CLÍNICA MÉDICA DO EXERCÍCIO E DO ESPORTE

DIFFERENCES IN PERIPHERAL BLOOD NATURAL KILLER CELL POPULATIONS BETWEEN ELITE KAYAKERS AND NON-ATHLETES

ARTIGO ORIGINAL

Grasiely Faccin Borges[1,4]
Ana Maria Botelho Miranda Teixeira[1]
Luís Manuel Lopes Pinto Rama[1]
Susana Pedreiro[3]
Amândio Manuel Cupido Santos[1]
Alain Guy Marie Massart[1]
Francisco Bessone Alves[2]
Artur Paiva[3]

1. Center for Sports and Physical Activity, Sport Sciences and Physical Education School, University of Coimbra, Coimbra, Portugal.
2. Human Kinetics School, Technical University of Lisbon, Lisbon, Portugal.
3. Center of Histocompatibility, Coimbra, Portugal.
4. Health and Biotechnology Institute, Federal University of Amazonas, Brazil.

Mailing address:
Ana Maria Botelho Teixeira
Estádio Universitário, Pavilhão 3,
Sta. Clara - 3040 Coimbra, Portugal
Gabinete: Pavilhão 3
E-mail: ateixeira@fcdef.uc.pt,
grasiely.borges@gmail.com

ABSTRACT

Introduction: Prolonged strenuous exercise has been associated a transient depression of immune function, with prolonged intense training schedules and competition able to lead to immune impairment in athletes." Para Introduction: Prolonged strenuous exercise and intense training schedules and competition could lead to immune impairment in athletes. Objetive: The objective of this study was to see if chronic training could produce sustained differences in the peripheral blood (PB) leukocyte subpopulations (granulocytes, monocytes, total lymphocytes, B and T lymphocytes, $CD4^+$ and $CD8^+$ T cells and Natural Killer cells) of elite kayakers when compared to non-athletes. Methods: The sample was composed of 13 elite male kayakers, 20± 3 years old, 75.0 kg ±7.9 weight and 177.3±7.1 cm stature. The VO_{2max} was 58.3±7.8 mL.kg.min^{-1}. The Non-athlete group was composed of 7 healthy males, aged 18±1 years old, 81.3±13.8 kg of weight and 171.9±4.5cm stature. The athlete's blood samples were collected at the beginning of the training season, after an off period of six weeks of training. Peripheral blood cell populations were identified by flow cytometry analysis. To verify the differences between the athlete and non-athlete groups the Mann-Whitney U Test was used. Results: No differences between the trained kayakers and the non-athletes were found at rest except for Natural Killer cells ($CD3^-CD56^+$) and the $CD3^-CD56^+CD8^+$ subset values that were lower in the athletes. Conclusion: Our study found that after six weeks without training only the NK $CD3^-CD56^+$ population and particularly the highly cytotoxic $CD3^-CD56^+CD8^+$ subpopulation had lower levels in the elite athletes when compared to the untrained men.

Keywords: athletes, natural killer cells, lymphocytes, T-lymphocytes, immunology.

INTRODUCTION

Prolonged and stressing exercise has been associated with transitory depression of the immune function[1], with prolonged intense training and competition could lead to immune impairment in athletes. However, it is not clear if there are substantial differences between the populations of elite athletes and non-athletes. The measurements of the T and B cell function do not seem to be affected by prolonged physical training [2]. However, the intrinsic immunological system responds differently to intense physical training. Reports of decreased oxidative activity of neutrophils [3] and decrease of the expression of cytokines in monocytes and dendritic cells [4], after high-volume and intensity training phases in swimmers at rest when compared to sedentary controls and leveled at age, were published. The effect of physical training over the number and function of natural killer cells (NK) is still discussed. While intervention or cross-sectional studies reported moderate increase in cytotoxicity of NK cells (NKCC) after moderate physical training [5], intense training has shown the capacity to alter subgroups of NK cells as well as to reduce NKCC[6,7].

The NK cells represent an intrinsic component of immunity which can destroy tumor and virus-infected cells with no previous sensibilization (i.e., non-MHC restricted)[8]. Approximately 40% of the NK cells ($CD3^-CD56^+$) in humans express CD8. The $CD3^-CD56^+CD8^+$ subpopulation in humans presented higher cytolytic activity when compared with the $CD8^-$ subpopulation both at rest[10] and after culture[11] and mediate autologous cytotoxicity of myeloid leukemia cells in patients with acute myeloid leukemia who were submitted to autologous bone marrow transplant[12].

It has been demonstrated that at rest, the immune function in athletes compared with non-athletes, presents more similarities than differences [13]. Despite of that , more studies specifying subset and relate them to specific training times are necessary. We became interested in examining a group of highly trained endurance athletes (kayakers) before the beginning of the training season and compare it with a group of non-athletes observing many immunological parameters. The aim of this study was to investigate the differences in the peripheral blood in the leukocytes counting, populations of B,T lymphocytes and in their subpopulations ($CD4^+$, $CD8^+$) and in the NK cells ($CD3^-CD56^+$) between athletes (kayakers) and non-athletes before the beginning of the training season and after the recovery period of six weeks.

MATERIALS AND METHODS

Participants' characteristics

The sample was composed of 13 elite kayak athletes, aged 20 ± 3 years, 75.0kg ± 7.9 weight and 177.3 ± 7.1cm height. The VO_{2max}

was 58.3 ± 7.8mL.kg.min^{-1}.The group of non-athletes consisted of seven men, aged 18.2 ± 1.1 years, 81.3 ± 13.8kg weight and 171.94 ± 4.51cm height.

All of them presented negative history of cardiovascular or hepatic diseases and presented normal values of clinical routine examination, including blood pressure values. None of the subjects made use of any medication during the study time.

After having received oral and written explanations, the consent form was provided and the experimental protocol was approved by the Ethics Committe in Research with Humans of the Faculty of Sports Science and Physical Education School of the University of Coimbra.

Venous blood samples

Blood samples were collected in a specific point in time (in November) after a six-week rest period and before the beginning of the training season for kayak athletes. The blood samples for the group of non-athletes were collected in an equivalent point in time.

Blood samples (15ml) were collected from the antecubital vein by venous punction, respecting a 48-hour rest pause after the last training session. A complete analysis of the leukocytes (LEU), granulocytes (GR), lymphocytes (Li) and monocytes (MO) counting was performed using a blood analyzer (Beckman Coulter T66, USA).

Flow cytometry

Lymphocyte subpopulations were determined by flow cytometry (FACSCalibur; BD, San Jose, CA, USA). The total number of T lymphocytes ($CD3^+$) and helper/inducer ($CD3^+CD4^+$), supressor/cytotoxic ($CD3^+CD8^+$), B Lymphocytes ($CD19^+$), natural killer cells ($CD3^-CD56^+$) and $CD3^-CD56^+CD8^+$ and $CD3^-CD56^+CD8^-$ subpopulations were included. These cells were stained with Lymphogram (CYT-C001, Cytognos,Spain) according to the manufacturer's recommendations. The stained cells were analyzed in a flow cytometer FACScan with the aid of the *CELLQuest* software (BD Biosciences). The data were analyzed by the computer program *Infinicyt* (Cytognos, Spain).

STATISTICAL ANALYSIS

Descriptive analysis was considered for the mean and standard deviation. Since the sample did not present normal distribution, Mann-Whitney U test was used to verify the differences between athletes and non-athletes. The significance level established was $P < 0.05$. The SPSS statistical program for *Windows* (version 16.0. SPSS Inc, Chicago) was used.

RESULTS

The results show that the number of leukocytes, lymphocytes, granulocytes and monocytes was similar and the groups did not present significant difference between each other (table 1).

T lymphocyte ($CD3^+$), helper/inducer lymphocyte T ($CD4^+$), supressor/cytotoxic T lymphocyte ($CD8^+$) and B lymphocytes ($CD19^+$) cells total counts and percentage did not evidence significant differences between the two groups (table 2). However, the trained subjects presented lower cell counting and NK percentage ($CD3^-CD56^+$) when compared with the non-athletes, specifically in the $CD3^-CD56^+CD8^+$ subgroup ($P < 0.05$, table 2).

Table 1. Total cell counts and percentage of peripheral leukocytes (LEU) populations in elite kayakers and untrained men. Values represent mean (± standard deviation).

Hematological parameters	Athletes (N = 13)	Untrained men (N = 7)	P
LEU de Leukocytes 10^3/µL	9.40 (1.63)	8.41 (1.88)	0.24
MO 10^3/µL	0.41 (0.16)	0.66 (0.69)	0.23
% MO	4.61 (1.88)	7.17 (6.63)	0.55
GR10^3/µL	5.60 (1.45)	5.31 (1.36)	0.32
% GR	63.03 (6.17)	63.10 (5.07)	0.96

MO: monocytes, GR: granulocytes.

Table 2. Total peripheral blood lymphocyte and subpopulations cell counts and percentage in elite kayakers and untrained men. Values represent mean (± standard deviation).

Variable	Athletes (N = 13)	Untrained men (N=7)	P
Lymphocytes (10^3/µL)	2.331.46 (454.59)	2.405.85 (685.09)	0.41
% Lymphocytes	25.17 (5.39)	28.70 (6.09)	0.91
$CD3^+$ (10^3/µL)	1.527.45 (467.32)	1.762.29 (466.96)	0.22
$\%CD3^+$	66.17 (16.21)	74.43 (11.65)	0.15
$CD3^+CD4^+$ (10^3/µL)	858.21 (280.30)	950.24 (282.30)	0.35
$\%CD3^+CD4^+$	56.33 (6.27)	53.74 (5.97)	0.22
$CD3^+CD8^+$ (10^3/µL)	535.07 (176.57)	633.40 (209.00)	0.22
$\%CD3^+CD8^+$	34.97 (5.53)	35.50 (3.78)	0.45
$CD4^+/CD8^+$ ratio	1.67 (0.43)	1.54 (0.29)	0.23
$CD3^+CD8^+CD4^+$ (10^3/µL)	10.04 (5.50)	17.64 (17.11)	0.32
$\%CD3^+CD8^+CD4^+$	0.64 (0.22)	0.95 (1.04)	0.49
$CD3^+CD8^-CD4^-$ (10^3/µL)	116.71(54.75)	135.00 (67.43)	0.44
$\%CD3^+CD8^-CD4^-$	7.46 (2.27)	8.29 (4.70)	0.50
$CD3^-CD19^+$ (10^3/µL)	402.13(139.58)	348.84 (133.50)	0.22
$\%CD3^-CD19^+$	17.19 (4.6)	14.40 (3.97)	0.10
NK $CD3^-CD56^+$ (10^3/µL)	59.62 (29.79)	96.12 (38.14)	0.02
% NK $CD3^-CD56^+$	2.46 (0.87)	5.28 (3.31)	0.02
NK $CD3^-CD56^+CD8^+$ (103/µL);	24.85^+(15.65)	63.27^+(45.52)	0.03
% NK$CD3^-CD56^+CD8^+$	43.15^+(13.11)	49.15^+(22.18)	0.29
NK $CD3^-CD56^+CD8^-$ (10^3/µL)	34.47^+(20.35)	67.54^+(55.67)	0.09
%NK $CD3^-CD56^+CD8^-$	56.24^+(13.04)	50.82^+(22.20)	0.10

NK: natural killer cells.

DISCUSSION

Our results show that the main differences between athletes and non-athletes at rest and after a period without training were found in the number of natural killer cells ($CD3^-CD56^+$) present in the peripheral blood. The $CD3^-CD56^+CD8^+$, subgroup specially presented values significantly lower than the ones found in the non-athletes (table 2).

Glesson et al.[6] reported that in athletes, in a swimming training season, there were not significant alterations in the number or percentages of B or T cells subgroups; however, significant decrease in the number and percentage of natural killer cells was observed. Conversely, a study by Pedersen et al.[14], in which trained cyclists were compared with untrained men, the percentage of NK cells was higher in the trained athletes, but the study does not report in which training phase the data were collected.

According to Gleeson and Bishop[15], the alterations occurred after a set of prolonged exercises may lead to natural suppression of the NK and T cells activity, which could potentially present benefits to the transplanted patients concerning rejection risk reduction. The effect of the exercise intensity in the cytolytic activity of NK cells

seems to present a dual-phase effect, with initial gain followed by delayed suppression[15,16]. The NK cells have behaved as the cells of highest response to a set of acute exercise [5]. Many studies support the increase in the NK cell counting in the circulation during progressive exercise, as well as fast recovery of post-exercise NK[5,17-19]. They are rapidly recruit to the peripheral blood probably, via shear stress due to increase in blood flow added with a catecholamine--induced[20] down regulation of adhesion molecule expression. This increase in the peripheral pool has been associated with increased immune surveillance[21].Despite of that, during prolonged exercise, circulating NK cells counting may decrease below the pre-exercise values[22], in which the cells leave the peripheral blood circulation possibly to enter sites of muscle damage[23]. Cells exit from circulation or inhibition of their entrance may mean that these cells, especially the more cytotoxic subgroup, are extra or transiting to sites where they are needed for immune or inflammatory function. Exercising does not seem to destroy NK cells; rather on the contrary, they are temporarily relocated to storage sites, such as peripheral veins walls, in response to the secretion induced by exercise of catecholamine and activation of adhesion molecules[24,25]. Reduction in NK cells counts reported to persist ut to seven days after exercise[26], a fact which seems to be corroborated by our results with time periods even longer.

CONCLUSION

Our data suggest that even after a six-week resting period and before the beginning of the training season, the number of NK cells in PB athletes is lower than the values found in non-athletes. This finding may reflect as chronic adaptation to training, suggesting that decrease in the number of $CD3^{-}CD56^{+}CD8^{+}$ cells may reflect in lower risk of autolog cells destruction and decrease of the inflammation status joined to the redistribution of NK cells in the tissues for imune surveillance.

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