Immune changes potentially associated with upper respiratory symptons in swimmers during a winter training season

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Introduction

The occurrence of immune changes in high-performance sports athletes with possible consequences for health and performance capacity is a known trend, Until recently few studies had found alterations in NK populations related to chronic exposure to regular training. In true rest situation athletes tend to show normal and similar values to those of sedentary and healthy people (Nieman, DC 2000). Although leukocytes have been shown to decrease after intense long duration exercise, 24 hours after they recover to normal numbers (Shephard, Rhind, & Shek, 1994). However it was suggested that moderate training intensity could increase NK cell count and NKCA in trained individuals (Shepard, et al 1994). Nevertheless, periods of heavy training with transitory impaired performance are associated with some innate and acquired immune depression which normally are reversible by tapering (Gleeson & Bishop, 2005).

There are few studies that address the chronic training effects on immune parameters, however the main ones reported are those that show alterations that occur with T cells, and include lower cells count values and diminished proliferative capacity (Gleeson, 2006). In a longitudinal study over a competitive season with high level Australian swimmers, no alterations were found for B or T cells, but a significant decrease on count and percentages of NK cells were noted (Gleeson et al 1995).

Recently the subsets of NK cells were considered based on their prevalence and function . The majority of NK cells (≈ 90%) are NKCD56dim with high cytotoxic capacity, and NKCD56bright (≈10%) with a higher affinity to IL-2 and a larger capacity to produce cytokines, namely IFN-γ, TNF-β, GM-CSF, IL-10, IL-13 after monokine stimulation (Cooper, 2001).

The striking sensivity of NK cells to exercise stress provides strong support that these cells my be implicated as a potential link between regular physical activity and overall health status (Timmons & Cieslaak, 2008)

Objectives

The aim of this study was to see how training load might influence salivary IgA values, the proportion and phenotypic features of circulating T cells subsets in peripheral blood of high competitive swimmers, and the monitoring of the two NK cells subpopulations, NK CD56bright and NK CD56dim and the possible association with the occurrence of URS episodes during a winter swimming season.

Methods

The sample consisted of a group of swimming athletes of high performance level 13 male (17.2 ± 1.3 years, 174.9 height ± 5.8 and 65.8 ± 6.8 kg weight) and 6 female (15.8 ± 0.8 years, height 163.0 ± 9.4 and 54.6 ± 5.0 weight). Training load was monitored by volume and intensity (Mujika et al 1995).

Blood and saliva samples were taken at rest on 4 characteristic moments of the preparation during a sports season (from September to April): before the start of the season and after a rest period of 5 to 6 weeks (t1) after 7 weeks of a period of gradual increase of training load (t2) after 6 weeks of an intense training cycle (t3) and 48 hours after the main competition (t4). Samples were always taken at the same moment of the day, in sitting position. A time lapse of 36 to 48 hours of rest after the last training session or competition was always respected.

Athletes upper respiratory symptoms (URS) episodes were monitored using daily log books (1 episode= repetition of more than two symptoms on consecutive days). A new episode was considered after an interval of 10 days (minimum) (Bishop, 2006). No differences were observed between genders which allowed putting them together for statistical analysis.

Total leukocyte counts and percentage were measured on an automatic counter (Coulter diff TM Analyser,). B, NK, T, γδ T cells, CD4 and CD8 T cells expressing HLA-DR, CD119 and CD126 T cells were determined by flow cytometry (FACSCalibur; BD, San Jose, C.A., USA).The identification and quantification of the two NK cell subsets was done based on the surface density of CD56 phenotype as follows: CD56dim/CD3-/CD8+/- and CD56bright/CD3-/CD8+/-. The expression of IFN-γ receptor CD119 (clone GIR-208; Pharmingen BD, San Diego, C.A., USA) and Granzyme B production was evaluated within the different NK cell subsets after an electronic gating in a lymphocyte region and an acquisition of 20.000 of total events. Data were analysed using “Infinicyt” software program (Cytognos, Spain).

Results are shown as the percentage of positive cells within each cell subset or/and their mean fluorescence intensity (MFI). Salivary IgA concentration was measured using an inhouse ELISA. Spearman rho for correlation analysis and Friedman tests

with "Dunn's Multiple Comparison Test“ was conduct for statistically analysis ($p<0.05$), preventing from not normal distribution and the low number of the sample subjects

Results and Discussion

Upper Respiratory Symptoms (URS)

The highest number of URS was found between t1 and t2 (53,8%) and also in t3 (43% of the athletes) which was concurrent with a flu peak.These were usually preceded by a lowering of the salivary IgA concentration and secretion rates.

In this study we found a mean of 2,1 ± 1,2 Episodes/athlete. The number of URS fall in line with those previously reported in available literature. The Australian average for general population is 2.5/year (Reid, V. L., Gleeson, M., Williams, N., & Clancy, R. L., 2004) and 3.5/year Episodes for athletes (Cox, A. J., Gleeson, M., Pyne, D. B., Callister, R., Hopkins, W. G., & Fricker, P. A. , 2008) (although there were fewer episodes we must consider that this study lasted only 7 months).

Salivary IgA

An inverse correlation between the mean weekly training volume and the mean salivary IgA concentration and secretation rate ($r=-0.15$, $p=0.001$; $r=-0.183$, $p<0.001$ respectively) was found.

A correlation between the pre-season sIgA concentration and the mean weekly sIgA concentration during the whole training season ($r=0.488$, $p=0.03$) was also found as well as an inverse correlation between de mean sIgA concentration registered during the training season and the number of URTI episodes ($p=-0.501$, $p=0.006$).

Leucocytes

During the season any important variation in WBC on PB was not found, which is in agreement with literature however a slight leukocytosis could be observed from the pre-season to the 2nd moment of the study (t2).

Total Lymphocytes

Although most reports of longitudinal studies in sport do not mention significant alterations in lymphocytes our data shows a trend towards a decrease in this leukocyte population, which is significant at the end of the competitive season after a taper period. Probably the 36 hours of rest after training was not enough to recover the normal values of this lymphocyte population, which seems to agree with Gleeson et al (1995) who found a similar decrease in a longitudinal study with swimmers. However a taper period with reduce training volume and 48 hours rest allowed for a recovery to normal NK values (Gleesson & Bishop, 2005).

T and B Lymphocytes

A reduction in both the percentage and the total number of T (CD3+) and B (CD19+) cells was observed at the end of the training season(t4).

CD4+ and CD8+ T Lymphocytes

Although a small decrease in the total number of CD4+ and CD8+ cells between the basal moment and the 2nd moment of evaluation was seen they were not statistically significant. No changes were observed for the CD4+/CD8+ ratio along the training season.

When looking at the added expression of other markers on the CD4+ T cells we found no changes in the percentage or in the mean Intensity fluorescence (MIF) of CD4+HLA-DR cells during the training season. The percentage of CD4+CD25+ decreased along the training season and showed a MIF peak in the 2nd moment of the study. The percentage of cells expressing the IFN-gR (CD4+CD119+) and the expression per cell of the receptor also decreased along the training season.

The expression of the IL-6R (CD126) at cell level, however was increased during the training season when compared to the basal levels. The percentage of CD4+CD126+ cells was also increased in the 2nd and 3rd moments of evaluation.

When looking at the CD8+ cells we found no changes in the percentage of HLA-DR positive cells during the training season but a peak of MIF was observed in the 3rd moment of the study.

The percentage of cells expressing Granzyme B was increased in the 2nd moment but decreased in the 3rd moment while the expression at the cell level was increase in this last moment of the study. Regarding the expression of the IFN-γR and the IL-6R the CD8+ cells followed the same pattern as the CD4+ cells.

Athletes that suffered more URS episodes during the training season had a lower number of regulatory T cells (CD25+) and a higher number of CD4+ T cells expressing the IFN-gR in the 3rd moment of evaluation that corresponded to an increase in the training load intensity.

γδT cells

A decrease from the 1st to the 2nd moment of the study was found for the total number of γδT cells but their percentage did not change during the training season. A decrease in the expression at the cell level for the IFN-gR was also found along the training season. No changes in the HLA-DR expression were found.

NK cells

Our data showed that the number and percentage of NK cells tended to exhibit a decrease in the total NK population specially on moments of intensified training (M3) $p<0.05$.

Looking at the NK subsets the data show a decrease in the cell numbers of the NK^{dim}, subset during the season, which was significant at t(2) corresponding to the first elevation on training volume. Looking at the percentage we found significantly reduced values more pronounced at t(2) and t(3) ($p<0,001$), that did not recover to the initial values at the end of competitive swimming season.

On the contrary the counts and percentage of the NK^{bright} subset showed an elevation at M2 and M3, which correspond to the heavy training mesocycles, remaining significantly elevated at the end of the season after taper and competition.

The NK population is considered the most responsive of the innate immune system, being cytotoxic and producing cytokines against target cells.

The results show a significant decrease in the percentage and number of NK cells, coinciding with periods of increased training load, never recovering to the initial values observed before the start of the season. In periods of more intense training there is a significant reduction of the $NKCD56^{dim}$ subpopulation which has greater cytotoxic capacity. The other subset, the $NKCD56^{bright}$ (≈10%), has a higher affinity to IL-2 and a large capacity to produce cytokines: IFN-γ, TNF-β, GM-CSF, IL-10, IL-13 after monokine stimulation (Cooper, 2001).

A significant increase in the $NKCD56^{bright}$ / $NKCD56^{dim}$ ratio was also found int(2) and t(3).

The expression of CD119 on subset $NKCD56^{dim}$ was investigated as they have a more cytotoxic role and tend to express the IFN-γ receptor. The % of the expression of CD119 and the expression per cell (MIF) is significantly lower during the training season which could denote a lesser citotoxic activity. The high affinity with IL-2, promotes a large production of INF-γ. When activated $NKCD56^{brigt}$ could be as cytotoxic as the $NKCD56^{dim}$ subset (Timmons & Cieslack, 2008)

Although the expression of Granzyme B at the cell level (MIF) did not exhibit significant alterations during the season, the percentage of cells expressing Granzyme B showed an elevation at the more intense training phases of the season when compared to the pre-season values. The elevated production of Granzyme B surrounding t(2) and t(3) corresponded to a higher incidence of URS around this period.

Conclusions

CD4+, CD8+ and γδ T cells down regulated the IFN-γR expression along the training season and increased the IL-6R expression from the pre-season to the 2nd and 3rd moments of the training season. Since the IFN-γ play an important role in activating the cellular adaptative immune response, decreasing it's production by the T cells may impair the activation of the B cells and the production of immunoglobulins (manly IgG2), possibly leading to an increase in susceptibility to disease.

The total number of NK cells in the peripheral blood (PB) fall in response to the most intense phase of training. We conclude that periods of high training load magnitude have a negative impact on innate immune cytotoxicity. However we were not able to confirm the association between URS occurrence and NK cells behaviour.

At the heavy training phases the $NKCD56^{dim}$ subpopulation showed reduced cell numbers, which could represent an impaired cytotoxic activity with consequences to immunity.

The $NKCD56^{bright}$ subset showed the opposite behaviour increasing at M2. This subpopulation is known to exhibits greater ability to produce regulatory cytokines and chemokines. It is also known that the $NKCD56^{bright}$ subset expresses high levels of adhesion molecules namely the CD62L, which facilitates the traffic of these cells to the lymph nodes and sites of inflammation, which could explain their increased mobilization during heavy training phases. This aspect may be related to redistribution phenomenon (Timmons & Cieslack,2008).

During the season there was a significant increase in the $NKCD56^{bright}$ / $NKCD56^{dim}$ ratio. This ratio had been used as a marker of immune depression, since an elevation of this ratio was in parallel with the reduced citolytic NK activity and associated with the fall in cytotoxicity (Suzui et al 2004).

The NK values tended to gradually recover to pre-season, possibly implying an adaptative mechanism. However there may exist, a compensatory mechanism associated with the fall in total NK and the more citotoxic subset, trough the increase in the percentage of the NK subpopulation that produces more regulatory cytokines.

When we compared athletes that didn't get any URTI, with those that had contracted a high number episodes (>3), we found a significant higher % of NK cells expressing Granzyme B ($p<0.05$) in the last the group.

We can argue that the lower % and MIF expression of CD119 on $NKCD56^{dim}$ throughout the season could represent an impaired immune response.

On the other hand it is possible to view this aspect as an immune adaptation mechanism associated with a down regulation of receptors in the presence of high availability of INF-γ produced by other cells, namely $NKCD56^{brigh}$ stimulated by IL-2 .

The most affected subset was the NKCD56^{brigh}. The real meaning of this behaviour, immune depression or adaptation process is still inconclusive.

However it seems possible that athletes that show a higher NKCD56^{bright} /NKCD56^{dim} ratio are more prone to URS. Our data does confirm that alterations in NK subpopulations may occur during training programs.

The main findings of this study point to an impact on immune function of progressive and light training loads at the initial phase of preparation, after long periods of rest, which should be taken into account by the coaches when adopting recovery strategies aimed at reducing the negative impact of training.

Our results also stress the importance of the use of daily logs or other strategies to monitor how athletes are adapting to workload, which could be useful in detecting early signs of difficulties in this process. For example, preventive measures like nutritional supplementation or training load adjustment could be implemented.

Also important is the medical care support at times of intensified training load, allowing for a rapid diagnosis and treatment of the episodes of illness recorded.

In summary we conclude that athletes subjected to long periods of intense training show alterations both in mucosal and systemic immune parameters.

Parameters related to both innate and acquired immunity show alterations after periods of intensified training volume and /or intensity.

Altered values usually return to basal levels after a tapering period.

It is possible that the sum of all these small alterations may compromise resistance to minor infections, like URS, especially during periods of heavy training. The real meaning of this response behaviour, immune depression or adaptation process, remains inconclusive.

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