AGGRESSIVE BEHAVIOR
Volume 00, pages 1–8 (2012)

Positive Correlation Between Serum Interleukin-1β and State Anger in Rugby Athletes

Mirko Pesce[1]*, Lorenza Speranza[1], Sara Franceschelli[1], Valentina Ialenti[2], Irene Iezzi[3], Antonia Patruno[1], Alessia Rizzuto[2], Claudio Robazza[2], Maria Anna De Lutiis[1], Mario Felaco[1], and Alfredo Grilli[2]

[1] Department of Medicine and Ageing Sciences, University G. D'Annunzio, Chieti, Italy
[2] Department of Psychological, Humanistic and Territorial Sciences, University G. D'Annunzio, Chieti, Italy
[3] Department of Biomedical Sciences, University G. D'Annunzio, Chieti, Italy

Recently, several studies reported a relationship between immune system activation and anger expression. Consequently, the aim of this study was to explore immunitary molecular mechanisms that potentially underlie anger expression. To this end, we applied the Frustration—Aggression Theory in a contact sport model, utilizing the nearing of sporting events to trigger anger feelings. In parallel, we evaluated the activation of immune system at mRNA levels. We enrolled 20 amateur rugby players (age ± SD, 27.2 ± 4.5) who underwent psychological assessment to evaluate anger, with the State-Trait Anger Expression Inventory-2 (STAXI-2), before rugby matches; at the same time blood samples were taken to analyze the variations of gene expression by microarray. During the 2 hr before each game, a significant increase was verified in the Rage State (RS) score compared to the score ascertained 72 hr before. At the same time, we found modulation in expression profile, in particular increased expression of gene that encodes interleukin l-β (IL-1β). In a regression analysis, RS score was related to IL-1β, and the potential risk factors age, body mass index, smoking, and drinking. The levels of cytokine were positively and independently related to RS score. Our results suggest that the nearing of sporting event can trigger anger state feelings and activate immune system in rugby players. We propose the IL-1β as a potential biological marker of anger. However, further research is necessary to clarify the correlation between cytokine and anger. Aggr. Behav. 00:1–8, 2012. © 2012 Wiley Periodicals, Inc.

Keywords: anger; IL-1β; immune system; CNS

INTRODUCTION

Anger as an emotional immediate response to a real or imagined event can be considered as a forerunner of aggressive behavior (Siegel, Suresh, Rekha, & Zalcman, 2007). Anger is frequently experienced and expressed as aggressive behavior in the athletic domain (Maxwell, 2004), especially in combative and collision sports such as ice-hockey, American football, boxing, karate (Isberg, 2000), and rugby (Robazza & Bortoli, 2007). Several studies have suggested that anger and rage occurring prior to competition (Gould, Greenleaf, & Krane, 2002) and their expression in this contest is influenced by psychological and biological variables (Parmigiani et al., 2009). Biological variables include neurological and endocrine mechanisms, however recent studies suggested a relationship between immune system activation and anger expression (Kiecolt et al., 2005; Kraus, Schafer, Faller, Csef, & Scheurlen, 2003; Suarez, Lewis, Krishnan, & Young, 2004).

The hypothesis of the Frustration–Aggression (Dollard, Doob, Miller, Mowrer, & Sears, 1967) postulates that the frustration, considered as an obstacle to the attainment of an objective, is the condition triggering for excellence the aggressive behavior. In the athletic context, some authors have considered the type of discipline practiced in an attempt to identify those structural features that make a sport more frustrating than another. The contact, for example, was considered the condition for excellence frustrating, because the opponent, in addition to violating the space and sometimes the athlete's body, can be an obstacle to the achievement of a goal.

On the assumption of Frustration–Aggression Theory, we have chosen the rugby as a sport that involves contact, proves more frustrating, and exposes

*Correspondence to: Mirko Pesce, Department of Medicine and Ageing Sciences, University G. d'Annunzio CH-PE, Via dei Vestini, 66100, Italy. E-mail: mirkopesce@unich.it

Received 15 March 2012; Accepted 22 October 2012

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ab.21457

© 2012 Wiley Periodicals, Inc.

the athlete to aggressive behavior. Therefore, to explore immunitary molecular mechanisms that potentially underlie anger expression, we have adopted an integrated psychobiological study approach that permits an evaluation of anger as objectively as possible.

We used the State-Trait Anger Expression Inventory-2 (STAXI-2 (Spielberger, 1994)) to evaluate anger expression 72 hr and then 2 hr before an official rugby match in 20 amateur players. Concomitantly, we measured gene expression profile of peripheral blood mononuclear cells (PBMCs) by microarray technique in order to investigate possible correlation between gene expression and anger scores.

PBMCs play a role in immune and inflammatory responses induced by a stimulus. Moreover, the leukocytes express specific receptors for stress mediators, such as hormones, neurotransmitters, growth factors, and cytokines (John & Buckingam, 2006).

Given the impossibility to take RNA samples from the concerned areas of the brain in healthy subjects, Sullivan, Fan, and Perou (2006) evaluated the comparability of the gene expression in the central nervous system (CNS) and in the peripheral blood, and found that in whole blood, the transcriptome showed significant similarities in the gene expression with several CNS tissues (Sullivan et al., 2006). It was not established whether the gene expression in the peripheral blood cells can act as a surrogate for the CNS gene expression, however the immune system communicates bidirectionally with the CNS in response to stimuli. Even if the gene expression profile in the PBMCs does not directly reflect the CNS response, in this study, we used it to indirectly evaluate the complex pattern of responses that characterizes anger expression.

MATERIALS AND METHODS

Participants

We recruited 20 male rugby athletes (20 men; age ± SD, 27.2 ± 4.5; body mass index [BMI], 23.9 ± 0.88). They were in good physical health, taking no medication, had no history of psychiatric or somatic diseases, and were undergoing the same training program.

The C-reactive protein (CRP) serum levels was measured as a nonspecific marker for inflammation and was utilized as exclusion criteria (Ablij & Meinders, 2002; Biasucci, 2004; Gabay & Kashner, 1999). The athletes recruited did not show CRP levels > 5 mg/L or statistical significant difference in CRP levels between 72 and 2 hr previous the official rugby matches ($P < .05$).

Information about the project was provided, and participants signed an informed consent. The study was approved by the Ethical Committee of the University "G. D'Annunzio" of Chieti-Pescara, Italy, and it has complied with APA ethical standards in the treatment of human sample.

TABLE I. Demographic Variables

	Mean	SD
Age	27.2	4.50
BMI	23.9	0.88
	n	%
Education		
Low	2	10
Medium	8	40
High	10	50
Smoking (Y/N)	7	35
Drinking (Y/N)	5	25

Questionnaires

Demographic information on age, BMI, education level, smoking, and alcohol use were filled out (Table I).

Anger Assessment

The anger level of each subject was evaluated by the STAXI-2 questionnaire administered 72- and 2-hr pre-official rugby match (Cornell, Peterson, & Richards, 1999; Dear, Watt, & Dockerill, 2003; Spielberger, 1994).

These measures were repeated at three independent sporting events (nonconsecutive). The athletes did not perform exercise 72 hr before sampling. Assessment was made in the competition phase of the seasonal training to render the biologic evaluations more specific for the psychological stimulus trigger of anger. This phase is, in fact, characterized by the potential optimum levels of performance and high emotional involvement of the athletes.

The STAXI-2 is a questionnaire made up of 57 items that measure the experience of anger intended as an emotional state characterized by subjective feelings of different intensities (state of anger), the tendency to perceive a great number of situations as annoying or frustrating (anger trait), and finally as an expression of the same (anger/out, anger/in, control/out, control/in). An index of anger expression can be derived to provide a summarizing measure of the expression and control of anger.

The raw scores of every scale were transformed in standardized scores, using tables of conversion from the scores obtained from the Italian normative sample (male, age 20–39 years) (Comunian, 2004).

Collection of Samples

At the same time of STAXI-2 questionnaire administration, venous blood was collected by phlebotomy in EDTA vacutainers (6 mL K_2EDTA, Becton Dickinson, Franklin Lakes, NJ, USA) and processed within 2 hr of procurement. Serum was isolated from the blood of all the athletes. PBMCs were isolated by density-gradient centrifugation through Ficoll/Hypaque (Pharmacia, Piscataway, NJ, USA) from the blood of athletes 72- and 2-hr pre-official rugby matches. After centrifugation (1500 × rpm; 4 °C; × 25 min), the interphase layer containing PBMCs was carefully removed, washed in phosphate buffer saline (PBS) (1×) followed by centrifugation (2500 × rpm; ×15 min). The cell pellet was placed into RNA later (Ambion, Austin, TX, USA) and immediately stored at 80 °C until further processing.

Microarray Analysis

RNA was isolated from PBMCs using SV Total RNA Isolation System (Promega, Madison, WI, USA). RNA concentration and purity were determined by measuring absorbencies at 260 and 280 nm, and a 260:280 ratio of 1.7 was considered acceptable for analysis. One microgram RNA of each subject was amplified using the "Ammino Allyl MessageAmp™ II aRNA Amplification kit" (Ambion), able to produce aRNA, containing 5-(3-amminoallyl)-UTP modified nucleotides. The obtained aRNA (5–20 μg) was labeled with Cys3 or Cys5 (Amersham, Pharmacia Biotech, Buckinghamshire, UK) and hybridized on the array. Cys5-aRNA prepared from each blood sample collected 2-hr pre-official rugby match was mixed with the equivalent amount of Cys3-aRNA from the blood samples collected 72-hr pre-official rugby match. Analysis was carried out using high-density array containing about 30,968 human genome probes (60-mer sense-strand polynucleotide probes; Eurogentec, Seraing, Belgium). Fluorescent signals were captured by ScanArray 5000 Packard laser scanning (Packard BioChip Technologies, Billerica, MA, USA) and normalized using the "ScanArray Express" software (Packard BioChip Technologies).

Semiquantitative Reverse-Transcriptase Polymerase Chain Reaction

Semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) was used to determine mRNA levels of the genes narrow down with microarray analysis on PBMCs. First-strand cDNA was generated by adding RNA (1 μg) to a mixture containing 1 mM deoxynucleoside triphosphates (d-NTP), 1 U/μL RNase inhibitor, 2.5 U/μL moloney murine leukemia virus RT, 2.5 μM oligo (dT), 5 mM $MgCl_2$, 10× PCR buffer in a final volume of 20 μL. RT was performed at 42°C for 1 hr followed by heat inactivation of RT at 92°C for 10 min. 18S was used as housekeeping gene and amplified from the same amount of RNA to correct for variation of different samples. PCR amplification was performed using a Programmable Thermal Controller (MJ Research, Watertown MA, USA). The PCR solution contained 10 μL of first-strand cDNA, 4 μL 10× PCR buffer, and 2 mM $MgCl_2$. 2 U *Thermophylus acquaticus* (*Taq*) DNA polymerase (Celbio, Milan, Italy), and water to a final volume of 50 μL. PCR cycling parameters (25–35 cycles) were chosen to ensure linear product formation over the amounts of RNA and other reagents described. The sequences of the gene-specific primers (MWG-Biotech AG, Milan, Italy) used to amplify the 18S, RPL32, RPS13, S100A8, UTX, RPL21, IL1B, S100A9, CYP1A2, NDUFA6, STX11, and COX7C transcripts along with their annealing temperatures are shown in Table II. Products were separated on 2% agarose gel electrophoresis and photographed after ethidium bromide staining under UV light. Bands on the gel were scanned using a computerized densitometric system (Bio-Rad Gel Doc 1000, Bio-Rad, Milan, Italy).

Measurements of CRP and Interleukin l-β

The amount of circulating CRP and interleukin l-β (IL-1β) was assayed using specific ELISA development systems (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Statistical Analysis

Data were analyzed using paired *t*-test statistic. Results are described as means ± SD for each assessment performed in triplicate. The level of statistically significant difference was defined as $P < .05$. Correlations between IL-1β and STAXI-2 scores were analyzed using the Spearman's rho correlation coefficient (ρ) and the corresponding probability value (*P*). Regression analysis was performed using the SPSS 18.0.1 (SPSS Inc., Chicago, IL, USA) for Windows.

The microarray obtained data were statistically analyzed using the SAM system (where SAM is significance analysis of microarray). In each experiment, a 1.5-fold change in the signal of each spot was considered as evidence of a different expression of the specific transcript. The false discovery rate <5% was considered. The Ingenuity Pathway Analysis Application (IPA; Ingenuity Systems, Mountain View, CA,

TABLE II. Sequences of the Gene-Specific Primers with Their Annealing Temperatures

Gene symbol	Primer sequence	Tmelting (Tm) (°C)
RPL32	*Forward 5′-CAACATTGGTTATGGAAGCAACA-3′*	58
	Reverse 5′-TGACGTTGTGGACCAGGAACT-3′	
RPS13	*Forward 5′-AACTGCAGCATATGGGTCGCATGCATGCTC-3′*	55
	Reverse 5′-AACTGCAGGGATCCTTATGCGACCAGGGCAGA-3′	
S100A8	*Forward 5′-ATTTCCATGCCGTCTACAGG-3′*	60
	Reverse 5′-TGGCTTTCTTCATGGCTTTT-3′	
UTX	*Forward 5′-AGACATTGAGGGAAGCTCTC-3′*	58
	Reverse 5′-TCAAGATGAGGCGGATGGT-3′	
RPL 21	*Forward 5′-TGGCCACATACATGCGAATC-3′*	55
	Reverse 5′-ATGCTGGGTGACATTGTAGACTCTT-3′	
IL-1β	*Forward 5′-ATGGCAGAAGTACCTAAGCTCGC-3′*	55
	Reverse 5′-ACACAAATTGCATGGTGAAGTCAGTT-3′	
S100A9	*Forward 5′-CAGCTGGAACGCAACATAGA-3′*	60
	Reverse 5′-CCACAGCCAAGACAGTTTGA-3′	
CYP1A2	*Forward 5′-CCACACCAGCCATTACAACCCTGCC-3′*	60
	Reverse 5′-TGCGCTGGCTCATCCTTGACAGTGC-3′	
NDUFA6	*Forward 5′-CAAGATGGCGGGGAGCGG-3′*	55
	Reverse 5′-GTATAGTGAGTTTATTTGTGCTC-3′	
STX11	*Forward 5′-CTCGCTCCCAGTCCAGGCAAAATG-3′*	58
	Reverse 5′-GCACAGGCTGGTTTGCAATTCTTG-3′	
COX7C	*Forward 5′-CCCTGGGAAGAATTTGCCA-3′*	56
	Reverse 5′-GGAACTGAAACATCCTTATG-3′	
18S	*Forward 5′-AGTCGCCGTGCCTACCAT-3′*	55
	Reverse 5′-GCCTGCTGCCTTCCTTG-3′	

USA) was used to identify biological organization of genes in cellular functions. Fisher's exact test was used to calculate a P-value determining the probability of the associations [www.ingenuity.com].

RESULTS

In Table I, a description of the group is shown. The BMI showed a normal distribution, with scores ranging from 22.2 to 26.7. Alcohol use was dichotomized into either less than 5 units/week (0) or greater than 5 units/week (1). Seventy-five percent ($n = 15$) reported no alcohol use at all.

STAXI-2 Scores

Anger was directly assessed using the STAXI-2. In 20 athletes, state anger (Rage State [RS] score), trait anger (RT score), and expression anger index scores (ER index) were performed for three nonconsecutive official rugby matches (event 1, event 2, and event 3; Table III).

These results show that the RS score significantly increased 2 hr compared to 72-hr pre-official rugby match. This increment is statistically significant in all three rugby matches considered ($P < .001$). The RT score and ER index measurement in all events did not show significant differences (Table III).

Identification of the Responsive Genes and RT-PCR

In this study, we evaluated the individual variation of the gene expression in PBMCs in response to an official rugby match, considering it as anger trigger stimulus.

The microarray analysis was carried out for each rugby player through the comparison of the mRNA purified from total RNA extract 72- and 2-hr pre-official rugby match, for a total of three

TABLE III. STAXI-2 Scores (Means ± SD)

	Event 1		Event 2		Event 3	
	72 hr Before	2 hr Before	72 hr Before	2 hr Before	72 hr Before	2 hr Before
RS score (mean ± SD)	54.7 ± 3.1	61 ± 2*	54.2 ± 3.0	60.5 ± 2.6*	54.3 ± 3.8	61.3 ± 2.2*
RT score (mean ± SD)	51.7 ± 2.9	52.7 ± 2.5	52.0 ± 2.8	53.0 ± 2.4	52.9 ± 2.2	52.7 ± 2.2
ER index (mean ± SD)	58.8 ± 3.1	60.7 ± 2.1	58.6 ± 3.1	60.5 ± 2.7	59.3 ± 2.3	60.7 ± 2.1

*$P < .001$ 2 hr versus 72 hr before three rugby matches.

TABLE IV. Fold Change of Genes Upregulated 2 hr versus 72 hr Before Rugby Match in PBMCs of Athletes

Gene symbol	Gene name	Fold change (mean ± SD)
RPL32	Ribosomal protein large subunit 32	2.28 ± 0.18**
RPS13	Ribosomal protein small subunit 13	2.13 ± 0.30**
S100A8	S100 calcium binding protein A8	1.84 ± 0.22**
UXT	Ubiquitously expressed transcript	1.73 ± 0.12**
RPL21	Ribosomal protein large subunit 21	1.98 ± 0.38**
*IL-1*β	Interleukin 1β	1.93 ± 0.38**
CYP1A2	Unspecific monooxygenase	1.62 ± 0.21**
NDUFA6	$NADH_2$ deydrogenase	1.85 ± 0.57**
S100A9	S100 calcium binding protein A9	2.13 ± 0.98**
STX11	Syntaxin 11	2.36 ± 0.10*
COX7C	Cytocrome c oxidase	2.36 ± 0.74*

$^{*}P < 0.05$, $^{**}P < .01$.

nonconsecutive events. Using SAM software, we identified 22 upregulated genes at 2 hr compared to 72 hr before the sport events. The analysis with the IPA software allowed us to extract 11 genes of known function, the transcription of which was at least 1.5 times increased in PBMCs extracted 2-hr pre-official rugby match for all three events (Table IV).

The Ingenuity software analysis of dataset molecules, showed that these genes (NDUFA6, COX7C) are involved in the production of energy on a mitochondrial level, in the protein synthesis (RPL13, RPL32, and RPL21), in the transport and secretion of proteins (STX11), and in the cytoskeletal reorganization on a microtubule level (product of the gene UXT). In particular, the increase in transcription of the calcium binding proteins (S100A8 and S100A9) and of the cytokine IL-1β, 2 hr before rugby matches, implicate an activation of the "Cell-to-cell signaling" and of the "Cell trafficking" functions in PBMCs at this time (Vandal et al., 2003). The semiquantitative evaluation through RT-PCR confirmed the increase in the presence of the transcripts at 2 hr compared to the 72 before the official rugby matches for all genes (Fig. 1).

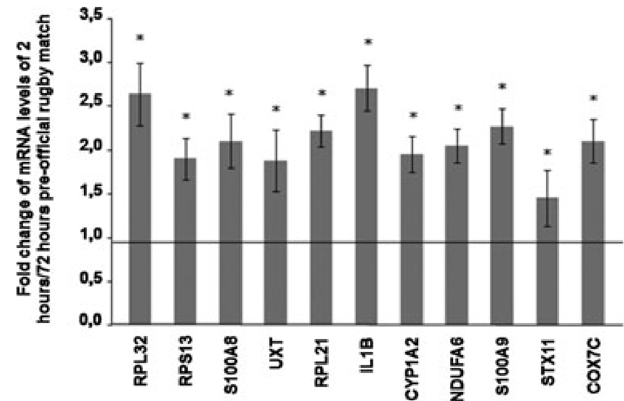

Fig. 1. Validation of genes responsive to an anger trigger stimulus. Total RNA extracted from PBMCs of 20 rugby players 2 hr and 72 hr before three official rugby matches and analyzed with semiquantitative RT-PCR. All mRNA were normalized to 18S mRNA expression. Values are expressed as means ± SD ($^{*}P < .05$).

Measurement of IL-1β Serum Levels

Among the products of the genes obtained from our analysis, several studies suggested a role in elicitation of anger strictly for IL-1β (Hassanain, Bhatt, Zalcman, & Siegel, 2005; Zalcman & Siegel, 2006). This molecule is a member of cytokines family and the explanations of its biological functions could contribute to clarify the relationships between anger expression and immune activation. In order to assess if upregulation of the transcript is realized in quantitative increase of protein secretion, we performed ELISA measurement of IL-1β levels in collected serum and

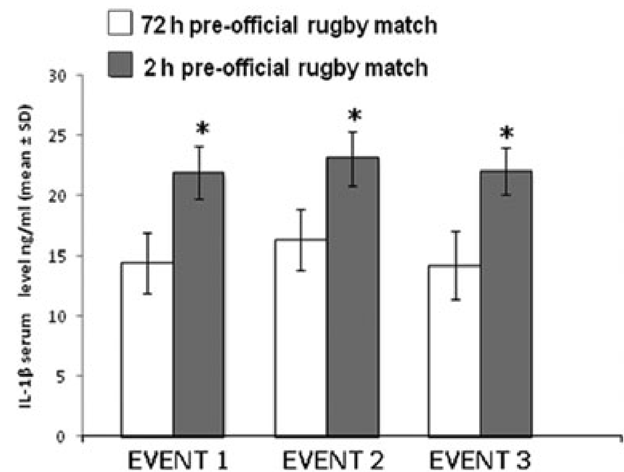

Fig. 2. ELISA measurement of serum IL-1β levels at 2 and 72 hr previous rugby matches. The serum levels of IL-1β increases in athletes nearing the rugby match ($^{*}P < .001$). Results are expressed in nanogram per milliliter (mean ± SD, $n = 20$).

compared data (Fig. 2). The ratio of serum IL-1β levels between samples collected 2 and 72 hr before a rugby match is positively for all three events (fold increase: 1.51 for event 1, 1.42 for event 2, and 1.55 for event 3, $P < .001$). This finding suggests that the product of *IL1B* gene, IL-1β, increases in circulating levels at 2 hr before an official sport event.

Relationship Between IL-1β and Anger

We explored the correlation between the anger score and IL-1β and demographic factors (age, BMI, smoking, and drinking). The IL-1β serum level at 2-hr pre-official rugby match showed significant and positive correlation with RS score at the same time in all three events evaluated ($\rho = .59$, $P < .01$, $\rho = .58$, $P < .01$,

TABLE V. Regression Analysis Results

	RS score Step 1						RS score Step 2					
	Event 1		Event 2		Event 3		Event 1		Event 2		Event 3	
Adjusted R^2	.250		.267		.304		.145		.218		.279	
F-value	7.33*		7.926*		9.316*		1.67		2.06		2.47	
Step 1: factors	β-value	t-value	β-value	t-value	β-value	t-value	β-value	t-value	β-value	t-value	β-value	t-value
IL-1β	0.54	2.7*	0.553	2.815*	0.584	3.05**	0.604	2.71*	0.611	2.146*	0.718	3.00**
Step 2: demographic												
Age	–		–		–		0.29	1.23	0.154	0.677	–0.18	–0.838
BMI	–		–		–		0.107	0.445	0.014	0.49	0.174	0.715
Smoking Y/N	–		–		–		0.001	0.005	0.032	0.130	0.194	0.770
Drinking Y/N	–		–		–		0.175	0.764	0.266	1.153	0.211	1.001

*$P < .05$; **$P < .01$.

$\rho = .55$, $P < .05$). No significant correlations were obtained for IL-1β serum level with RS score at 72-hr pre-official rugby match, RT score, ER index, BMI, and age at both sampling times in any official rugby matches considered.

Linear regression analysis was used to determine whether the variance in RS score at 2-hr pre-official rugby match could be explained by IL-1β production, while controlling for BMI, age, drinking, and smoking. To determine the relative contribution of each factor to RS score at 2-hr pre-official rugby matches, the factors were introduced stepwise. The results are showed in Table V. In the first step, the IL-1β serum level factor was introduced. A higher IL-1β cytokine level was related to increased anger (RS score at 2-hr pre-official rugby match), which explained 25% (event 1), 26.7% (event 2), and 30.4% (event 3) of the variance in RS score. After introducing the demographic variables (age, BMI, smoking, and drinking), the model revealed that they were not correlated to a high RS score. The relative contribution of each variable in the model was determined by comparing the standardized coefficient β (β-value). When comparing the β-value of the demographic variable, the value did not change significantly, showing that both factors had an independent relation with anger (Table V). Interestingly, the pro-inflammatory cytokine IL-1β showed a significant positive relation with anger (RS score at 2-hr pre-official rugby match) in all three events as well. An increased serum level of IL-1β was related with a higher RS score.

DISCUSSION

The objective of this study was to evaluate anger and analyze the gene expression profile in PBMCs of rugby players close to a sport event. Findings showed the presence of anger feelings in the 2 hr preceding an official rugby match; in particular, the anger state component (RS) was significantly increased, while the anger trait component (RT) did not show significant changes. In agreement with the Frustration–Aggression hypothesis, which postulates that competitive situation, understood as a condition of conflict of interests between two parties who want to achieve a benefit not obtained simultaneously from both, is a frustration in itself, these results suggest that a salient stimulus for an athlete, coming closer as for an official rugby match, triggers anger state feelings. As expected within the frameworks of the Frustration--Aggression hypothesis, study findings showed the tendency of contact sport competitors (such as rugby players) to feel anger symptoms.

From the analysis of the gene expression profile emerged an increased expression of 11 genes of known functions at 2 hr compared to 72-hr pre-official rugby match. These genes are involved in the production of energy, in the protein synthesis and transport, in the cytoskeletal reorganization and in particular, in intercells communication (Vandal et al., 2003). These findings suggested a nonpathologic activation in the PBMCs in coincidence with the sport events, in assonance with the arousal state in which the athlete necessarily finds himself a short time before playing. Recently, studies on the relation between anger and immune system, described a role for IL-1β in tuning anger expression (Hassanain et al., 2005; Kiecolt et al., 2005; Suarez, Lewis, & Kuhn, 2004). The ELISA measurement of this cytokine reported a presence in circulation of a notable quantity at 2 hr compared to 72 hr before the official rugby matches, parallel to the increase in the points for RS induced by the same events. Noteworthy, regression analysis showed that state anger, expressed as RS score, was significantly and positively related to pro-inflammatory cytokine IL-1β in nearing of sport event. This relation

remained intact after controlling for the significant confounders age, BMI, drinking, and smoking. A relatively large proportion (25–30%) of the total variance in state anger was explained. These data led to argue a role for this cytokine in sporting events triggering for anger, and they fitted with studies that reported an association of IL-1β with anger expression. Studies on felines and rodents showed that this cytokine can act directly on a cerebral level, building up anger behavior or acting on a peripheral level modulating the turnover of various neurotransmitters implicated in the biogenesis of anger episodes (Anisman, Gibb, & Hayley, 2008; Kiecolt et al., 2005; Zalcman & Siegel, 2006). In addition, it has been shown that peripheral IL-1β is able to activate the hypothalamic pituitary adrenal (HPA) axis through the stimulation of the vagal afferents involved in the response to physical and emotional stressors (Goehler et al., 1999; Grinevich et al., 2001; Schmidt, Aguilera, Binnekade, & Tillders, 2003).

In this study, the psychometric test used is mainly psychological state test (i.e. measuring the individual psychological response to a specific situation) and the observed correlation between psychological and biological factors suggests the interesting hypothesis that psychometric tests might predict immune system responses in specific context, such as agonistic interactions. But the reverse might also be true and so the immune system responses may be used to understand the psychological state of an individual and the response in a specific situation.

In conclusion, it is possible to consider an imminent sport event as a stimulant for inducing anger, and such condition is associated in PBMCs with an increase of the genes expression. In particular, the serum levels increase IL-1β and their significant and positive correlation with RS score nearing sport matches may contribute to explain the already evident connection between the expression of anger and immune system activation. However, it is not possible, at present, to hypothesize a correlation between IL-1β and the quality of the performance, in that this cytokine is associated with other components of emotivity, in particular that of anxiety, the symptoms of which may induce a decrease in sport performance.

Future research should aim at furthering the assessment of the potential role of the IL-1β and other immune activation mediators as predictors of athletic performance in relation to the expression of anger and other aspects of emotivity (e.g. anxiety and fear).

CONFLICT OF INTEREST

No conflict of interest.

ACKNOWLEDGMENTS

The Italian Ministry for University and research is acknowledged for financial support.

REFERENCES

Ablij, H. C., & Meinders, A. E. (2002). C-reactive protein: History and revival. *European Journal of Internal Medicine 13*, 412–422.

Anisman, H., Gibb, J., & Hayley, S. (2008). Influence of continuous infusion of interleukin-1β on depression-related processes in mice: Corticosterone, circulating cytokines, brain monoamines, and cytokine mRNA expression. *Psychopharmacology*, *1999*, 231–244.

Biasucci, L. M. (2004). CDC/AHA workshop on markers of inflammation and cardiovascular disease: Application to clinical and public health practice. Clinical use of inflammatory markers in patients with cardiovascular diseases: A background paper. *Circulation*, *110*, 560–567.

Comunian, A. L. (2004). *Italian adaptation of STAXI-2 State-Trait Anger Expression Inventory-2*. Firenze, Italy: OS Giunti.

Cornell, D. G., Peterson, C. S., Richards, H. (1999). Anger as a predictor of aggression among incarcerated adolescents. *Journal of Consulting and Clinical Psychology*, *67*, 108–115.

Dear, G. E., Watt, B. D., & Dockerill, J. (2003). Factor structure of the Spielberger Anger Expression scales when used with Australian prisoners. *Psychological Reports*, *92*, 617–620.

Dollard, J., Doob, L., Miller, N., Mowrer, O., & Sears, R. (1967). *Frustration and aggression*. Firenze, Italy: Giunti-Barbera Universitaria.

Gabay, C., & Kashner, I. (1999). Acute phase proteins and other systemic responses to inflammation. *New England Journal of Medicine*, *340*, 448–454.

Goehler, L. E., Gaykema, R. P. A., Nguyen, K. T., Lee, J. E., Tilder, F. J. H., Maier, S. F. . . . Watkins, L. R. (1999). Interleukin-1 beta in immune cells of the abdominal vagus nerve: An immune to nervous system link? *Journal of Neuroscience*, *19*, 2799–2806.

Gould, D., Greenleaf, C., & Krane, V. (2002). Arousal-anxiety and sport behavior. In T. S. Horn (Ed.), *Advances in sport Psychology* (2nd ed., pp. 207–241). Champaign, IL: Human Kinetics.

Grinevich, V., Ma, X. M., Herman, J. P., Jezova, D., Akmayev, I., & Aguilera, G. (2001). Effect of repeated lipopolysaccharide administration on tissue cytokine expression and hypothalamic-pituitary-adrenal axis activity in rats. *Journal of Neuroendocrinology*, *13*, 711–723.

Hassanain, M., Bhatt, S., Zalcman, S., & Siegel, A. (2005). Potentiating role of interleukin-1 beta (IL-1 beta) and IL- 1 beta type 1 receptors in the medial hypothalamus in defensive rage behavior in the cat. *Brain Research*, *1048*, 1–11.

Isberg, L. (2000). Anger, aggressive behaviour and athletic performance. In Y. L. Hanin (Ed.), *Emotions in sport* (Vol. 1, pp. 13–33). Champaign, IL: Human Kinetics.

John, C. D., & Buckingam, J. C. (2006). Cytokines: Regulation of the hypothalamic-pituitary-adrenal response during crowding stress. *Journal of Physiology and Pharmacology*, *57*, 463–477.

Kiecolt, J. K., Loving, T. J., Stowel, J. R., Malarkey, W. B., Lemeshow, S., Dickinson, S. L. . . . Glaser, R. (2005). Hostile marital interactions, proinflammatory cytokine production, and wound healing. *Archives of General Psychiatry*, *62*, 377–384.

Kraus, M. R., Schafer, A., Faller, H., Csef, H., & Scheurlen, M. (2003). Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa- 2b therapy. *Journal of Clinical Psychiatry*, *64*, 708–714.

Maxwell, J. P. (2004). Anger rumination: An antecedent of athlete aggression? *Psychology of Sport and Exercise*, *5*, 279–289.

Parmigiani, S., Dadomo, H., Bartolomucci, A., Brain, P. F., Carbucicchio, A., Costantino, C. . . . Volpi, R. (2009). Personality traits and endocrine response as possible asymmetry factors of agonistic outcome in karate athletes. *Aggressive Behavior*, *35*, 324–333.

Robazza, C., & Bortoli, L. (2007). Perceived impact of anger and anxiety on sporting performance in rugby players. *Psychology of Sport and Exercise*, *8*, 875–896.

Schmidt, E. D., Aguilera, G., Binnekade, R., & Tillders, F. J. (2003). Single administration of interleukin-1 increased corticotrophin releasing hormone and corticotrophin releasing receptor mRNA in the hypothalamic paraventricular nucleus which paralleled long-lasting (weeks) sensitization to emotional stressors. *Neuroscience*, *116*, 275–283.

Siegel, A., Suresh, B., Rekha, B., & Zalcman, S. S. (2007). The neurobiological bases for development of pharmacological treatments of aggressive disorders. *Current Neuropharmacology*, *5*, 135–147.

Spielberger, C. D. (1994). *STAXI (State Trait Anger Expression Inventory)*. Firenze, Italy: OS Giunti.

Suarez, E. C., Lewis, J. G., Krishnan, R. R., & Young, K. H. (2004). Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women. *Psyconeuroendocrinology*, *29*, 1119–1128.

Suarez, E. C., Lewis, J. G., & Kuhn, C. M. (2004). The relation of aggression, hostility and anger to lipopolysaccharide-stimulated tumor necrosis factor (TNF)-alpha by blood monocytes of normal men. *Brain Behavior and Immunity*, *16*, 675–684.

Sullivan, P. F., Fan, C., & Perou, C. M. (2006). Evaluating the comparability of gene expression in blood and brain. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*, *141B*(3), 261–268.

Vandal, K., Rouleau, P., Boivin, A., Ryckman, C., Talbot, M., & Tessier, P. A. (2003). Blockade of S100A8 and S100A9 suppresses neutrophil migration in response to lipopolysaccharide. *Journal of Immunology*, *1715*, 2602–2609.

Zalcman, S. S., & Siegel, A. (2006). The neurobiology of aggression and rage: Role of cytokines. *Brain Behavior and Immunity*, *20*, 507–514.