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Case Study

Immunological Alterations During a Kayaking Season. A Case Study with A Paddler Presenting with Asthma

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Abstract

Asthma is not rare in elite athletes being more frequent in endurance sports practiced outdoors. In three moments of the season, we studied an elite kayaking marathoner with recurrent crises of exercise-induced bronchoconstriction aggravated when training hard in a cold environment.

Blood collection was realized at the beginning of the season (M1), after the winter championships (M2), and after the summer championships (M3). Total leukocyte, neutrophils, and lymphocyte counts decreased from M1 to M2 and tended to recover in M3 while monocytes showed the opposite behavior. The two lineages of T cells (αβ and γδ) showed slight variations during the season. From a very low initial value, CD19+ B cells increased progressively during the season. In M2, memory cells (CD45RO+) increased while naïve cells (CD45RA+) decreased. These changes were reverted in M3. The CD4+/CD8+ ratio, despite the variations seen during the season, was always below the laboratory reference values for healthy subjects.

We report the variations of the immunophenotype profile during the season, in an elite endurance athlete.

Keywords: Immune system; Sport; Kayak; Training; Asthma

Introduction

In athletes, strenuous bouts of prolonged exercise are associated with depressed immune cell function while light to moderate exercise seems to enhance the immune response [28,33]. In general, immune function depression induced by exercise is most pronounced when exercise is continuous, prolonged, of moderate to high intensity, and performed in a fasting state [12]. Intense and prolonged exercise may induce a transitory period of immune frailty ("open window") which may last between 3 and 72 hours, and increase athletes' susceptibility to infection [33]. Recurrent periods of intensified training can result in marked depression of the immune function. Although, elite athletes often show no clinical signs of immune suppression, frequent intense physical loads may lead to an altered immune profile that may increase the susceptibility to Upper Respiratory Tract Infections (URTI) [40] which can be augmented when environmental conditions are not favorable [24]. This study aimed to evaluate the changes in the immune system during a competitive season in a marathon kayaker.

Methods

Subject

A 30-year-old elite paddler specialized in marathon races which integrates the Portuguese team in international competitions as the World and European marathon championships was studied. The evaluation was performed in three moments: in September at the beginning of the season after 15 days of resting (M1), in March, after the completion of the Winter National Championship (M2), and in July after the Summer National Championship (M3). This study was conducted in accordance with the policy statement of the Declaration of Helsinki, adopted by the World Medical Association, regarding the ethical principles for medical research involving human subjects and approved by the Ethical Committee of the Faculty of Sport of the University of Porto, Portugal. The subject was informed of the risks associated with their participation before giving voluntary written consent.

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Clinical Status

The kayaker participant in this study showed recurrent crises of Exercise-Induced Bronchoconstriction (EIB) during training in cold environments. He was otherwise healthy, showing excellent physical conditioning, as assessed by specific (kayaking) and unspecific (physical conditioning) testing.

The first moment of the evaluation was performed after 15 days of complete rest following the end of the precedent competitive season that finished with the withdrawal during the World Marathon Championship due to acute upper respiratory constraints provoked by the dramatic fall (- 20° Celsius) of the air temperature on the racing day. The kayaker had never been under treatment for the bronchial complaints and his symptoms only occurred during strenuous exercise. He was prescribed salbutamol 100 µg (4 puffs) bid and during exacerbations budesonide 200 id.

Training Program

The kayaker clearly defined two peaking phases, the first ending with the Winter Championships (WC), and the second ending with the Summer Championships (SC). WC consists of 5000 m races in K1, K2, and K4. SC consists of a 35 km K1-marathon that usually selects participants for international competitions, namely European and World Championships.

Training Microcycle (basal model) at the beginning of the season

All training sessions began with a specific warm-up period (e.g. calisthenics for strength training and low-intensive pace for water sessions) usually lasting between 10 and 20 min.

Monday

Morning (M) - Rest

Afternoon (A) – 30' running at a moderate pace + Endurance strength training (Circuit training. 10 exercises, 40% of maximal load, 5 circuits, 1'/1' work/rest ratio, 5' total rest after each circuit.

Tuesday

M – Water. 1 h of low-intensity steady-state kayaking (70% of maximal heart rate (MHR))

A – Water. 12 x 10"maximal sprints with full recovery

Wednesday

M – Water. 1h10'. Long intervals. 5 x 2000m (80% MHR)

A – 30' running at a moderate pace + Strength training. Extensive hypertrophy. 6 exercises (bench press; semi-squat; biceps curl; French curl lying down; bench pull; pull-ups), 12-15 Repetitions Maximum (RM). 5 sets each exercise, 2-3' of rest between exercises.

Thursday

M – 1h of mountain bicycle in the wild

A – Water. VO_2max intervals. 2 x 8 x (400m at 90% MHR), 60 s and 3 min recovery between intervals and sets, respectively.

Friday

M – Water. 1 h of low-intensity steady-state kayaking (70% of maximal heart rate (MHR)).

A – 30' running at a moderate pace + Strength training. Extensive hypertrophy. 6 exercises (bench press; semi-squat; biceps curl; French curl lying down; bench pull; pull-ups), 6-8 Repetitions Maximum (RM). 5 sets each exercise, 2-3' of rest between exercises.

Saturday

M – Water. 1h15' fartlek. Pacing variations are determined by the athlete's motivation and physical conditioning.

A – 45' running at moderate pace + 30' general calisthenics

Sunday

M – 20-25 km at a moderate pace (70% of maximal heart rate)

A – Rest

Training microcycle (basal model) after the Winter National Championship

- Monday

M - Rest

A – 30' running at a moderate pace + Endurance strength training (Circuit training. 10 exercises, 40% of maximal load, 5 circuits, 1'/1' work/rest ratio, 5' total rest after each circuit.

- Tuesday

M – Water. 2 h of moderate-intensity steady-state kayaking (80% of maximal heart rate (MHR)) ending with 8 x 10"maximal sprints with full recovery

A – Water. 1 h of low-intensity steady-state kayaking (60% MHR)

- Wednesday

M – Water. 1h30'. Long intervals. 7 x 2000m (80-85% MHR), recovery 3' at very slow pace

A – Strength training. Extensive hypertrophy. 6 exercises (bench press; semi-squat; biceps curl; French curl lying down; bench pull; pull-ups), 12-15 Repetitions Maximum (RM). 5 sets each exercise, 2-3' of rest between exercises.

- Thursday

M – 1h of running. Speed training. Several skipping exercises + 8 x 30 m (100%), with full recovery

A – Water. VO2max intervals. 2 x 10 x (400m at 90% MHR), 60 s and 3 min recovery between intervals and sets, respectively.

- Friday

M – Water. 1 h of low-intensity steady-state kayaking (70% of maximal heart rate (MHR)).

A – 30' running at a moderate pace + Strength training. Extensive hypertrophy. 6 exercises (bench press; semi-squat; biceps curl; French curl lying down; bench pull; pull-ups), 6-8 Repetitions Maximum (RM). 5 sets each exercise, 2-3' of rest between exercises.

- Saturday

M – Water. 1h35' fartlek. Pacing variations are determined by the athlete's motivation and physical conditioning.

A – 45' running at moderate pace + 30' general calisthenics

– Sunday

M – 35 km at a moderate pace (70-80% of maximal heart rate)

A – Rest

Blood Sampling

Blood collection was performed after overnight fasting and following a 2-day period of physical training avoidance. Venous blood samples (5 ml) were drawn by puncture of the antecubital vein with the subject in a seated position, after 15 min of complete resting and into ethylenediaminetetraacetic acid (EDTA) vacutainers and processed within six hours.

Analytical Procedures

A Complete Blood Count (CBC) was obtained using an automated blood counter (XE-5000. Sysmex Corporation, Kobe, Japan) and lymphocyte subset immunophenotypes were characterized by multiparametric flow cytometry study (FACSCanto II, Becton Dickinson Biosciences (BD), San Jose, CA, USA). Immunophenotype data analysis was performed using the INFINICYT (Cytognos, Salamanca, Spain) software program.

Total leukocytes and differential count for five populations were obtained using standard procedures (Max M - Coulter Electronics ®).

Immunophenotyping

Mouse monoclonal antibodies were used and directed against leukocyte cell surface antigen and conjugated to Fluorescein Isothiocyanate (FITC) or Phycoerythrin (PE). Cluster, clone, fluorescent stain, origin, and antibody specificity are summarized in Table 1.

Samples Preparation for Flow Cytometry

Lymphocyte subsets were determined through direct immunofluorescence. EDTA-treated blood was incubated (20 min, 4ºC, dark) with 15 µl of monoclonal antibodies conjugated with FITC or PE. Subsequently, erythrocytes were lysed for 10 min with 2 ml of *Facs Lysing Solution* ® (BD). Finally, the cells were washed in PBS, centrifuged at 1500 r/min, and maintained again in PBS. List mode files were acquired within two hours in a flow cytometer (FACScan,BD).

Table 1: Cluster, clone, fluorescent stain, origin, and antibody specificity.

Cluster	Fluoro-chrome	Clone	Origin	Main cell reactivity
CD3+	FITC	SK 7	BD*	T cells
CD4+	PE	SK 3	CT **	Helper/inducer T cells
CD4+CD45RA+	FITC	2H4	CT **	CD4 "naive"
CD8+	PE	SK 1	BD *	Cytotoxic/suppressor T cell
CD57+/CD8+	FITC/PE	HNK - 1, SK 1	BD *	T cells, not restri. to MHC
CD3-CD16+&56+	FITC/PE	SK 7, B 73.1, My 31	BD *	Natural Killer cells
CD19+	FITC	4 G 7	BD *	B cells
TCR γδ	PE	11 F2	BD *	Cytotoxic T cells CD4-CD8-

Legend: *BD, Becton Dickinson, San José, California, USA; ** CT, Coulter Electronics.; fluorescein isothiocyanate (FITC); phycoerythrin (PE); Isotype controls from BD and Coulter were used.

Table 2: Immune changes during a competitive kayaking season.

Cells	M1	M2	M3
Leukocytes [a]	6.27	4.96	5.5
Lymphocytes [a]	2.41	1.77	2.23
Lymphocytes (%)	38.4	35.7	40.5
Neutrophils [a]	3.1	2.5	2.8
Neutrophils (%)	49.2	50.2	50.3
Neut/Lymp. ratio	1.28	1.41	1.25
Monocytes [a]	0.4	0.6	0.3
Monocytes (%)	7	11.1	5.8
Eosinophils [a]	0.3	0.1	0.1
Eosinophils (%)	5.1	2.4	2.6
Basophils [a]	0.02	0.03	0.04
Basophils (%)	0.3	0.6	0.8

[a] (x 10^9/L); M1 – Basal values, after 15 days of resting; M2 – After Winter National Championship; M3 – After Summer National Championship

Data Collection and Analysis

It used the software for research FACScan Lysis II 1.1. (BD), drafting graphics of points for lymphocyte separation using Frontal dispersion windows (FSC) versus lateral dispersion windows (SSC) and confirmed by the gate in CD45 versus SSC, with windows in the cells with the highest fluorescence intensity in CD45. One thousand events were analyzed. For the multipliers FL1 and FL2, 600 and 581 volts were applied, respectively with a linear amplification for FL1 and a spectral compensation for FL2. To confirm the analyzed population, the lymphocyte percentage obtained was compared with the different values of the five subpopulations, and the result was obtained with the first graphic of points – FL1 versus SSC – using CD45/CD14 (Leucogate - BD). Cells showing the double mean fluorescence intensity compared to the negative control were considered positive.

Results

A clear variation in absolute leukocyte counts was seen during the season (Table 1), with leukocyte, lymphocyte, and neutrophil absolute count values decreasing 32%, 26%, and 19% respectively, and monocyte absolute counts increasing 50% between M1 and M2. In M3, the tendency was to return to initial values. Basophils increased during the season while eosinophils decreased between M1 and M2 and remained stable in M3.

Immunophenotype also changed throughout the season (Table 2), with some T cells (CD3+) subpopulations decreasing (CD3+ 7%, CD3+ αβ 6.9%, CD3+ γδ 34%, CD8+ 18%, CD45RA 36.4%) or increasing (HLA-DR 1088%, CD4+ 28.1%, CD25+ 24%, CD45RO 136.3%, and CD4+/CD8+ 66%), and an increase in B cells (CD19+ 136.8%) between M1 and M2. In M3, almost all indicators tended to return to initial values.

Discussion

This study aimed to analyze the alterations induced by training and competitions on the immune profile of an elite marathon kayaker with the recurrent crisis of exercise-induced airway hyperresponsiveness. Elite kayakers, particularly marathoners, are characterized by long periods of strenuous daily training workouts that can induce marked acute immune changes. These changes are transient and usually revert after 24 hours [23,26]. However, training continuity can impair complete immune recovery eliciting chronic adaptations, which can alter the immune status of the athletes. The alterations observed in this study, are almost all within the laboratory reference values, which makes clinical validation difficult. It seems that except for

Table 3: Lymphocyte subsets change during a competitive kayaking season.

Cells: x 10^9/L (% of lymphocytes)	M1	M2	M3
CD3+	1.91 (79.4%)	1.31 (73.8%)	1.69 (76.2%)
CD3+CD16+/56+	3%	0.7%	2.3%
CD3+αβ	1.72 (71.7)	1.18 (66.7)	1.55 (69.7)
CD3+γδ	0.098 (4.1)	0.047 (2.7)	0.082 (3.7)
CD3+HLA-DR	0.031 (1.3)	0.42 (23.6)	0.035 (1.6)
CD3+CD25+	0.27 (11.3)	0.25 (14.3)	0.30 (13.9)
CD4+	0.68 (28.4)	0.64 (36.4)	0.67 (30.3)
CD4+CD45RA	0.32 (13.3)	0.39 (22.3)	0.33 (14.8)
CD4+CD45RO	0.41 (17.1)	0.32 (18.1)	0.36 (16.2)
CD4+CD25+	0.26 (10.7)	0.21 (11.9)	0.24 (11.0)
CD8+	1.28 (53.2)	0.77 (43.6)	1.11 (50.0)
CD8+CD45RA	1.03 (42.7)	0.35 (19.9)	0.89 (40.0)
CD8+CD45RO	0.19 (7.9)	0.50 (28.5)	0.23 (10.6)
CD3-CD8+	8.5%	11.3%	8.7%
CD16+/56+	0.56 (24.3)	0.37 (21.2)	0.49 (22.0)
CD19+	0.05 (1.9)	0.07 (4.5)	0.16 (7.1)
CD25+	13.2%	16.4%	17.2%
CD56HLA-DR	0.1%	2.6%	0.2%
CD94+	0.37 (15.2)	0.37 (20.9)	0.31 (14.0)
CD94HLA-DR	0.1%	5.4%	0.2%
HLA-DR	0.07 (2.7)	0.57 (32.1)	0.18 (8.0)
CD45RA	1.73 (71.8)	0.80 (45.7)	1.63 (73.0)
CD45RO	0.65 (27)	1.12 (63.8)	0.64 (28.6)
CD4+/CD8+ ratio	0.50	0.83	0.60

salivary IgA, clear and consistent markers of immunodepression in athletes remain elusive [38].

In general, all immunological indicators that changed between M1 and M2 tend to return to their initial values in M3, which is in line with the results found by Bobovcak et al. [4] in top athletes in different moments of the season. Total leukocyte counts markedly decreased, which was accompanied by the concomitant decrease in neutrophils and lymphocytes. Low basal leukocyte and neutrophil counts are common in athletes engaged in stressful exercises either due to the eventual translocation to peripheral sites of potential antigen encounter (e.g. lungs, gut) or due to clearance of muscle debris by phagocytosis [11,16,34]. Lymphocyte decrease in our study conflicts with the results obtained by Hatch-Mcchesney et al. [16] after 22 weeks of military training who showed the opposite behavior. The marked increase in monocytes may be related to arduous training. A high basal number of monocytes increases the production of prostaglandins resulting in eventual immunosuppression [48]. These biomarkers return to initial values in M3. As training volume increased after M2 we can speculate that this improvement is the outcome of less aggressive climatic conditions reducing exercise-induced bronchoconstriction and the subsequent inflammatory processes [44,47]. Asthmatic patients are characterized by high levels of eosinophils [15]. The decrease seen in M2 can be attributed to the efficacy of the anti-asthma drugs which eventually attenuated allergic reactions. Basophils ranged within laboratory normal ranges and showed a slight increase during the season. T lymphocytes (CD3+) decreased from M1 to M2 increasing in M3 which is partially supported by Baj et al. [2] who verified a significant decrease in the absolute number of CD3+ cells throughout a cycling season but conflict with Rodrigues dos Santos et al. [41] who verified high stability in these immune cells. T cells in our kayaker are markedly higher than those seen in the young military before and after 22 weeks of military training [16].

Both, CD3+αβ and CD3+ γδ cells showed slight variations which can be considered an index of good immune surveillance [5,10,41]. CD3+ γδ cells which have a broad spectrum of immune functions are clearly mobilized during exhaustive exercise but return to baseline values within 15 min [1].

In humans, 2–4% of CD4+ cells express CD25+. Our athlete displayed higher values presumably associated with his clinical status [15], improved prevention against autoimmunity [6], or increased immune alertness [31].

Training does not significantly affect immune cells expressing HLA-DR [21] whose mean clinical reference value is 2.2% (1.3 – 4.0). In our study, the dramatic peak in M2 can be related to the elevated number and percentage of activated monocytes [9] and other immune cells which is a characteristic of trained subjects. High values of cells expressing HLA-DR can be deleterious to the immune status [34].

After both moderate (50% VO2max) or intense (80% VO-2max) exercise NK cells count increases dramatically, but returns to basal levels 3.5 h post-exercise [35]. As a chronic adaptation, NK cell count may decline during prolonged intense physical training [27]. In M1 our paddler showed high basal values, above the highest laboratory reference value, that remained stable throughout the season and were similar to those found in marathon runners [34] but completely different from military trainees [16 whose basal values (6.4±4.3%) increased (9.2±6.7%) after 22 weeks of military training. Can be raised the hypothesis that our kayaker's respiratory condition involves both allergen-specific T helper type 2 cells i.e. adaptive immunity [49] and innate immunity.

The cluster of differentiation CD94+ is a cell surface molecule involved in MHC I recognition by NK and activated/memory CD8+ cells [14]. The percentage of cells expressing CD94+ did not change after exercise [17] and showed longitudinal stability in highly trained athletes [39]. Our results confirm these assumptions.

CD4+ cells (M1: 684; M2: 644; M3: 675 cells/μL) varied slightly during the season which is in line with the study of Hunt et al. [19] in healthy active subjects but conflicts with Makras et al. [28] who showed a significant increase in CD4+ cells after 4 weeks of intermittent moderate exercise while Weiss et al. [50] showed a significant decrease in CD4+ cells after 4 weeks of anaerobic training (weight lifting and interval training). The exercise mode and physical status of the subjects can be the reasons for this discrepancy.

CD8+ cells respond to diverse antigens presented by MHC class I molecules by proliferating, secreting cytokines and chemokines, and directly lysing infected cells [3]. In a cohort of 273 healthy Chinese mean values for CD8+ T cells were 515±27.2 cells/ μL [51], and in active healthy subjects, after 30 min of cycling, values increased from 338±120 cells/μL to 512±214 cells/ μL [19]. In trained athletes, the CD8+ cell percentage is higher (33±5% of total lymphocytes) than in athletic inactive persons [8], however, it was found lower values in athletes [7,46]. The high values in this study conflict with other studies. High values of CD8+ T cells are important to cope with intramuscular inflammation and help to facilitate muscle tissue regeneration [37] which fits well with the arduous training that characterizes this elite kayaker. Moreover, urges to highlight the contribution

of CD8+ T cells to the suppression of airway hyperresponsiveness and airway inflammation [45]. In athletes, B-lymphocytes account for 11±3% of circulating lymphocytes [8]. Albeit B cell counts are very stable [41] and did not vary during a swimming season [13], periods of strenuous training can decrease CD19+ cells [29,43] what conflicts with our results. The initial value (M1) is very low and clearly out of the reference values [32], and can be related to the migration of B cells to the upper respiratory tract tissues and subsequent apoptosis [36], situation progressively reverted in M2 and M3 possibly due to anti-asthma therapeutic or improved adaptive immune system.

The number of CD4+CD45RO (memory) and CD4+CD45RA (naïve) T cells showed slight alterations during the season. While CD8+CD45RA experienced a marked decrease (-114%) in M2 and recovered the initial values in M3, CD8+CD45RO showed the opposite behavior: increased 260% and tended to recover initial values in M3. The increase verified in M2 of CD8+CD45RO T cells can be related to the fight against allergic inflammation and airway sensitivity [25] problems that normally increase during winter in outdoor athletes. Conflicting with our results, Woods et al. [52] showed a tendency for the percentage and number of CD4+ and CD8+ naive cells (CD45RA+) to increase and for CD4+ memory cells (CD45RO+) to decrease after 6 months of moderate aerobic training. This discrepancy may result from differences in the level of training and health status in the two studies.

The normal CD4/CD8 ratio in healthy people is not completely established but varies roughly in a 2:1 ratio [22]. Basal values of CD4+/CD8+ ratios in young active subjects are normally greater than 1.5 [20]. An inverted CD4+/CD8+ ratio (<1.0) is usually associated with some diseases. However, studies conflict with the morbidity and mortality rates associated with low CD4+/CD8+ ratios [30]. CD4+/CD8+ ratio decreases after exercise returning to basal values within 60 min [18]. After a kayaking ultramarathon, the CD4+/CD8+ ratio decreased, increasing further during the recovery period [41] which conflicts with the starting value of our marathoner which is similar (0.5) to some values found in cancer patients with the worst recovery diagnosis [42]. The slight increase in the CD4+/CD8+ ratio verified in M2 does not clearly means a significant improvement in the immune response but a circumstantial decrease in CD8+ T cells.

Conclusion

The immune status of the kayaker analyzed in this study showed evident signals of immunosuppression at the beginning of the season eventually related to his clinical status and reflected in low neutrophils count, very low CD4+/CD8+ ratio, and low CD19+ cells. It is difficult to discriminate the relative influence of training and URTI on immune cell changes. The low CD4+/CD8+ ratio during the season can be a signal of chronic inflammation in line with the health status of the athlete.

Increased count of CD19+ cells in M2 points to the improvement of the adaptive immune system and a higher degree of immune alertness which is reinforced by the increase in cells expressing CD45RO. However, other immune changes (elevation in monocyte count and cells expressing HLA-DR) contradicted this improvement. After summer competitions the kayaker tended to recover starting values. The magnitude of the immune alterations verified during the season showed an overall immune instability that can be deleterious for health and physical performance.

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