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Effects of AM3 (Inmunoferon®) on increased serum concentrations of interleukin-6 and tumour necrosis factor receptors I and II in cyclists

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Abstract

The aims of this study were to examine the changes in plasma concentrations of inflammatory cytokines induced by training and competition in professional cyclists. We report the serum concentrations of interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), tumour necrosis factor receptors I and II (TNFR-I and -II) in a prospective, randomized, double-blind trial involving the administration of AM3 (Inmunoferon®), an oral booster immunomodulator, or placebo to 16 professional cyclists ($n = 8$ in each group) for 65 consecutive days. Serum was collected just before treatment began (baseline), at the end of pre-competition training, before the mountain stage of the competition (60 days), 4 h after finishing this stage (62 days), and 18 h after the fifth and last day of competition (65 days). To determine the normal levels of cytokines and soluble TNF receptors, individual samples from 14 moderately trained healthy controls were studied. After 60 days of training, the serum concentrations of IL-6 did not differ significantly from those at the beginning of the study for either group of cyclists (placebo and AM3). A significant rise was seen in IL-6 concentrations in both the AM3 and placebo groups at 62 days, 4 h after finishing the mountain stage. The increase was significantly greater in the placebo group than in the AM3 group. At 65 days of treatment, 18 h after the fifth and last day of competition, IL-6 concentrations were similar to those recorded at the end of the training, but were significantly higher in the placebo group than in the AM3 group. At the end of training, serum TNFR-I concentrations in both groups of cyclists were significantly lower than at baseline. The concentrations of serum TNFR-I and -II both 4 h after finishing the mountain stage and 18 h after the fifth and last day of competition were significantly higher than those recorded after training in both groups. Professional cycling competition is associated with increases in serum IL-6 and TNFR-I and -II concentrations. Inmunoferon treatment reduced significantly the concentrations of IL-6 but not those of TNFR-I and -II.

Keywords: *AM3, cytokines, inflammation, Inmunoferon, exercise, sport therapy*

Introduction

Cytokines are a complex network of molecules with the capacity to modulate the functions of the cells of several systems (Oppenheim & Feldmann, 2001). Their spectrum of target cells is wide, including hepatocytes, striated muscle and endothelium (Aggarwal, Samanta, & Feldmann, 2001a; Oppenheim & Feldmann, 2001), and they play an important role in regulating the activation and proliferation of immune system cells.

Though the cells of the immune system are the main cytokine producers, endothelial, vascular smooth muscle and striated muscle cells may also have an important role in their secretion (Aggarwal *et al.*, 2001a; Penkowa, Keller, Keller, Jauffred, & Pedersen, 2003; Smith, 2000). The cytokines mainly manifest their biological activity though paracrine and autocrine mechanisms, but the biological and clinical importance of the systemic endocrine effects of at least some cytokines has also been reported (Aggarwal *et al.*, 2001a; Matsuda & Hirano, 2001; Smith, 2000).

Cytokines can be classified according to different structural and functional criteria (Oppenheim & Feldmann, 2001). According to their effects on the regulation of the immune-inflammatory response, they are classed as either pro- or anti-inflammatory.

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Tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are crucial inflammatory-reactive cytokines mainly but not exclusively produced by monocytes (Córdova & Alvarez-Mon, 2001; Starkie *et al.*, 2001a). In contrast, IL-10 and IL-4 are pivotal anti-inflammatory cytokines (Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999). The functional complexity of the cytokine network is expanded by the involvement of soluble receptors of these molecules and others such as IL-1Ra and cortisol (Aggarwal, Samanta, & Feldmann, 2001b; Dinarello, 1998; Drenth, Krebbers, Bijzet, & van der Meer, 1998). A clear example of the balanced regulation of this system is that TNF-α has two soluble receptors, serum TNFR-I and serum TNFR-II, both with the ability to block its biological activity (Aggarwal *et al.*, 2001b).

Interleukin-6 is produced by different immune system cells, including T and B lymphocytes, monocytes and accessory cells (Matsuda & Hirano, 2001, Starkie *et al.*, 2001b). It is also secreted by mesenchymal and endothelial cells (Matsuda & Hirano, 2001; Ostrowski *et al.*, 1999). The target cell spectrum of IL-6 is wide, including immune system and parenchymatous cells such as hepatocytes and endothelium (Matsuda & Hirano, 2001; Ostrowski *et al.*, 1999). Tumour necrosis factor alpha, one of the molecules with the greatest pro-inflammatory activity, is also secreted by different cell populations, including those that make IL-6 (Aggarwal *et al.*, 2001a; Beyaert & Fiers, 1998; Smith, 2000). Tumour necrosis factor alpha has intense modulatory effects on immunocompetent cells as well as parenchymatous cells, such as those of striated muscle, the liver and endothelium (Aggarwal *et al.*, 2001a; Beyaert & Fiers, 1998; Smith, 2000). Tumour necrosis factor alpha also has regulatory effects on nervous and endocrine systems (Aggarwal *et al.*, 2001a; Pedersen *et al.*, 2001; Smith, 2000). Together with IL-6, TNF-α is thought to be one of the main mediators of the asthenia and weight loss associated with the inflammatory processes (Beyaert & Fiers, 1998). The production kinetics of cytokines are only partially understood (Aggarwal *et al.*, 2001a, 2001b; Matsuda & Hirano, 2001). The production of these molecules is thought to be limited in time, with that of TNF-α being very brief and that of IL-6 somewhat more prolonged (Aggarwal *et al.*, 2001a; Matsuda & Hirano, 2001).

Biologically, competitive sport is a highly demanding activity. Modifications in cytokine production have been associated with competitive sport (Ostrowski *et al.*, 1999; Ostrowski, Schjerling, & Pedersen, 2000; Starkie *et al.*, 2001a), with increases in the plasma concentrations of both IL-6 and TNF-α (Ostrowski *et al.*, 2000; Smith *et al.*, 2000; Starkie *et al.*, 2001a). The functional and potentially clinical importance of cytokine alterations induced by competitive sport remains unclear. However, various authors have reported high concentrations of these molecules to be correlated with reduced physical performance due to over-training (Ostrowski *et al.*, 2000; Smith, 2000; Starkie *et al.*, 2001a). Furthermore, immune system disturbances appear to be involved in the high incidence of infections contracted by professional sportspersons (Pedersen & Hoffman-Goetz, 2000; Smith, 2000).

The regulation of the immune system is an important preventive and therapeutic aim in inflammatory diseases (Losy & Michalowska-Wender, 2002; Moreland & Misischia, 2002). Medical interventions include the administration of anti-inflammatory cytokines and the clinical use of inflammatory cytokine blockers. The balanced modulation of cytokine-producing and cytokine-responding active immune cells is a promising medical field (Dinarello, 2003). AM3 (Inmunoferón®), a polysaccharide/protein compound, is a commercially available oral immunomodulator of natural origin, with regulatory effects on the production of certain cytokines (Brieva, Guerrero, Alonso-Lebrero, & Pivel, 2001). It has been shown that AM3 can reduce the production of pro-inflammatory cytokines in different experimental and clinical models of inflammation (Brieva *et al.*, 2001; Perez-Garcia *et al.*, 2002). Simultaneously, AM3 is able to enhance the effector activity of immune cells involved in the response against infectious agents (Prieto *et al.*, 2001). The clinical use of this immunomodulator is safe; it has no detrimental side-effects (Perez-Garcia *et al.*, 2002; Prieto *et al.*, 2001). Recently, investigations conducted into the effect of AM3 on the alterations of the immune system associated with high-performance sport have shown it to have a protective action with regard to the stress response (Córdova, Martin, Reyes, & Alvarez-Mon, 2004).

Given the potential contribution of changes in pro-inflammatory cytokine concentrations to an increased risk of infections and reduced physical performance in competitive sportspersons, the effect of AM3 on serum concentrations of IL-6, TNF-α, TNFR-I and TNFR-II was examined in a population of professional cyclists during training and competition. The study design was that of a randomized, placebo-controlled, double-blind clinical trial. To identify competitive cycling-related changes in these cytokine concentrations, and the ability of AM3 to modulate them, sex- and age-matched healthy controls were also monitored.

Methods

Participants

The participants were 16 male professional cyclists aged 21 – 26 years. All cyclists belonged to

professional cycling teams (Kelme-Costa Blanca and Relax Fuenlabrada).

Inclusion criteria. Cyclists were included if they had more than 3 years' experience of professional cycling competition and had followed a similar training programme since the beginning of the professional cycling season. The protocol was approved by the local ethics committee and followed the principles of the Declaration of Helsinki. Before entry into the study, all participants were fully informed of the procedures, were given the opportunity to ask questions, and provided their witnessed, informed written consent.

Exclusion criteria. Cyclists unable or unlikely to comply with the study protocol were excluded, as were those with chronic diseases, those who in the 3 months before the study had received immunosuppressants, immunomodulators, cimetidine or other medications that might modify the immune response, those who had admitted to taking any kind of drug, those who had taken any quantity of alcohol or who had smoked at all in the 6 months before the study, and those who had suffered an acute infection in the month before the study began. Evidence of intestinal malabsorption, alterations in calcium metabolism, autoimmune disorders or tumour disease was also grounds for exclusion.

Protocol

In a double-blind clinical trial, 16 professional cyclists who met the above criteria were randomized to receive orally either AM3 ($n=8$) or an indistinguishable placebo ($n=8$) (Table I). Clinical studies at admission included a review of participants' medical histories, physical examination, a complete blood test including the determination of sodium, potassium, chloride, calcium, urea, glycaemia, total cholesterol, HDL cholesterol, triglyceride, thyroid-stimulating hormone, C-reactive-protein, ferritin, yGT, ALAT (GPT), ASAT (GOT), total bilirubin, alkaline phosphatase, creatine phosphokinase, creatinine, albumin, total testosterone and basal cortisol concentrations, sedimentation rate, full blood and reticulocyte counts, and an electrocardiogram according to the programme of obligatory examinations by the International Cycling Union (UCI) (medical monitoring programme for trade teams I and II) (http://www.uci.ch/english/health_sante/docs_2002/uci_examinations_2002. pdf).

Table I. Physical characteristics of the cyclists ($n=16$).

Age (years)	26.2 ± 1.3
Body mass (kg)	69.8 ± 1.9
Height (m)	1.80 ± 0.01
Body fat (%)	7.1 ± 0.2
Maximal oxygen uptake ($ml \cdot kg^{-1} \cdot min^{-1}$)	81.1 ± 1.7
Heart rate at the anaerobic threshold ($beats \cdot min^{-1}$)	170.6 ± 2.2
Oxygen consumption at the anaerobic threshold ($ml \cdot kg^{-1} \cdot min^{-1}$)	66.1 ± 1.2

Note: The anaerobic threshold was determined as the intensity corresponding to a blood lactate concentration of $4\,mmol \cdot l^{-1}$. Values are mean $\pm$ standard error of the mean.

A week before the race started, and to check their performance, the cyclists underwent a continuous and progressive maximal test to exhaustion. The test was performed on a cyclosimulator (Cateye CS-1000, Cateye Co. Ltd., Osaka, Japan), upon which their own competition bicycle was fixed. The test started at $32\,km \cdot h^{-1}$ with increments of $1\,km \cdot h^{-1}$ every minute, with continuous electrocardiograph monitoring (electrocardiograph, Schiller AG, Baar, Switzerland). The laboratory experimental conditions (temperature 21°C), schedule and warm-up were the same for all cyclists. On the day of the experiment, the participants arrived at the laboratory at 08:00 h. It was recommended that they have a light training session and that their diet was high in carbohydrates the day before the test. The cardiac response was also monitored telemetrically throughout the test using the cyclists' own heart rate monitor (Polar Xtrainer Plus, Polar Electro Oy, Kempele, Finland). Respiratory gases were measured using an expired gas analyser (Medical Graphics System CPX-Plus de Medical Graphics Corporation, St. Paul, MN), which was calibrated before each measurement. The anaerobic threshold was determined as the intensity corresponding to a blood lactate concentration of $4\,mmol \cdot l^{-1}$. At baseline, similar physical characteristics between the placebo and AM3 groups were observed.

The race (Mallorca Challenge) was characterized by five consecutive stages covering 749.1 km in total. The mountain stage of 167.1 km contained four second category climbs. The average heart rate during this stage was $156 \pm 4.3\ beats \cdot min^{-1}$. The race was cycled in a Mediterranean climate, which is characterized by a moderate temperature throughout the year (mean = 16.5°C), moderate to minimal annual rainfall and northerly winds. All cyclists consumed a high carbohydrate diet (20% fat, 65% carbohydrate and 15% protein) during the whole period.

Peripheral venous blood was collected at the start of the study from all cyclists to assess serum cytokines. In both the AM3 and placebo groups, peripheral venous blood was collected to assess serum cytokines before starting the treatment (baseline), after 60 days before the mountain stage of the competition (60 days), 4 h after finishing this stage

(62 days) and 18 h after the fifth and last day of competition 65 days). The blood samples were obtained from an antecubital vein using serum separating tubes (BD Vacutainer^{TM} System) with clot activator (Becton Dickinson, Belliver Industrial Estate, Plymouth, UK). All sampling was done between 09:00 and 09:30 h with the participant seated, with the exception of the samples obtained 4 h after finishing the mountain stage. After 20 min of clotting at room temperature, tubes were centrifuged at 3000 rev $\cdot$ min^{-1} for 20 min on Multifuge 3 S-R (Heraeus, Hanau, Germany) and the serum aliquoted and frozen at -80°C for later use.

Fourteen age-matched, healthy, non-smoking, moderately trained males with a mean age of 23 years (range 21 – 25) formed the control group. To properly control potential time-dependent variations in the immune parameters analysed, peripheral venous blood from the healthy control group was collected.

Treatments

Inmunoferon® (AM3; I.F. Cantabria, Madrid, Spain), a polysaccharide/protein compound purified from C*andida utilis*, is a commercially available booster immunomodulator with a low toxicity profile. Previous dose-finding and kinetic studies with AM3 in pilot studies showed 3 g $\cdot$ day^{-1} to be the optimal dose for maximum immunostimulation without side-effects (Perez-Garcia *et al.*, 2002; Prieto *et al.*, 2001). Six capsules (two 500 mg capsules three times a day) of either AM3 or placebo were orally administered daily for 65 consecutive days. The compliance of the participants was monitored by one of the members of the sport medical service.

Cytokine assays

Interleukin-6, TNF-α, TNFR-I and TNFR-II were measured by commercially sandwich ELISA (R&D Systems, Inc. MN, USA) in duplicate wells, following the manufacturer's recommendations. The intra-assay coefficients of variation were 4.3, 5.6, 5.7 and 3.9%, respectively. The lower limits of detection were 0.09, 0.12, 3.00 and 1.00 pg $\cdot$ ml^{-1}, respectively.

Statistical analyses

All analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between the control and treatment groups were tested by the Mann-Whitney *U*-test and Kruskal-Wallace test. Comparisons of serological parameters within treatment groups at different instants during the study were undertaken using the non-parametric Friedman test and Wilcoxon rank test. Statistical significance was set at $P < 0.05$.

Results

The race provoked a significant increase in IL-6 which was prevented by AM3

Serum IL-6 concentrations were measured at baseline, before the beginning of the treatment with either the placebo or AM3, and again after 2 months of pre-competition training (Figure 1). At baseline, no significant differences were found in IL-6 either between the two groups of cyclists or between these and the moderately trained healthy controls. Nor were there any differences between the placebo and AM3-treated groups after 2 months of pre-competition training. Four hours after the finishing mountain stage, there was a significant rise in serum IL-6 in both the placebo ($P = 0.009$) and AM3 group ($P = 0.014$) compared with baseline, and in the placebo group after 60 days of pre-competition training ($P = 0.0018$). However, serum IL-6 was significantly higher in the placebo group than the AM3 group ($P = 0.05$). These differences were also observed 18 h after the fifth and last day of competition ($P = 0.05$). We did not observe any significant differences in hematocrit concentrations before and after each stage of the race. There were no significant differences in IL-6 serum concentrations in the group of moderately trained healthy controls.

The race was not associated with increased serum TNF-α, but was associated with increased concentrations of soluble receptors I and II

Figure 2 shows the results of the TNF-α analysis. Two months of pre-competition training made no significant difference to the serum TNF-α concentrations of either the placebo or AM3 groups. Four hours after finishing the mountain stage, significant reductions were registered in both groups ($P = 0.05$) compared with baseline, although there were no differences between the two experimental groups. Eighteen hours after the fifth and last day of competition, the serum TNF-α concentrations were similar to those at baseline. No significant differences were seen between the two experimental groups.

For both placebo and AM3 experimental groups, serum TNFR-I concentrations fell significantly after 2 months (end of pre-competition training) ($P = 0.017$ and $P = 0.021$ respectively) (Figure 3). After the end of the second race stage (the mountain stage), both serum TNFR-I and TNFR-II concentrations were significantly higher than those observed at the end of the pre-competition training period (60 days) in the placebo and AM3 groups (TNFR-I:

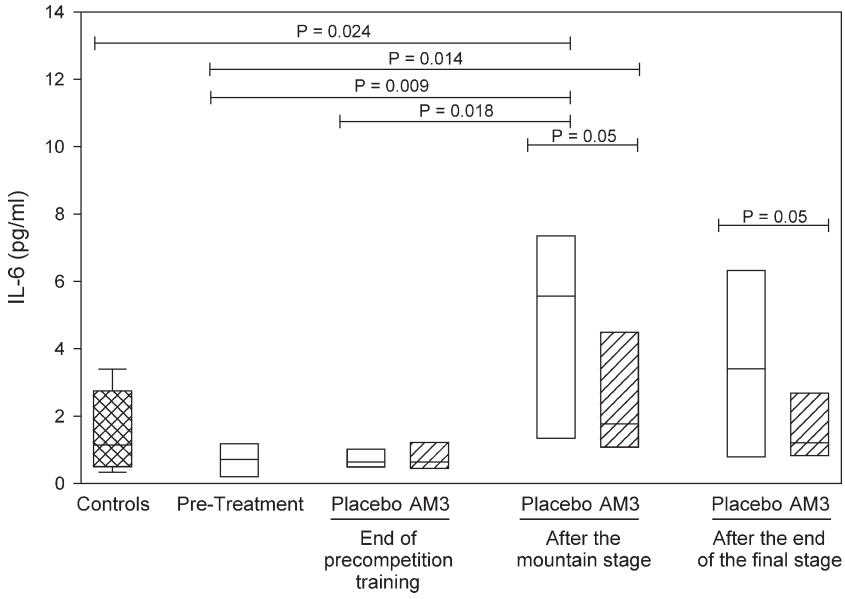

Figure 1. Serum IL-6 concentration in moderately trained healthy controls and the two groups of cyclists. Serum was collected just before treatment began (baseline), at the end of pre-competition training, before the mountain stage of the competition (60 days), 4 h after the end of the mountain stage (62 days), and 18 h after the fifth and last day of competition (65 days). Data represent median and interquartile range.

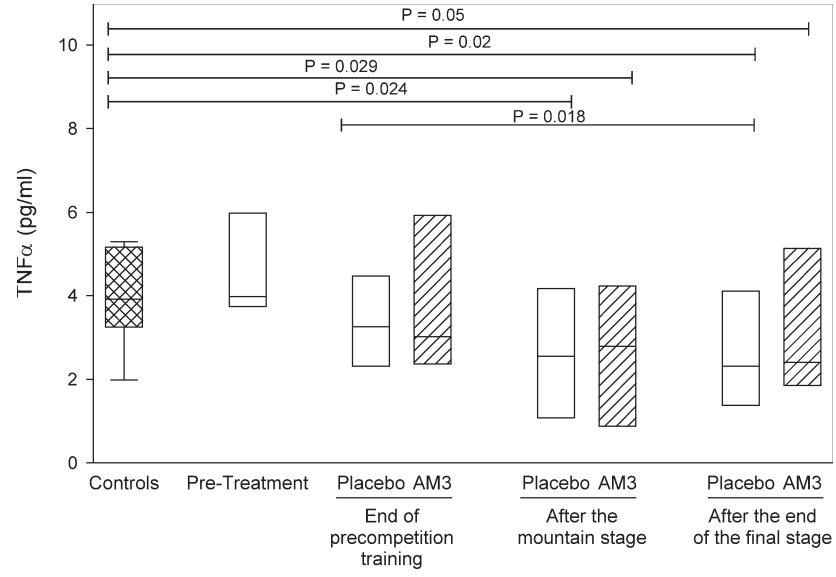

Figure 2. Serum TNF-α concentration in moderately trained healthy controls and the two groups of cyclists. Serum was collected just before treatment began (baseline), at the end of pre-competition training, before the mountain stage of the competition (60 days), 4 h after the end of the mountain stage (62 days), and 18 h after the fifth and last day of competition (65 days). Data represent median and interquartile range.

$P = 0.018$ and $P = 0.012$ respectively; TNFR-II: $P = 0.018$ and $P = 0.012$ respectively) (Figures 3 and 4). Furthermore, serum TNFR-I concentrations in both experimental groups were signifi- cantly higher 4 h after finishing the mountain stage than at 18 h after the fifth and last day of competition (placebo: $P = 0.017$; AM3: $P = 0.036$), although no significant differences were observed between the two groups at any time. The concentrations of both TNF receptors were significantly higher in both experimental groups 18 h after the fifth and last day of consecutive daily racing, and were significantly higher than those found before the start of the competition (TNFR-I: $P = 0.036$ and $P = 0.017$ respectively; TNFR-II: $P = 0.018$ and $P = 0.012$ respectively). No significant differences in serum TNF-α or TNFR-I and -II were observed for the group of moderately trained healthy controls.

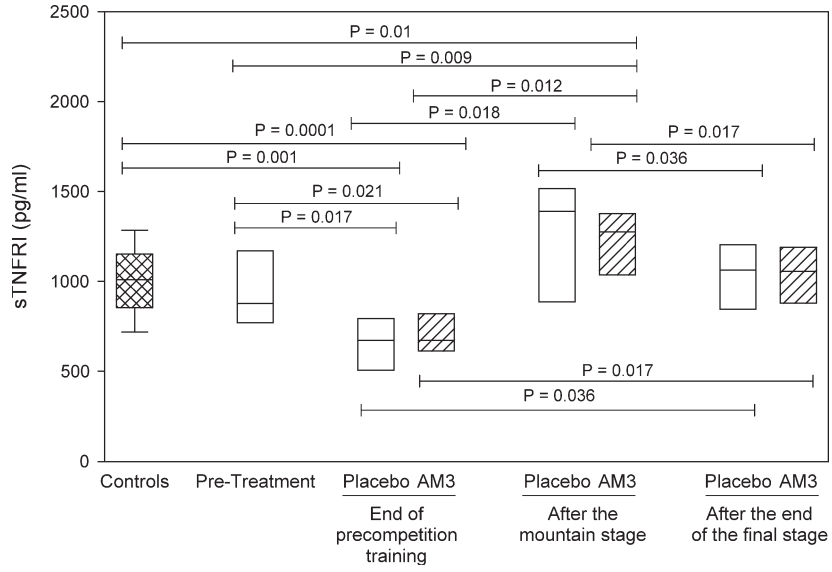

Figure 3. Serum TNFR-I concentration in moderately trained healthy controls and the two groups of cyclists. Serum was collected just before treatment began (baseline), at the end of pre-competition training, before the mountain stage of the competition (60 days), 4 h after the end of the mountain stage (62 days), and 18 h after the fifth and last day of competition (65 days). Data represent median and interquartile range.

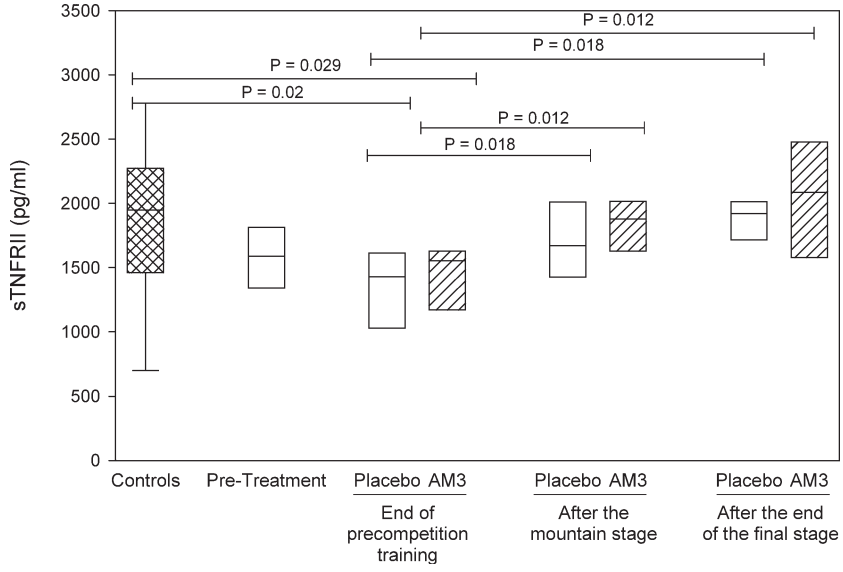

Figure 4. Serum TNFR-II concentration in moderately trained healthy controls and the two groups of cyclists. Serum was collected just before treatment began (baseline), at the end of pre-competition training, before the mountain stage of the competition (60 days), 4 h after the end of the mountain stage (62 days), and 18 h after the fifth and last day of competition (65 days). Data represent median and interquartile range.

Discussion

This results of this study show that the immunomodulator AM3 can prevent raised serum IL-6 concentrations induced by competition cycling (measured 4 h after the end of the race). Furthermore, pre-competition cycling training is associated with a reduction in the concentrations of the soluble receptors of TNF-α, whereas competition induces a significant increase in their concentrations but no concomitant increase in TNF-α itself.

The physical exercise of competition is stressful to the body (Northoff, Weinstock, Berg, 1994; Smith, 2000; Smith *et al.*, 2000). Among the cellular systems that experience increased demand during sporting competition are the neuroendocrine and

muscular systems (Pedersen *et al.*, 2001; Smith, 2000). Also, it has been demonstrated that IL-6 has many biological roles, including induction of lipolysis, suppression of TNF production and stimulation of cortisol production (Pedersen *et al.*, 2004a). Secondary to the physiological adaptations of their nervous and endocrine systems, and with the potential participation of products derived through the stress suffered by striated muscle, athletes experience acute and chronic modifications to their immune systems (Ostrowski *et al.*, 1999; Prieto *et al.*, 2001; Smith, 2000). When moderately trained healthy controls are subjected to intense cycling for 2 h, there is a significant rise in the concentration of plasma cytokines such as TNF-α (Haahr *et al.*, 1991; Pedersen & Hoffman-Goetz, 2000).

Other sports are associated with increased concentrations of IL-6, including marathon running (exercise with concentric and eccentric components). In pure concentric exercise, Akerstrom *et al.* (2005) showed a marked IL-8 mRNA and protein expression within muscle fibres, without changes in plasma concentrations of IL-8 (IL-8 acts as an angiogenic factor in human microvascular endothelial cells). These concentrations fall again, however, just a few hours after the end of the race (Northoff *et al.*, 1994). It should be noted that the site of origin of this extra IL-6 is not clear, although it can reasonably be discounted that it is due to increased production by lymphocytes and monocytes in the peripheral blood (Starkie *et al.*, 2001a). It has also been suggested that it may come from cells of the immune system situated in the secondary lymphoid tissues and/or other cell lineages (Matsuda & Hirano, 2001; Smith, 2000). Reaction to the physiological stress of exercise might commit endothelial or striated muscle cells to the production of IL-6 (Fischer *et al.*, 2004; Pedersen *et al.*, 2001, 2004b; Penkowa *et al.*, 2003). On the other hand, IL-6 production can be altered by carbohydrate ingestion during exercise (Starkie *et al.*, 2001b). However, in our study all cyclists followed the same diet and the same planning of provisioning of food during the race. The present results clearly show that cycling training led to no significant rise in serum IL-6 concentrations probably due to muscular adaptations to the training. However, 4 h post-exercise, after the mountain stage of the competition, there was a marked rise in this cytokine. Concentrations were still high 18 h after the end of the final stage. Interestingly, the administration of the immunomodulator AM3 significantly reduced this increase in serum IL-6 during competition to the extent that concentrations were normal again 18 h after the end of the last day of the race. The exercise increased the circulating concentrations of stress hormones, and therefore increased IL-6 concentrations (Smith, 2000; Smith *et al.*, 2000). As the stimulus of exercise, the workload and the conditions during the competition were the same for all cyclists, it could be that AM3, at least in part, can prevent tissue damage. This immunomodulatory effect of AM3 on IL-6 production has previously been established in mouse cellular stress models (peritoneal macrophages), with significant reductions in serum IL-6 production being observed (Brieva *et al.*, 2001). Brieva and colleagues found reduced concentrations of IL-6 in a group of mice treated with patented glycoconjugate (the same molecule as in AM3). In the treatment group, they observed a decreased infiltration of polymorphonuclear cells, which limited the tissue injury produced by secretion of aggressive mediators and the effector functions of these cells.

The concentrations of TNF-α, another pro-inflammatory cytokine, whose have been reported to be raised after physical exercise (Starkie *et al.*, 2001a; Ullum *et al.*, 1994). With respect to cycling, no variations in the serum concentrations of this cytokine have been reported (Ullum *et al.*, 1994). The present study found no increase in serum concentrations of this molecule during training for cycling competition independent of the treatment received. After the very demanding mountain stage, however, significant reductions were seen. Although it has recently been demonstrated that AM3 has a clear inhibitory effect on TNF-α production in mice (peritoneal macrophages) (Brieva *et al.*, 2001), no differences were found between the present treatment groups. In this study, we also monitored the serum concentrations of the TNF-α soluble receptors I and II. The results clearly show that during pre-competition training there is a fall in these receptors, while competition cycling notably increased their concentrations, in line with the findings of Ostrowski *et al.* (1999). In the present study, there were no significant differences between the AM3- and placebo-treated groups with respect to increases in either receptor.

The origin of the increased concentrations of serum TNFR-I and TNFR-II has yet to be established. It has been reported that demanding physical exercise is associated with activation of the lymphomonocyte system (Smith, 2000), and it is known that T cells and activated members of the monocyte – macrophage system can secrete soluble receptors as an anti-inflammatory mechanism (Aggarwal *et al.*, 2001b; Dinarello, 1998; Ullum *et al.*, 1994). Therefore, it might be that the origin of the raised serum concentrations seen in the cyclists in this study are secondary to an activation of the immune system. It should be pointed out that TNF-α soluble receptors I and II in the plasma are capable of binding TNF-α (Moreland & Misischia, 2002). Furthermore, the detection of TNF-α by

ELISA measures both free and receptor-bound TNF-α. Therefore, since there was no simultaneous increase in the concentration of TNF-α and those of its soluble receptors, demanding physical exercise is associated with the net reduction of free pro-inflammatory molecules.

Inmunoferon (AM3) can prevent increases in the circulating concentrations of creatine kinase and aldolase associated with competition athletics (Córdova *et al.*, 2004). It can also normalize the alterations to natural killer and monocyte populations in obstructive lung disease patients and also in those suffering infection complications (Prieto *et al.*, 2001). Inmunoferon also normalizes the response of immunocompromised patients (Perez-Garcia *et al.*, 2002; Prieto *et al.*, 2001). In addition, AM3 seems to have no clinically adverse side-effects (Perez-Garcia *et al.*, 2002; Prieto *et al.*, 2001). Taken together, these data suggest that the serum IL-6 response induced during competition sport can be reduced through immunomodulators. The use of these drugs could reduce the associated systemic, metabolic and muscular problems.

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