T-lymphocyte function was more marked in asthmatic groups. However, PHA-pulsed T-lymphocyte proliferation significantly decreased ($P < 0.05$) 2 and 4 h after exercise challenge only in asthmatic subjects with EIA (group 2). This inhibitory effect was maximal at the fourth hour. At this moment, the mean inhibition (percentage of the basal values) of PHA-pulsed T-lymphocyte proliferation was, respectively, $10 \pm 7\%$ in group 1, $30 \pm 13\%$ in group 2 ($P < 0.05$), and $19 \pm 12\%$ in group 3. Twenty-four hours after the challenge, basal values of T-lymphocyte proliferation were again reached in the three groups. The same type of result was noted when we observed the effect of challenge on spontaneous T-lymphocyte proliferation in our three groups (Fig. 3).

Table 1. Mean leukocyte (total and differential) count before and after exercise in normal volunteers ($n = 10$)

	Before	After exercise	After exercise		
			+10 min	+30 min	+2 h
WBC/mm ⁻³	6100 $\pm$ 1050	7050 $\pm$ 1000	7200 $\pm$ 1220	6650 $\pm$ 1200	6250 $\pm$ 1100
% Neutrophil	73 $\pm$ 16	74 $\pm$ 18	76 $\pm$ 15	76 $\pm$ 17	75 $\pm$ 15
% Lymphocyte	23 $\pm$ 9	23 $\pm$ 11	23 $\pm$ 11	22 $\pm$ 13	22 $\pm$ 9
% Basophil	1 $\pm$ 2	1 $\pm$ 1	0	1 $\pm$ 2	1 $\pm$ 1
% Eosinophil	1 $\pm$ 1	0	0	0	0
% Monocyte	2 $\pm$ 2	2 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 2

WBC: white blood cells.

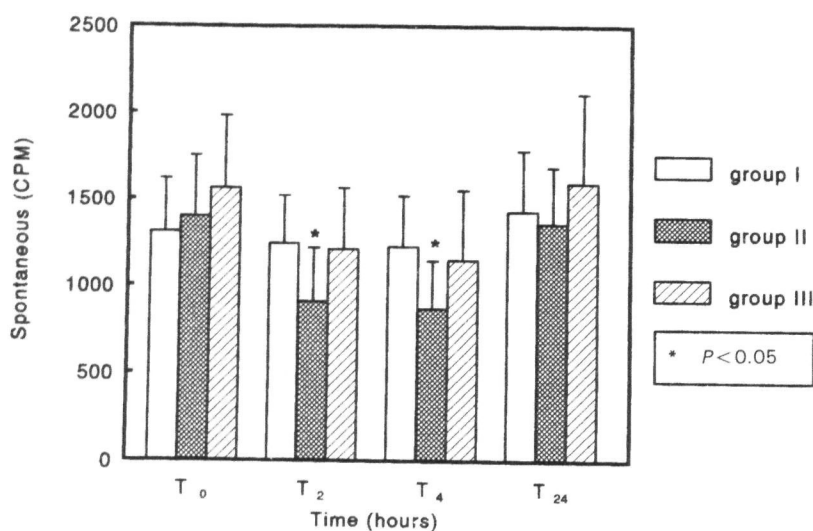


Fig. 3. Effect of exercise on spontaneous T-lymphocyte proliferation in normal and asthmatic subjects. Each bar represents mean $\pm$ SD for 10 subjects.

Discussion

Much attention has been focused on exercise as a potent suppressor of the immune response. Immune system alterations in response to acute and moderate exercise are generally characterized by a transient mild leukocytosis and lymphocytosis (6, 21, 24). Both B- and T-lymphocyte numbers rise, the percentage increase being stronger for B cells. There is also some evidence to suggest that lymphocyte function can be temporarily impaired during and after exercise (28, 36, 41). The assessment of lymphocyte function after exercise has usually involved the "in vitro" response of peripheral leukocytes to mitogens and antigens. Several researchers have reported that immediately after an acute or moderate effort, T-lymphocyte proliferation may be suppressed (22, 32). However, on this last point, there are conflicting data in the literature (37, 41).

In our study, we investigated the effect of exercise on T-lymphocyte proliferation in normal subjects, and asthmatic subjects with or without EIA. We have observed a significant difference between normal subjects and asthmatic subjects with EIA. In contrast to normal subjects, we observed a large decrease of spontaneous and PHA-pulsed T-lymphocyte proliferation 2 and 4 h after exercise challenge in our asthmatic subjects with EIA. This decrease of lymphocyte function is not associated with a significant change of leukocytosis.

Various hypotheses can explain the decrease of T-lymphocyte proliferation observed after EIA. Firstly, during exercise, corticosteroids and catecholamines are secreted, compounds which are known to modulate the immune system. *In vitro*, glucocorticosteroids have been shown to inhibit mitogen- and antigen-induced proliferation responses (16, 38). T-lymphocyte proliferation has also been reported to decrease after epinephrine

administration (15). In the present study, an eight-fold increase in plasma epinephrine and a 12-fold increase in plasma norepinephrine during exercise were found in our three groups.

Secondly, it is also possible that exercise is associated with changes in lymphocyte subpopulations. Indeed, various authors have described in normal subjects a decrease in the number of T-helper cells with no change in the suppressor cell count a few minutes after strenuous exercise (30, 32). Our preliminary results do not show a relationship between lower thymidine uptake and variations in the number of CD4 and CD8 cells (data not shown). Further studies are needed to determine the proliferative activity of each subset individually. In previous studies, we have shown that intravenous administration of histamine may decrease, in man, T-lymphocyte proliferation for many hours (11, 13). In this study, we have measured plasma histamine with a sensitive and specific RIA, and we have observed, only in asthmatic subjects with EIA, a significant increase of histamine 10 min after the challenge. This increase of plasma histamine is followed 2 and 4 h later by a significant decrease in T-lymphocyte proliferation. The same prolonged action of histamine can be seen when purified neutrophils are incubated *in vitro* with histamine (10^{-7} mol), washed, and then tested 2 h later in a Boyden chamber (14). Little is known about the affinity of histamine receptors for their agonists in inflammatory cells, but the stimulation of H_2 -receptors can cause a rise in intracellular cAMP which persists for at least 1 h (31). We think that the inhibition of lymphocyte function observed can be partly explained by a direct effect of histamine on lymphocytes. Indeed, it is well documented that histamine exerts, *in vitro*, via H_2 -receptor activation, an inhibitory effect on inflammatory cells (23, 34, 40). *In vitro* studies on animals and recently even in man have shown that exogenous histamine can also depress T-lymphocyte proliferation by the stimulation of H_2 -receptors (1, 4, 13).

This study demonstrates that in asthmatic patients with EIA a significant increase in plasma histamine occurs a few minutes after the challenge. Histamine release has previously been reported in EIA by other authors (3, 26, 27). The cellular origin of this plasma histamine increase remains controversial. In our study, we have not observed a significant basophilia after the challenge. Moreover, we have documented that, although asthmatic subjects with or without EIA had an equivalent basophilia after exercise, only asthmatic subjects with EIA had an increase in plasma histamine. In our sense, these findings suggest that the cellular origin of plasma histamine increase during EIA depends, at least partly, on pulmonary mast cells.

In conclusion, we have observed, only in EIA, a

rapid and transient increase in plasma histamine. This is followed 2 and 4 h later by a significant decrease of T-lymphocyte proliferation. The relationship between these two phenomena is not clear. However, the pathogenesis of EIA is still in dispute, and the observed decrease of T-lymphocyte function could be a consequence of histamine release by pulmonary mast cells.

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