Overtraining and immune system: a prospective longitudinal study in endurance athletes

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ABSTRACT

GABRIEL, H. H. W., A. URHAUSEN, G. VALET, U. HEIDELBACH, and W. KINDERMANN. Overtraining and immune system: a prospective longitudinal study in endurance athletes. *Med. Sci. Sports Exerc.*, Vol. 30, No. 7, pp. 1151–1157, 1998. A prospective longitudinal study investigated for 19 ± 3 months whether immunophenotypes of peripheral leukocytes were altered in periods of severe training. Leukocyte membrane antigens (CD3, CD4, CD8, CD14, CD16, CD19, CD45, CD45RO, and CD56) of endurance athletes were immunophenotyped (dual-color flow cytometry) and list mode data analyzed by a self-learning classification system in a state of an overtraining syndrome (OT; $N = 15$) and several occasions without symptoms of staleness (NS; $N = 70$). Neither at physical rest nor after a short-term highly intensive cycle ergometer exercise session at 110% of the individual anaerobic threshold did cell counts of neutrophils, T, B, and natural killer cells differ between OT and NS. Eosinophils were lower during OT, activated T cells ($CD3^+HLA\text{-}DR^+$) showed slight increases (NS: 5.5 ± 2.7; OT 7.3 ± 2.4% $CD3^+$ of cells; means ± SD; $P < 0.01$) during OT without reaching pathological ranges. The cell-surface expression of CD45RO ($P < 0.001$) on T cells, but not cell concentrations of $CD45RO^+$ T cells, were higher during OT. OT could be classified with high specificities (92%) and sensitivities (93%). It is concluded that OT does not lead to clinically relevant alterations of immunophenotypes in peripheral blood and especially that an immunosuppressive effect cannot be detected. Immunophenotyping may provide help with the diagnosis of OT in future, but the diagnostic approach presented here requires improvements before use in sports medical practice is enabled. **Key Words:** STALENESS, IMMUNOPHENOTYPES, CD45RO, LYMPHOCYTES, FLOW CYTOMETRY, DIAGNOSIS, EXERCISE, ENDURANCE TRAINING

The leukocytosis of exercise has been known since the end of the last century (32). By investigating immune cells in peripheral blood, the only cell line that with some certainty to be impaired after strenuous exercise are the neutrophils (34). Reports about reduced cytotoxicity of natural killer (NK) cells, impaired *in vitro* proliferative responses of T and B lymphocytes, and altered functions of the monocyte/macrophage system are contradictory (26,29,33). Furthermore, so far the clinical relevance in healthy individuals of the measured effects in peripheral blood cells have not yet been uncovered. The difference between *in vitro* effects and clinically detectable phenotype contrasts with epidemiological findings. These findings show increased incidences of self-reported symptoms of upper respiratory tract infections (URTI) after strenuous endurance exercise (11,25,30). Also, personal experiences of athletes, coaches, and team physicians after single bouts of exercise under extremely hard conditions and or during periods with high training loads and/or increased frequency of competitions, especially if other stressors (psychological distress, malnutrition, weight loss, drugs, and disturbance of biological rhythms) coincide, suggest that incidence of infections—especially URTI—is higher than in other training periods (4). These experiences contrast with results of studies about overtraining/overtraining syndrome that cannot prove the hypothesis of increased URTI (5,15,19,21,22,28,31,43).

The present prospective longitudinal study investigated the impact of the overtraining syndrome on immune (presented here) and other (40,41) parameters. Cell line–specific and function-related surface receptors were measured to find parameters for diagnostic purposes. Common laboratory methods (immunophenotyping and flow cytometry) combined with a new self-learning diagnosis system served to help with the diagnosis of an overtraining syndrome.

MATERIALS AND METHODS

Study Design

Approximately 3–5 months apart, each individual (12 cyclists, 3 triathletes; age: 23.4 ± 6.7 yr, height 178 ± 7 cm, body mass 68.9 ± 7.0 kg, body fat 12.5 ± 2.1%, heart volume 14.0 ± 1.7 $mL \cdot kg^{-1}$, $\dot{V}O_{2max}$ 61.2 ± 7.5 $mL \cdot min \cdot kg^{-1}$) was investigated five times. Each of these investigations consisted of standardized tests over 2 separate days. The total time of the study was 19 ± 3 months. In

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Submitted for publication January 1997.
Accepted for publication July 1998.

TABLE 1. Absolute cell counts of leukocyte and lymphocyte subpopulations.

	NS	OT
Leukocytes	5131 ± 811	4954 ± 735
Neutrophils	3101 ± 788	2993 ± 770
Eosinophils	278 ± 176	211 ± 176*
Monocytes	345 ± 101	360 ± 150
Lymphocytes	1499 ± 342	1479 ± 414
$CD4^+CD45RO^-$	293 ± 132	278 ± 116
$CD4^+CD45RO^+$	319 ± 121	306 ± 102
$CD8^+CD45RO^-$	312 ± 123	286 ± 110
$CD8^+CD45RO^+$	155 ± 82	177 ± 91

*$P < 0.001$ compared with NS.
Means and SD; NS: normal status; OT: overtraining syndrome.

agreement with the individual training and competition program of each athlete, a period opportune for induction of OT was chosen, although the procedure of induction was not strictly defined. All investigations were performed on the same time of the day after an overnight fast. Before laboratory testings, training sessions were recorded for 2 wk, and most of the training sessions were monitored for heart rates. On the day before each testing, only regenerative training sessions were allowed. The last intensive or longer lasting training was at least 36 h before testing. Each individual gave informed written consent before the start of the study, which was approved by the Faculty of Medicine of the University of the Saarland.

The first day of each investigation comprised the following tests: present clinical and training history, physical examination, anthropometric measurements (45), resting ECG, incremental graded spiroergometry with ECG, and indirect measurement of blood pressure. In addition, on the first day of the first investigation, heart volume was measured by combined one- and two-dimensional echocardiography (modified Simpson rule (2); Vingmed CFM 700, Sonotron Inc., Norway). On the second day of each investigation, 3–7 d later, present history was taken again, a standardized psychological questionnaire was filled in and a highly intensive short-endurance exercise to exhaustion ("stress test") was performed to take repeated blood samples for determination of immunological parameters.

Two experienced physicians independently diagnosed OT by exclusion of other reasons, e.g., organic diseases. Classical symptoms as decrease of performance (reduction of results at recent competitions, unexpectedly premature interruption of training or competition), decreased subjective performance capacity and early fatigue with training going along with more or less severe vegetative symptoms (13,14,17,20). At the time of diagnosis, no subject suffered from infectious disease or diminished iron stores, determined by clinical examination and routine laboratory parameters.

Ergometry

All exercises were performed on electrically braked cycle ergometers in the upright position. An incremental graded exercise was conducted to subjective exhaustion as described in detail before (6,12,35).

The stress test consisted of an endurance exercise 10% above the maximal lactate steady state performed to subjective exhaustion (6,39). In 10-min intervals during the stress test, the athletes estimated their subjective rating of exertion using the Borg scale (1). At least 15 min after insertion of an catheter into an antecubital vein and after 15 min of quiet supine rest, the first blood sample was taken, the second at the end of the 10th min of exercise, and the third and fourth immediately and 1 h after exercise, respectively.

Immunophenotypes

One- and two-color indirect immunofluorescence technique was used to determine leukocyte and lymphocyte subpopulations in whole blood as described in detail before (42). Cell concentrations were corrected for plasma volume changes (3). Linear FSC and SSC scatter signals in combination with four-decade logarithmic FITC and PE fluorescence signals of lymphocytes, monocytes, and granulocytes were collected with a FACScan flow cytometer (BD) and were stored as FCS1.0 list mode files (6). The detailed procedures of processing the FCS1.0 list mode data are provided in Valet et al. (42), and CLASSIF1 program system (Partec, Münster, Germany) was used for calculations.

Statistics

Data are shown as means and standard deviations. Medians were calculated for each individual in case of not-overtrained normal state (NS). Statistical comparisons between NS and OT were made using the Wilcoxon test for matched pairs. The level of the significance was set at 2.5% ($P < 0.025$).

RESULTS

General Aspects and Performance

In 15 of 85 examinations, OT was diagnosed. Within these 15 OT cases, 6 appeared during the competition pe-

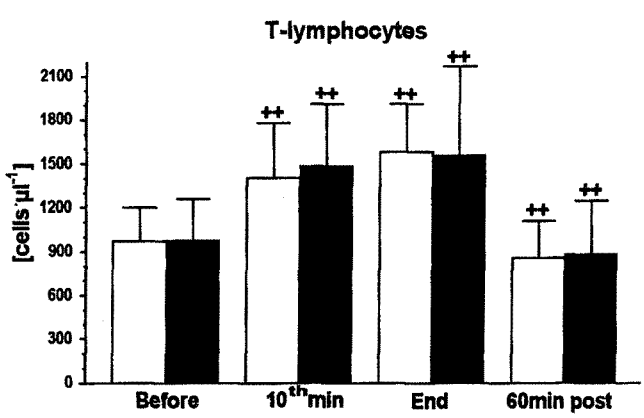

Figure 1—T lymphocyte ($CD3^+$) counts in overtraining (OT; *black bars*) and normal conditions (NS; *open bars*) of endurance athletes before, at the end of the 10th minute, at the end, and 60 min after a highly intensive endurance exercise to volitional exhaustion at 110% of the individual anaerobic threshold (OT: 16 ± 6 min; NS: 23 ± 10 min). Means ± SD; + $P < 0.025$, ++ $P < 0.01$ in comparison to values before exercise; *$P < 0.025$, ** $P < 0.01$, *** $P < 0.001$ between NS and OT; $N = 15$.

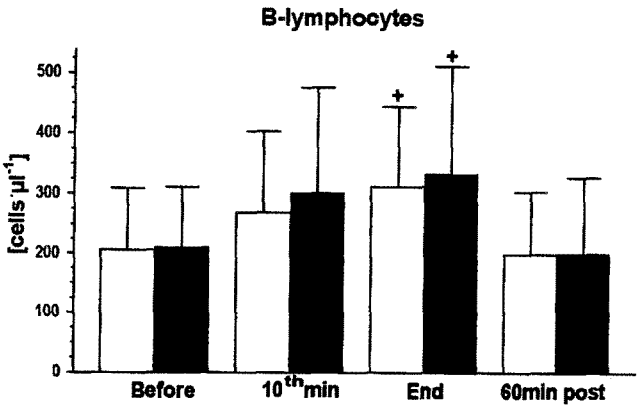

Figure 2—B lymphocyte (CD19$^+$) counts. For further information see Figure 1.

riod. OT was experimentally induced in 12 cases, mostly by a substantial increase of high-intensive training during 2–3 wk without the usual regenerative days, or by prolonging the competition period or a training camp. Weekly training volume before OT was not different from NS (approximately 10 (OT) and 9 (NS) h). The athletes had significantly increased their amount of training at an intensity above or within the range of the individual anaerobic threshold before OT (approximately 4.5 h per week) in comparison to NS (approximately 1.5 h per week). The athletes complained about typical OT symptoms: the feeling of heavy muscles of the lower limbs at modest exercise intensities, 13 athletes complained about intense daily fatigue and lack of concentration, 11 reported sleeping disorders, 4 a diminished appetite, and 3 an increased sweating rate. These complaints had started 13 ± 4 d before the examination date and lasted for 24 ± 10 d.

Borg-scale values were significantly higher after 10 min of the stress test during OT (OT 16.3 ± 1.5; NS: 14.3 ± 1.3; $P < 0.01$) without differences to NS at the end of this exercise test (all ratings >18). The following factors of the self-condition scale according to Nitsch (27) were altered significantly during OT: mean of all 14 binary factors as a measure for the global mood profile, fatigue, recovery, strain, sleepiness, and satisfaction (41).

Time to exhaustion of the stress test was significantly less by 27% during OT (16 ± 6 min) in comparison to NS (23 ± 10 min; $P < 0.01$). The maximal lactate concentration in the incremental graded exercise test was significantly decreased during OT (7.5 ± 2.7 mmol·L^{-1}; NS: 9.1 ± 2.4 mmol·L^{-1}; $P < 0.01$) (40,41).

Symptoms of URTI

Five of 15 athletes (33%) reported URTI symptoms during the 4 wk before the investigation dates. No athlete complained about severe generalized symptoms like chills or fever, but symptoms were localized to the URT (sore throat, rhinitis with clear secretion, mucosal swelling of the nose) in all but one case. One athlete reported a productive cough for a few days. Before the 70 investigations without exhibition of OT on 17 occasions, athletes reported URTI symptoms (24%). Three cases with fever and predominant symptoms of the URT were observed. During the last 2 wk before the investigations, severe infections were not recorded.

Immunophenotypes: Cell Counts

Neither percentages nor absolute cell counts of the major cell lines (neutrophils, monocytes, B, total T, $T_{helper/inducer}$, $T_{suppressor/cytotoxic}$, and NK cells) showed differences before, during, or 60 min after the stress test. Particularly, the exercise induced mobilization of cells undergoing greatest fluctuations were not different (Table 1, Figs. 1-3). Among leukocyte subpopulations, eosinophils were lower during OT. HLA-DR$^+$ T cells (activated T cells) were slightly, but significantly, increased during OT (Fig. 4). Also, the percentage of CD16/CD56$^+$ among T cells was higher (Fig. 5). Cell counts of CD45RO$^+$ T cells, either CD4$^+$ or CD8$^+$, were not different between NS and OT (Table 1).

Immununophenotypes: Surface Antigen Contents

Among all surface antigens only CD45RO on both CD4$^+$ and CD8$^+$ T cells were higher during OT (Fig. 6). HLA-DR expression on T cells tended to be lower during OT, but differences to NS were not significant ($P = 0.089$). All other surface receptors did not show differences between OT and NS (Table 2).

Diagnosis of OT

The relative antigen content of CD45RO on both CD4$^+$ and CD8$^+$ T cells was the decisive data column to classify OT successfully. All other parameters were eliminated during repetitive iterations of the learning procedure. By using the 10th and 90th percentiles of normal samples, diagnosis of NS was correct in 84.3% and OT was recognized in 66.7% (Table 3, classification 1). Negative and positive predictive values were 96% and 44%, respectively. Subsequently, it was hypothesized that an increased expression of CD45RO indicates OT. A second classification tested how far the "overexpression" of CD45RO could recognize OT. The normal expression was associated with NS in 93.2% of

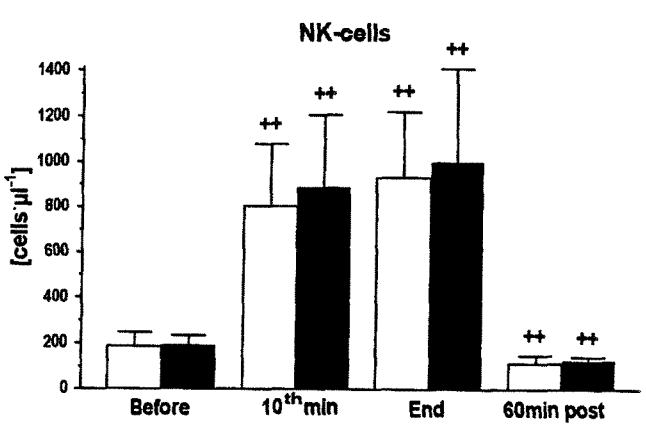

Figure 3—NK-cell (CD3$^-$CD16/CD56$^+$) counts. For further information see Figure 1.

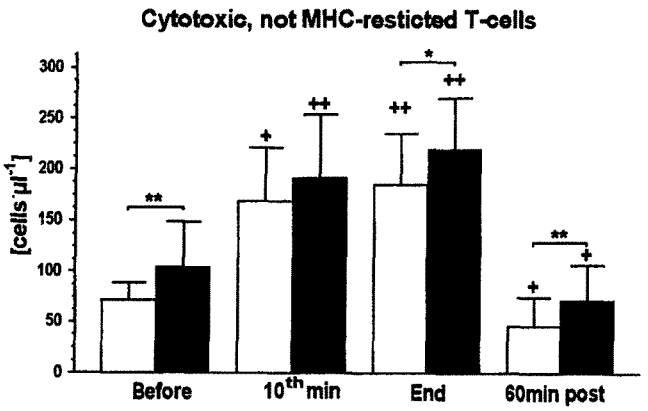

Figure 4—Cytotoxic, non-MHC-restricted T-cell counts ($CD3^+CD16/CD56^+$). For further information see Figure 1.

the cases and increased expression with OT in 93.3% (Table 3, classification 2; negative/positive predictive value: 96/67%). The third independent and prospective approach classified 19 unknown samples, which were independent from those of the study were classified. Eleven samples were from healthy athletes who were not overtrained. The other eight athletes suffered from an overtraining syndrome (clinical diagnosis). Ten of 11 normal samples and 5 of 8 OT samples were diagnosed correctly (Table 3, classification 3; negative/positive predictive values: 77/86%).

DISCUSSION

On the one hand, the present study provides information about unaltered distributions of all major cell lines. Only eosinophils showed reduced, activated T cells ($CD3^+HLA\text{-}DR^+$); and cytotoxic, non-MHC-restricted T cells ($CD3^+CD16/CD56^+$) moderately increased cell counts. On the other hand, new diagnostic aspects of OT by analyzing flow cytometrically achieved list mode data from immunophenotypes by using a new self-learning classification system were shown, although this classification procedure requires improvements for use in sports medical practice.

Contradicting results exist about total leukocyte counts during OT. Lehmann et al. (19) found reduced cell counts in eight overloaded middle/long distance runners, and Fry et al. (5) could not show altered leukocyte concentrations in five overtrained elite soldiers. Matvienko (23) presented reduced cell counts for athletes with stagnating performance, but without OT symptoms. On the basis of the present results, a reduction of the total leukocyte concentration is improbable. Also, in the here presented study, cell counts of all major cell lines detectable in peripheral blood, namely neutrophils, monocytes, B, total T, $T_{helper/inducer}$, $T_{suppressor/cytotoxic}$, and NK cells, were not altered during OT. In this context, the standardization of blood sampling conditions and laboratory methods are particularly important. The reason for reduced cell concentrations of eosinophils remains unclear. The mean eosinophil count of the five overtrained athletes reported by Fry et al. (5) was lower before starting the intensified training program. The immunological function of eosinophils are chemotaxis, adherence, phagocytosis, degranulation, production of lipid mediators, and reactive oxygen species (10). Activation of eosinophils leads to an increased production of cells in the bone marrow (e.g., in bronchial asthma) and induces an enhanced migration into inflamed tissues. Perhaps the phenomenon of a reduced eosinophil count during OT could be interpreted as an increased migration out of circulation but must remain without substantial proof at present.

The unaltered NK-cell counts presented here contradict literature findings. Fry et al. (5) reported about a decrease of NK cell concentrations under resting conditions during OT. This effect was likely due to relative high levels at the beginning of the study of one or two of the five subjects under investigation, if the high standard deviation is considered (mean 600, SEM 60 cell·μL^{-1}), and it remains questionable why. Such values are suspicious to have pathological or methodological reasons. It therefore is assumed, that no valuable effect on NK-cell numbers could be shown. In addition NK cells were determined as $CD56^+$ cells by using a single color immunofluorescence technique, which may have considerable overlap with $CD3^+CD56^+$ T cells and exclude $CD3^-CD56^-CD16^+$ NK cells. This and other papers presenting data about immunophenotypes in training studies showed further weak points from a methodological point of view. Single-marker studies (5,31,43) or incomplete description (15) of the use of marker combinations for T cells and T-cell subpopulations, and membrane activation markers such as HLA-DR, make it most difficult to interpret the results correctly. The CD4/CD8 ratio is easily influenced by $CD8^+$ NK cells, if single marker studies are used, and therefore does not necessarily represent a "helper/suppressor T-cell ratio" (15,43). Furthermore, there is no substantial reason to believe that this ratio plays "an important role in immunosurveillance" and a "ratio" below 1.5 being indicative of an increased susceptibility to infection (15,16). Cell separation methods, immunophenotyping protocols, standardized measurements, combination of monoclonal antibodies, and list mode data analysis were not directly comparable in many of the investigations (9) but have significant influence on the proportion of the one to the other cell subset.

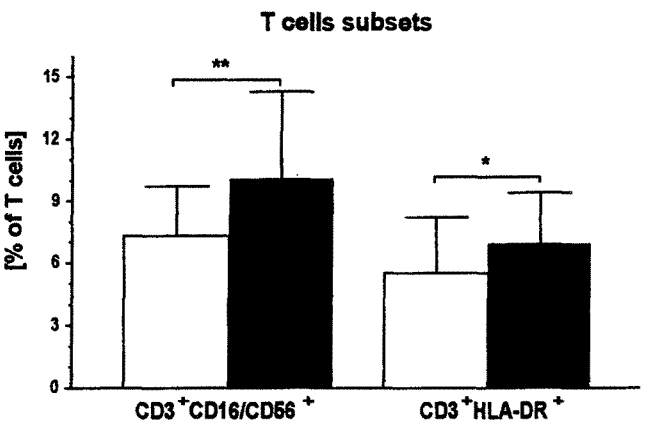

Figure 5—Cell counts of $HLA\text{-}DR^+$ or $CD16/CD56^+$ cells in percentage of $CD3^+$ T cells. For further information see Figure 1.

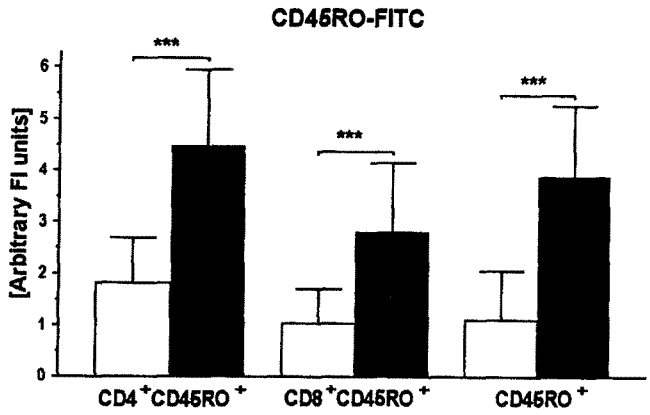

Figure 6—Mean fluorescence intensity (FI) of anti CD45RO-FITC on $CD4^+$, $CD8^+$, and total T cells ($CD3^+$). For further information see Figure 1.

If results of overtraining studies are regarded together with the present results, it can almost be excluded that overload training periods and an overtraining syndrome of relatively short duration leads to significant alterations of the distribution of the important immune cell lines detectable in peripheral blood. Cell concentrations of all major cell lines were unaltered during OT at physical rest. Furthermore, the mobilization into circulation and recirculation patterns after acute bouts of exercise during overtraining does not seem to be affected. This might be interpreted as an unimpaired trafficking of immune cells through the organism.

If the small increases of percentages of activated T cells expressing HLA-DR or interleukin-2 receptor (5) and unchanged HLA-DR expression on total lymphocytes (B cells + T cells) are regarded, the effect of intensified training and overtraining under the investigated conditions on the T cells must be regarded as marginal, especially if compared with activations that are induced by infections or other inflammatory processes in the organism. This is confirmed by studies of Verde et al. (43) showing trends of increased phytohemagglutinin- and concanavalin-stimulated lymphocyte proliferation, whereas others found a slightly impaired mitogenic-stimulated lymphocyte proliferation in four of five subjects (5).

The relatively high upregulation of CD45RO on T cells indicated a change in T-cell function, but it seems unlikely that this effect was specific for an OT. CD45RO is an isotype of the transmembranous tyrosine phosphatase CD45 (leukocyte common antigen (36)) and has its task with the T-cell receptor-mediated activation of lymphocytes (37,44). After immunogenic stimulus, T cells proliferate and express CD45RO instead of CD45RA, which is regarded as a late sign of activation (24). The intermediate population from $CD45RA^+$ to $CD45RO^+$ cells are $CD45RA^+CD45RO^+$ and expresses more interleukin-2 receptors than "naïve" $CD45RA^+$ T cells (46). One week after an 12-h duration endurance competition, this intermediate population is increased by 104% and indicates a moderate activation of T cells (7). Furthermore, the percentage of $CD45RO^+$ cells within total T cells increase with age (8). Viral infections, e.g., with Epstein-Barr virus, lead to a great increase of $HLA\text{-}DR^+$ and $CD45RO^+$ and $CD8^+$ (suppressive) T cells (18,38). The results of the present study show on the one hand an upregulation of CD45RO, but on the other hand a stable percentage of $CD45RO^+$ T cells, either $CD4^+$ or $CD8^+$. Obviously, a slight activation of T cells takes place but is not strong enough to increase the pool of circulating $CD45RO^+$ T cells. This effect considered together with the small increase of $HLA\text{-}DR^+$ T cells indicate a fine upregulation of the T-cell function, which at present is not seen as clinically relevant. On the other hand, an influence of URTI during the week before is unlikely, because seven athletes reported symptoms like sore throat or rhinitis, but this group did not show different expression densities for CD45RO or percentages of $HLA\text{-}DR^+$ T cells compared with those who did not report infectious symptoms ($N = 8$) during periods before OT. A further aspect might be that none of the athletes showed a high percentage of activated T cells or T cells expressing HLA-DR at high levels. This indicates that OT does not lead to a pathological enhancement of the T-cell function and the stimulus "OT" is only minimally immunogenic. Furthermore, it remains unclear which is the concrete immunogenic stimulus.

Although the immunologic meaning of the upregulated expression of CD45RO seems to be of minor importance, this upregulation enabled a differentiation between NS and OT. It was the first successful attempt of an diagnosis by using a self-learning classification on the basis of lymphocyte immunophenotypes (42). Percentages of immune cell lines of subpopulations determined by staining the activation receptors HLA-DR and CD45RO did not contribute to the diagnosis of OT. In general and beyond the aims of this specific hypothesis of the study, it must strongly be recommended to look at surface membrane contents (receptor densities) in addition to percentages and absolute cell counts of immune cell populations to achieve optimal results for clinical diagnoses. The present classification based on flow cytometric list mode data indicates that the clinical diagnosis of an OT could be confirmed with a sensitivity of about 67% (specificity: 84%). It seems speculative to regard an upregulation of CD45RO as a criterion for OT, but under the

TABLE 2. Membrane antigen contents in arbitrary fluorescence intensity units.

mAb-fluorescent dye	Lymphocyte subpopulation	Fluorescence intensity (arbitrary units)	
		NS	OT
antiCD3-FITC	$CD3^+$	2.26 ± 0.57	2.39 ± 0.64
antiCD19-FITC	$CD19^+$	1.05 ± 0.26	1.19 ± 0.20
antiCD16-PE and	$CD3^-CD16/CD56^+$	2.32 ± 1.12*	2.50 ± 1.04*
antiCD56-PE	$CD3^+CD16/CD56^+$	0.63 ± 0.29	0.65 ± 0.17
antiCD4-PE	$CD4^+CD45RO^-$	10.16 ± 3.19	10.16 ± 2.90
	$CD4^+CD45RO^+$	12.26 ± 3.62	11.74 ± 2.70
antiCD8-PE	$CD8^+CD45RO^-$	14.40 ± 3.75	14.11 ± 3.95
	$CD8^+CD45RO^+$	14.77 ± 4.37	15.02 ± 4.56
antiHLA-DR	$CD3^-HLA\text{-}DR^+$	5.35 ± 2.75*	4.68 ± 2.50*
	$CD3^+HLA\text{-}DR^+$	1.67 ± 1.40	1.32 ± 0.77
IgG-FITC	lymphocytes	0.05 ± 0.02	0.05 ± 0.01
IgG-PE	lymphocytes	0.05 ± 0.01	0.03 ± 0.01

*: $P < 0.001$ in comparison to value directly below.
†$P < 0.001$ in comparison to corresponding value at NS.
Means and SD; FITC: fluorescein isothiocyanate; PE: phycoerythrin; mAb: monoclonal antibody; NS: normal status; OT: overtraining syndrome.

TABLE 3. Confusion matrices for flow cytometric classification and clinical diagnosis of normal status (NS) and overtraining syndrome (OT).

		Flow Cytometrical Classification	
		NS	OT
Classification 1 Clinical diagnosis	NS (N = 51)	84.3	25.5
	OT (N = 15)	13.3	66.7
Classification 2 "Overexpression" of CD45RO	NS (N = 51)	92.2	13.7
	OT (N = 15)	13.3	93.3
Classification 3 Clinical diagnosis	NS (N = 11)	90.9	9.1
	OT (N = 8)	37.5	62.5

Classification 1: original data set; classification 2: hypothesis: overexpression of CD45RO indicates OT; classification 3: prospective classification of 19 unknown samples.

hypothesis that this would be the case, the upregulation of CD45RO and the clinical diagnoses of NS or OT could be done with very high sensitivity and specificity. The prospective approach of 11 NS and 9 OT (Table 4, classification 3) is more important for the confirmation of the results. Three interpretations are possible why "only" about 60% of OT was recognized. Probably the receptor upregulation of CD45RO differs interindividually, which means that each subject might not react at the same extent than others. Furthermore, OT might not always go along with an upregulation of CD45RO. Finally, it must be taken into account that a gold standard to diagnose OT does not exist and the clinical diagnosis of an OT need not inevitably be correct, although the long personal experience of two independent physicians provides a high probability for a correct clinically based diagnosis.

The present study was conducted under the prediction to induce an overtraining syndrome in endurance athletes over a time period over about 1.5 yr. Before drawing conclusions from the results, some aspects have to be included as follows. The diagnosis of OT was subjective but took into account recommendations from the literature. A gold standard to diagnose an OT does not exist. Furthermore, it is impossible to standardize time schedule for training and competitions over 1.5 yr. Protocols of training inclusively monitoring of heart rates had to serve as estimates for the performed physical loads. Also, results and conclusions must be restricted to endurance athletes at present and cannot be extended to other athletes. Last but not least, immunophenotypes of leukocytes derived from peripheral blood provide only limited information about the actual balance of the immune system of the whole organism.

From the present study the following conclusions are drawn. OT does not lead to clinically relevant alterations of immunophenotypes in peripheral blood. On the one hand, the moderate activation of T cells as shown by slight increases of the percentage of HLA-DR$^+$ T cells and the upregulation of CD45RO on T cells indicate an enhanced functional state of T cells. On the other hand, pathological ranges are not achieved, which excludes a significant activation of this part of the immune system. Unaltered exercise-induced mobilization and redistribution patterns of leukocyte and lymphocyte subpopulations indicate an unchanged flexibility to transport immune cells through the blood from one site to another. In consideration of the present results and the few other studies about overtraining and immune functions effects on immunophenotypes, *in vitro* proliferation response of lymphocytes to mitogens, secretory immunoglobulins, and also plasma glutamine levels cannot serve as parameters explaining the experiences of coaches, physicians, and athletes themselves of an increased susceptibility to infections in overtraining periods. So far, anecdotal reports about an increased susceptibility to infections cannot be confirmed by overtraining studies published up to date. It might help as an explanation for this apparent contradiction that most studies did not investigate top athletes in situations particularly likely to induce an overtraining syndrome like psychological stress, malnutrition, and postinfectious periods. Experimentally induced overtraining might not necessarily reflect a comparable situation. Last but not least, immunophenotyping of lymphocytes provided help with the diagnosis of OT in this study and will probably support the diagnosis of OT in future. This may be seen as an innovative and promising part of the present study, which requires improvements before it can be used in the daily routine of the sports medical practice.

The authors thank B. Weiler for excellent technical assistance.
This study was supported by grants from the "Bundesinstitut für Sportwissenschaft" in Cologne, Germany.
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