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Reduced thymic output in elite athletes

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ABSTRACT

Athletes undergoing intensive training schedules have chronic exposure to stress-induced hormones such as cortisol that can depress immune function. We compared the circulating levels of T cell receptor excision circles (TREC), a marker of recent thymic emigrants, as well as the levels of naïve and memory subsets in a group of elite endurance athletes and in controls. The athletes showed a reduction in absolute numbers of naïve T cells, particularly in CD4 T cells. In contrast, memory cells were increased. TREC levels in the athletes were significantly reduced compared to age-matched controls. Such changes resemble premature ageing of the T cell component of the immune system. Since thymic production of T cells naturally decline with age, these results raise the concern that prolonging high intensity exercise into the 4th decade of life may have deleterious consequences for athletes' health.

1. Introduction

The activity of the thymus, an essential organ for the production of T cells, declines with age (Fallen et al., 2003b; Gray et al., 1975; Jamieson et al., 1999; Kurashima et al., 1995; McFarland et al., 2000). Consequent upon this decline is a change in the distribution of cells in the peripheral circulation, with naïve T cells decreasing with age while memory and effector cells increase. Such changes are more extreme either when the thymus has been removed early in life (Gress and Deeks, 2009), or when there is profound lymphopenia requiring reconstitution of the peripheral lymphoid compartment, as after haematopoietic stem cell transplantation (Fallen et al., 2003b). In this latter case it is clear that in patients over 30 years of age the contribution of the thymus to repopulation of the peripheral T cell pool is significantly impaired and distortions in the distribution of different T cell subsets can be maintained for some years after the transplant itself (Fallen et al., 2003a). Such changes in the distribution of T cell subsets may not be reflected in the peripheral T cell numbers, since a constant level of T cells may be maintained through expansions and contractions of different subsets. Measurement of thymus activity is thus essential to understand the relative contributions of the thymus versus peripheral expansion in maintaining T cell numbers. Signal-joint T cell receptor excision circles (sjTREC) are a marker of the commitment of T cells to the αβ lineage during intrathymic development (Koup et al., 1998); thus the concentration of sjTREC in peripheral T cells can provide an indication of the activity of the thymus.

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2. Materials and methods

2.1. Donor cohorts

The athlete cohort comprised a group of 19 national standard triathletes training at Loughborough University; each individual trained for a minimum of 6 days a week and for a minimum of 1.5 h per session, swimming, running and cycling between 280 and 400 km per week plus 8 h per week of weight, interval or skills training. The age range in this cohort was 18–36, with a median age of 20.7 years.

The age-matched control cohort comprised students from the Universities of Bath, Edinburgh and University College, London selected only on the basis of an age between 18 and 36 years at the time of sampling, with a median age of 21. A second control group, drawn largely from volunteer co-workers, had an age range from

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30 to 55. While healthy, the control donors reported no systematic endurance training programs.

2.2. Blood samples

20–40 ml samples of peripheral blood were taken by sterile venepuncture after informed consent from each donor under approval by the relevant institutional ethics committees. Samples were taken from the athletes on a rest day, at least 18 h after the last exercise session.

Absolute cell counts were obtained from fresh unseparated blood using BD TruCount tubes and the BD MultiTest CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC reagents; data were collected and analysed using BD Multiset software on a BD FACScan flow cytometer (all BD Biosciences, San Jose, CA).

Peripheral blood mononuclear cells were prepared by density gradient centrifugation with a lower layer of Lympholyte (Cedarlane Laboratories Limited, USA) on which PBMC float. These were recovered, washed twice in RPMI1640, resuspended in 10 ml RPMI 1640 and the cell yield counted using an haemocytometer (Neubaur Chamber, Weber).

2.3. Flow cytometry

Cells stained with fluorochrome-conjugated antibodies were visualised using flow cytometry using FACSCalibur flow cytometer and CellQuest software (BD Bioscience, San Jose, California, USA). FloJo (Tree Star Inc., Ashland, Oregon, USA) was used for analysis of stained subsets. The antibodies used for analysis were CD3-APC (clone HIT3a), CD4-PerCP (clone SK3), CD8-PerCP (clone SK1), CD27-FITC (clone M-T271), CD45RA-FITC (clone L48) and CD45R0-PE (clone UCHL1) (all BD Bioscience, San Jose, California, USA).

2.4. Selection of CD4 and CD8 subsets from PBMC's

Isolation of CD4 + and CD8 + T-cells was carried out using EasySep positive magnetic separation (Stem Cell Technologies, Vancouver, Canada) according to the manufacturer's instructions and positive selection MS+/RS + columns with a maximum capacity of 1×10^7 labelled cells.

2.5. Quantitative PCR measurement of TREC numbers

Real-Time PCR was carried out using an ABI/PRISM 7500 sequence detection system and SDS software (Applied Biosystems, Foster City, CA) to quantify sjTREC in cell lysates from donor and athlete samples. The 5′ primer, 3′ primer and probe sequences were CACATCCCTTTCAACCATGCT, GCCAGCTGCAGGGTTTAGG and FAM-ACACCTCTGGTTTTTGTAAAGGTGCCCACT-TAMRA, respectively.

PCR reactions were set up in 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA); sealed using Optical Adhesive Covers (Applied Biosystems, Foster City, CA, USA) and transferred to the ABI/PRISM 7500 Sequence Detector for analysis.

To quantify sjTREC a series of standard dilutions of plasmid containing the signal-joint breakpoint was created following a standard protocol (Douek et al., 2000). Each sample was run in triplicate along with the dilution series of TREC plasmid and 'no template' control (NTC).

The PCR was carried out in 25 μl reactions containing 5 μl of cell lysates or 5 μl of the standard, and a final concentration of 1 U Platinum Taq (Invitrogen Ltd., USA), 3.5 mM $MgCl_2$, 0.25 mM dNTPs, 12.5 mM of each primer, 3.75 mM fluorescent labelled probe, 2.5 ml Platinum buffer and 0.5 ml of ROX reference dye. One cycle of denaturation (95 °C for 5 min) was performed, followed by 40 cycles of amplification (95 °C for 30 s, 60 °C for 1 min).

2.6. Statistical analysis

Statistical analyses were carried out using Prism (Graphpad Software Inc., La Jolla, CA, USA). Comparison between the athlete and individual control groups was made using the non-parametric Mann–Whitney test. In addition, comparison between all groups was made using the Kruskal–Wallis test and adjusted with Dunn's multiple comparison test. A p value of 0.05 was taken as the threshold for significance.

3. Results

3.1. Peripheral CD3 T cell counts in athletes are the same as those in controls yet thymic output is significantly reduced

As exercise has been shown to induce apoptosis of thymocytes (Concordet and Ferry, 1993), we sought to evaluate the impact of chronic exercise stress on the output of new T cells. Previous studies have shown that exercise causes transient changes in lymphocyte numbers – a lymphocytosis followed by a lymphopenia that resolves over 3–12 h. To determine whether this might influence the results obtained, we measured the levels of CD3 + cells (T cells) in the peripheral circulation of the athletes on a rest day (at least 16 h after exercise) and compared the values obtained to those of normal, healthy, age-matched controls, as well as to an older healthy control group. None of the values obtained – means of 1456 ± 420 CD3 + cells per ul of blood for the athletes versus 1411 ± 467 for the matched controls and 1421 ± 742 for the older controls – are significantly different from any other.

To ask whether there was a differential contribution of thymic output to the peripheral T cell populations, we compared the sjTREC concentration in the peripheral blood of the athletes with that of age-matched controls, as shown in Fig. 1. Although there is no significant difference in the number of T cells in the peripheral circulation comparing athletes with age-matched controls, we observed a striking and highly significant ($p < 0.0001$) reduction in the sjTREC number in the athletes. The median sjTREC count in the control cohort is 3.20×10^4/ml versus 0.13×10^4/ml in the athlete cohort. In normal, healthy individuals, values as low as those seen in the athletes are not generally observed until at least 60 years of age (Jamieson et al., 1999; McFarland et al., 2000).

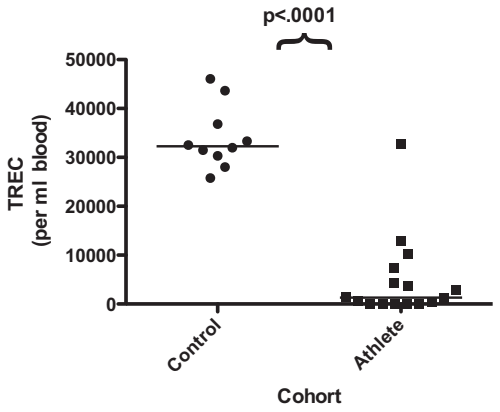

Fig. 1. Thymic function is significantly diminished in athletes versus age-matched controls. qPCR measurements of sjTREC numbers were obtained for the athletes and age-matched controls. Symbols represent individual values (means of triplicates) while the horizontal bars show the mean values obtained (control: $3.4 \times 10^4 \pm 0.65 \times 10^4$; athlete: $0.5 \times 10^4 \pm 0.8 \times 10^4$). The two cohorts differ significantly ($p < 0.0001$).