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Reprint

Differential Expression of Interleukin-2 Receptor Alpha and Beta Chains in Relation to Natural Killer Cell Subsets and Aerobic Fitness

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Abstract

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Immunophenotyping by dual parameter flow cytometry was used to compare the expression of interleukin-2 receptor α and β chains on lymphocyte subsets in the peripheral blood of 7 trained and 6 untrained volunteers (respective $\dot{V}O_2max$ 57.0±6.1 and 39.0±4.5 ml·kg^{-1}·min^{-1}). Venous blood samples were collected at least 36 h after the most recent exercise session. The trained subjects had higher circulating counts (10^9·l^{-1}) of total leukocytes (5.80±0.83 vs. 4.63±0.21, $p<0.05$), granulocytes (3.14±0.72 vs. 1.90±0.30, $p<0.05$), and NK cells (CD16$^+$, 0.32±0.14 vs. 0.16±0.05, $p<0.05$; CD56$^+$, 0.41±0.14 vs. 0.21±0.03, $p<0.01$), but lower lymphocyte counts than their sedentary peers (1.90±0.22 vs. 2.26±0.25, $p<0.05$). Counts for T cells (CD3$^+$) and B cells (CD19$^+$), and the CD4$^+$/CD8$^+$ ratio did not differ between the two subject groups.

The p55-IL-2 receptor α expression (CD25$^+$: 0.63±0.11 vs. 0.69±0.17) was unrelated to training, but the p70–75-IL-2 receptor β expression was higher in the active group (p70/Mik-β$_1$$^+$: 0.42±0.09 vs. 0.20±0.06, $p<0.001$; p75/TU27$^+$: 0.36±0.08 vs. 0.17±0.07, $p<0.005$). Beta chain co-expression was also higher on NK cell subsets ($p<0.001$) in trained than in sedentary subjects. Aerobic power was strongly correlated with IL-2Rβ expression ($r=0.914$, $p<0.001$ for Mik-β$_1$; $r=0.884$, $p<0.005$ for TU27). We conclude that physical conditioning is associated with an increase in IL-2 receptor β expression on lymphocytes as assessed by the proportion of circulating p70–75-IL-2Rβ positive NK cells. Such changes could enhance protection against both infectious diseases and cancer. Longitudinal studies are now needed to explore the causal nature of this association.

Key words

Aerobic power, cytokines, cancer, immune function, natural immunity, training

Introduction

Both acute exercise and physical conditioning modulate functions of the immune system (37,40). Depending on the intensity and duration of effort, a single acute bout of physical activity can enhance, suppress or provoke no change in immune reactivities (41). Similarly, exercise training can induce favorable or unfavorable changes in resting immune function, with potential consequences for resistance to viral infections (6, 9) and cancer (13,36,50). Key elements in such changes are interleukin-2 (IL-2), the interleukin-2 receptor (IL-2R), and the number and activity of circulating natural killer (NK) cells.

IL-2 is one of several lymphokines that are produced by antigen- or mitogen-activated CD4$^+$ T helper (T$_H$) lymphocytes following stimulation through the T cell receptor (TcR) (21). IL-2 exerts regulatory effects on virtually all cell types participating in immune responses and plays a pivotal role in regulating the proliferation, differentiation and secretory capacity of T and B cells, monocytes, NK cells and LAK (lymphokine-activated killer) cells (42). Like polypeptide hormones, IL-2 exerts its biological effects through a specific transmembrane receptor system composed of three distinct non-covalently associated glycoprotein subunits: an α chain with a molecular weight of 55 kilodalton (kDa) recognized by the monoclonal antibody (mAb) anti-CD25; a β chain with a molecular weight estimated to be 70–75 kDa identified by Mik-β$_1$ and TU27 mAbs; and a recently cloned 64-kDa γ chain (47). The α subunit, which binds IL-2 with low affinity and has a short cytoplasmic tail of 13 amino acids (aa) is unable to transduce IL-2 dependent signals in the absence of the β subunit. Thus, the α chain appears to be functionally limited to increasing the IL-2 binding affinity of the β chain (42). The β chain contains a large intracytoplasmic domain of 286 aa which is essential for internalization (34) and intracellular transduction

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of the IL-2 signal (45) with resultant lymphocyte activation and proliferation (51). Preliminary functional analysis of the IL-2Rγ chain (86 intracytoplasmic aa) suggests that it also modulates the IL-2 binding affinity of the β chain by decreasing the IL-2 dissociation rate (33). In addition, the γ chain associates with the β chain to form an intermediate-affinity IL-2 binding site which is itself capable of signal transduction (39,52). A fully functional high-affinity IL-2 receptor thus consists of a heterotrimeric complex resulting from covalent association between the three chains; this configuration allows signal delivery at greatly reduced IL-2 concentrations (45).

The IL-2Rα chain is normally constitutively expressed by 30–35% of unstimulated peripheral blood mononuclear cells (PBMC) (100–500 sites/cell) (15,55). The IL-2Rβ is expressed by only 10% of circulating PBMC (500–1000 sites/cell) (48,55), the majority of these being NK cells (25). Fewer still resting lymphocytes express high affinity IL-2 receptors (~150 sites/cell) (26,55). In fact, the vast majority of unactivated resting T and B lymphocytes express neither intermediate nor high affinity IL-2R binding sites and are largely unresponsive to IL-2 (4,42). Antigen- or mitogen-stimulation of resting T cells induces a considerable (30–100-fold) but transient upregulation of IL-2Rα chains (peaking at 48–72 h). However, there is only a modest induction of β chains. This results in the expression of many more α chains (>10,000 excess chains/cell) than β chains on the surface of activated T cells and the formation of 2000 to 3000 high-affinity binding sites (45); in essence, the resultant number of high-affinity IL-2 binding sites is limited by the relatively invariant number of intermediate-affinity subunits.

NK cells are large granular lymphocytes (LGL) which constitutively express nonspecific cytolytic activity against a number of susceptible viral and tumor target cells (54). NK cells normally constitute up to 15% of human PBMC and comprise a heterogeneous cell population in terms of cell density, cell surface expression of CD16 and CD56, and responsiveness to IL-2 (35). Most peripheral blood $CD16^+$ NK cells co-express CD56 at low density (the $CD16^+/CD56^{dim}$ phenotype); however, a subset (about 5–10% of NK cells) express the CD56 antigen in high density (the $CD16^-/CD56^{bright}$ phenotype) (16,22). In contrast to T cells, the majority of freshly isolated NK cells constitutively express intermediate-affinity IL-2 receptors (25,52). $CD56^{dim}$ NK cells express only intermediate affinity βγ heterodimers, whereas $CD56^{bright}$ NK cells express high-affinity heterotrimers composed of α, β and γ subunits (22,47). Thus, $CD56^{bright}$ cells proliferate vigorously and exhibit enhanced cytolytic activity in response to 10–100 pM concentrations of IL-2 (42), whereas $CD56^{dim}$ NK cells require 100-fold higher concentrations of IL-2 to induce an equivalent response (1,5). As such, $CD56^{bright}$ cells constitute the precursors to LAK cells (39). Once activated, these cells are directly oncolytic to nascent or metastasizing neoplasms (35) and unlike T cells which express high-affinity IL-2 receptors for only a limited time after activation, NK cells express such receptors and are responsive to IL-2 for a prolonged period (5).

In view of the significance of IL-2-receptor-mediated lymphocyte reactivities and fitness-related changes in immune functions, this study was undertaken to examine a possible relationship between aerobic fitness and IL-2-receptor expression in a cross-sectional comparison of endurance trained and sedentary individuals. Specifically, we analyzed the difference in the expression of α (p55) and β (p70–75) subunits of the IL-2 receptor on various lymphocyte subsets, including $CD16^+$ and $CD56^+$ NK cells.

Materials and Methods

Subjects

Thirteen healthy volunteers were recruited from the University of Toronto student population under conditions approved by the University Committee on the Ethics of Human Experimentation. They comprised 7 endurance trained men (mean±SD; age: 21.9±2.1 years, mass: 73.2±6.2 kg, height: 179.2±4.8 cm, maximal heart rate 186.0±5.4, and maximal oxygen consumption: 57.0±6.1 $ml \cdot kg^{-1} \cdot min^{-1}$) and 6 untrained men (age: 23.4±3.3 years, mass: 75.6±7.6 kg, height: 176.3±5.5 cm, maximal heart rate 189.0±3.2, and maximal oxygen consumption: 39.0±4.5 $ml \cdot kg^{-1} \cdot min^{-1}$). After a routine medical examination to exclude allergic conditions and other significant pathology, volunteers were given a detailed explanation of the experimental protocol and completed a written consent form. Subjects were asked to refrain from intensive, or prolonged physical activity during the 36 h period prior to testing, since many reports have indicated that exercise-induced alterations in NK function usually revert to normal within 24 h (18). Furthermore, subjects were instructed to maintain their usual diurnal patterns of eating, sleeping and light exercise throughout the test period. All subjects were non-smokers and did not consume any food or drugs on the morning of testing.

Determination of maximal oxygen intake ($\dot{V}O_2max$)

Subjects were tested on a Cardiogenics cycle ergometer at 60 pedal r.p.m. After a 4 min warm-up at 60 W, the work rate was increased by 30 W every 2 min to voluntary exhaustion, reached in no more than 12–15 min. Criteria for attainment of $\dot{V}O_2max$ included: a plateau of oxygen consumption (an increment <150 $ml \cdot min^{-1}$ with a further increase in work-rate); a heart-rate close to the individuals age-related maximum and not augmented by a further increase in work-rate; signs of exertional intolerance (fatigue and an inability to maintain the required pedal rhythm) and/or a respiratory gas exchange ratio (R) value >1.15.

Expired gas was collected and analyzed using a Sensormedics Metabolic Measurement Cart (System MTS 4400; Alpha Technologies, Anaheim, CA). This system incorporates a helical flowmeter, a digital volume transducer, an infrared carbon dioxide analyzer and a zirconium dioxide electrochemical cell oxygen analyzer. Autocalibration was carried out using precision-analyzed cylinder gas mixtures of 24% O_2, 8% CO_2 and 100% N_2, 0% O_2, 0% CO_2, and a 3 liter syringe.

Blood sampling

Samples of peripheral blood were collected from the median cubital vein, one week after the determination of maximal oxygen intake. Subjects rested for 30 min in an upright position prior to venipuncture. All samples were obtained between 0800 and 0900 h, to minimize circadian effects. Blood samples of 10 ml were drawn into sterile liquid ethylenediaminetetra-acetic acid (EDTA) glass vacutainers (Becton-Dickinson, Oakville, ON). Samples were kept at room temperature, mixed continuously with a Coulter mixer (Coulter Elec-

Table 1 CD* nomenclature of monoclonal antibodies (mAbs) used in this study.

CD Antigen	mAb	Surface Receptor/ Major Cellular Reactivity	Isotype
CD3	anti-Leu-4	All mature T cells (TcR-CD3 Complex)	IgG_1
CD4	anti-Leu-3a	T helper/inducer cells, monocytes	IgG_1
CD8	anti-Leu-2a	T cytotoxic/suppressor, NK cell subset	IgG_1
CD14	anti-Leu-M3	Monocytes	IgG_{2b}
CD16	anti-Leu-11a	NK cells, neutrophils; low affinity $Fc\gamma RIIIA$	IgG_1
CD19	anti-Leu-12	B cells, pre-B cells	IgG_1
CD25	anti-Tac	IL-2Rα/p55, activated T_{H1} lymphocytes	IgG_1
CD122	anti-Mik-β_1	IL-2Rβ/p70, NK cells and monocytes	IgG_{2a}
CD122	anti-TU27	IL-2Rβ/p75, NK cells and monocytes	IgG_1
CD56	anti-Leu-19	NK cells, T cell subset	IgG_1

* C.D., cluster of differentiation

tronics, Hialeah, FL) and analyzed within 2 h of collection. Prior tests on control subjects have established test-retest reliability of .92.

Leukocyte and differential counts

Total cell counts and differential leukocyte counts were carried out using an automated Coulter JT haematology analyzer (Coulter Electronics). This instrument uses aperture impedance, volume, conductivity and light scattering measurements to determine cell characteristics, and standard spectroscopy to determine haemoglobin concentrations.

Immunophenotyping of leukocyte and lymphocyte subpopulations

Monoclonal antibodies

The properties of the monoclonal antibodies (mAbs) used in this study are set out in Table **1**. All reagents were conjugated to one of two markers fluorescing in the visible spectrum: fluorescein isothiocyanate (FITC) or phycoerythrin (PE) fluorochromes. The mAbs Mik-β_1 and TU27 were purchased from Endogen (Boston, MA) and Janssen Biochemica (Geel, Belgium), respectively. Mik-β_1 is an IgG_{2a} FITC-conjugated mAb which recognizes the p70 chain of the human IL-2R (49). TU27 is specific for the p75 chain and is an IgG_1 FITC-conjugated mAb (46). All other reagents were obtained from Becton Dickinson (Mountain View, CA). The p55 α chain of the IL-2R was detected using IgG_1 mAb CD25. Non-specific staining was tested by controls, which included cells without staining or autofluorescence, and cells stained with fluorochrome-conjugated, isotype-matched antibodies (mouse IgG_1 and IgG_{2a} FITC or PE) which do not bind to human cells. The control samples were used to determine gating parameters and cut-off points. Anti-CD14 was used to detect any monocyte contamination (<5%) of the electronically gated lymphocyte cluster (>95% lymphocytes), and the percentage of lymphocytes was adjusted accordingly.

Two-colour immunofluorescence staining procedure

Whole blood was used rather than isolated peripheral blood mononuclear cells (PBMC), since separation of PBMC prior to mAb staining is time consuming and can lead to a selective cell loss (38). Samples of 100 µl of EDTA-blood were combined with saturating concentrations of selected mAbs, in the desired dual staining combinations. Reagent tubes containing the anti-coagulant-treated whole blood and mAbs were gently vortexed for 3 s and then incubated on ice for 30 min in the dark. Next, 2 ml of FACS lysing solution (Becton Dickinson) were added to lyse red cells and partially fix the white cells. The mixture was vortexed and left in the dark for 10 min at room temperature. Subsequently the samples were centrifuged for 6 min at 4 °C and 500 × g, the supernatant was aspirated to 50 µl and the pellet was resuspended in 2 ml of cold phosphate-buffered saline (PBS) with 0.1 % sodium azide. After vortexing and a second centrifugation (for the same time and at the same force), the pellet was resuspended in 300 µl of cold 0.5 % paraformaldehyde in PBS with 0.1 % sodium azide. The fixed cells were stored in the dark at 4 °C for subsequent flow cytometric analysis.

Flow cytometric analysis

The mAb stained cell suspensions were analyzed for fluorescence on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Inc, Mountain View, CA) equipped with a 488-nm 15-mW argon laser source. For cell subpopulation analysis, a minimum of 10,000 events per blood sample was acquired and stored using Consort 30 software (Becton Dickinson). Digitized data were later analyzed on a Hewlett Packard 310 microcomputer system using LYSIS software (Becton Dickinson). The flow cytometer was calibrated and optimized before analysis, using a mixture of monosized FITC- and PE-conjugated and unconjugated latex particles (4.8 µm CaliBRITE beads, Becton Dickinson) in conjunction with AutoCOMP software. Fluorescence detectors were optimized using an isotype negative control; fluorescence compensation was adjusted using a CD4/CD8 dual-stained sample to minimize spectral overlap between the FITC and PE emissions.

Calculation of percentages and absolute counts

The FACScan indicates the percentage of cells bearing the specific fluorochrome label within the gated lymphocyte region. Absolute counts for a given antigen were calculated from the total leukocyte count, using the following expression:

$$\frac{\text{Total leukocytes}(10^9 \cdot l^{-1}) \times \%\ \text{lymphocytes} \times \%\ \text{antigen-positive subset}}{10{,}000}$$

The fraction of a given lymphocyte subset co-expressing the IL-2R was calculated using the formula:

Table 2 Leukocyte and lymphocyte subset cell concentrations in untrained and trained subjects[a].

	Cell Concentration	
	Untrained	Trained
	× 10^9 cells · l^{-1} ± S.D.	
Leukocyte subsets		
Total leukocytes	4.63 ± 0.21 (100 %)	5.80 ± 0.83 (100 %)*
Granulocytes	1.90 ± 0.30 (41.0 %)	3.14 ± 0.72 (54.1 %)*
Lymphocytes	2.26 ± 0.25 (48.8 %)	1.90 ± 0.22 (32.8 %)*
Lymphocyte subsets		
Total lymphocytes	2.26 ± 0.25 (100 %)	1.90 ± 0.22 (100 %)*
T cells ($CD3^+$)	1.73 ± 0.25 (76.5 %)	1.33 ± 0.16 (70.0 %)
B cells ($CD19^+$)	0.27 ± 0.12 (11.9 %)	0.21 ± 0.05 (11.1 %)
NK cells ($CD16^+$)	0.16 ± 0.05 (7.1 %)	0.32 ± 0.14 (16.8 %)*
NK cells ($CD56^+$)	0.21 ± 0.03 (9.3 %)	0.41 ± 0.14 (21.6 %)**
IL-2Rα ($CD25^+$)	0.69 ± 0.17 (30.5 %)	0.63 ± 0.11 (33.3 %)
IL-2Rβ (Mik-β_1^+, p70)	0.20 ± 0.06 (8.9 %)	0.42 ± 0.09 (22.1 %)**
($TU27^+$, p75)	0.17 ± 0.07 (7.5 %)	0.36 ± 0.08 (18.9 %)**

a Untrained subjects (n = 6); trained subjects (n = 7)
Each bracketed number represents the corresponding percentage of total leukocytes or lymphocytes.
* $p < 0.05$, **$p < 0.01$ (comparison between the mean values of trained and untrained subjects)

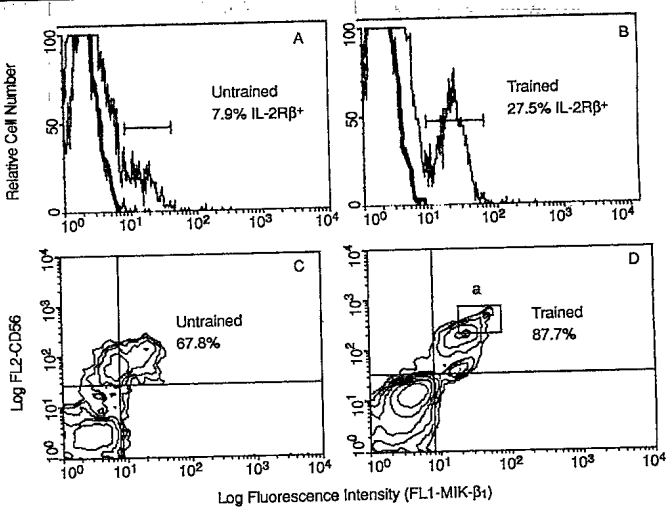

Fig. 1 Histograms **A** and **B** show the percentage of peripheral blood lymphocytes expressing the IL-2Rβ chain in an untrained versus a trained subject. Superimposed traces show the appropriate negative isotype controls. Contour plots **C** and **D** display the co-expression of Mik-β_1 (IL-2Rβ chain) by $CD56^+$ lymphocytes in an untrained (C) versus a trained subject (D). The percentage of $CD56^+$ lymphocytes expressing the IL-2Rβ is indicated in the upper right-hand corner of panels C and D. Region **a** (panel D) represents $CD56^{bright}$ lymphocytes. Bright cells refer to cells with fluorescence intensity greater than channel number 160. Markers defining quadrants were positioned to include > 98 % of negative isotype-stained cells in the lower left quadrant.

$$\frac{\%\ \text{double-labeled cells}}{\%\ \text{double-labeled cells} + \%\ \text{FITC-only labeled cells}} \times 100$$

The fraction of $IL\text{-}2R^+$ cells expressing a given lymphocyte subset marker was calculated using a similar formula, except that the percentage of FITC-only labeled cells was replaced by PE-only labeled cells.

Statistical analysis

Results are expressed throughout as means ± standard deviations (SD). One-way analysis of variance was used to determine the significant differences, with the alpha level set at $p = 0.05$. The association between continuous variables (Pearson product-moment correlation) was calculated by the least squares procedure. Two-tailed unpaired Student's t-tests were also used to assess the significance of differences between means (44).

Results

Leukocyte and lymphocyte subset counts and their relationship to maximal oxygen intake

Intergroup differences in the concentration and proportions of leukocyte and lymphocyte subsets are summarized in Table 2. The trained subjects showed higher percentages and absolute counts of total leukocytes, granulocytes, and NK cells, but lower counts for lymphocytes. Counts for T cells ($CD3^+$), and B cells ($CD19^+$), and the $CD4^+/CD8^+$ ratio (1.8 ± 0.6 vs. 1.7 ± 0.2 for trained subjects) did not differ significantly between untrained and trained subjects.

The percentage of lymphocytes expressing IL-2Rα (CD25) was unrelated to training status (range 18.1–38.7 %, mean 33.3 % for trained subjects and 30.5 % for untrained subjects). In contrast, the IL-2Rβ expression of p70 (Mik-β_1) (Fig. 1) and p75 ($TU27^+$) receptors was significantly higher in the active group (Table 2). The overall range was 7.9–27.5 % for Mik-β_1 and 4.8–24.4 % for TU27.

In addition, there was a strong association between $\dot{V}O_2max$ and expression of the IL-2Rβ, (Mik-β_1 and TU27, Fig. 2 **A** and **B**) as well as between $\dot{V}O_2max$ and NK cell subsets (CD16 and CD56, Fig. 2 **C** and **D**). All leukocyte and lymphocyte values fell within their normal ranges (32).

Expression of IL-2Rα on T lymphocytes and NK cells

Trained subjects showed a significantly higher percentage of $CD4^+$ (8.4 % higher) lymphocytes expressing the IL-2Rα (CD25) than did untrained subjects (Table 3). A slight trend to higher levels of CD25 co-expression on $CD8^{bright}$, CD16 and CD56 positive lymphocyte populations in trained subjects was not statistically significant (Table 3).

In contrast to $CD4^+$ cells, $CD8^+$ lymphocytes consist of two phenotypically and functionally distinct subpopulations ($CD8^{bright}$ and $CD8^{dim}$ cells) which are distinguished by their fluorescence staining intensity (see legend, Fig. 4). $CD8^{dim}$ cells are a subset of NK cells with the phenotype $CD3^-/CD16^+$, unlike $CD8^{bright}$ cells which are $CD3^+/CD16^-$ cytotoxic/suppressor cells of the T cell lineage (16,27).

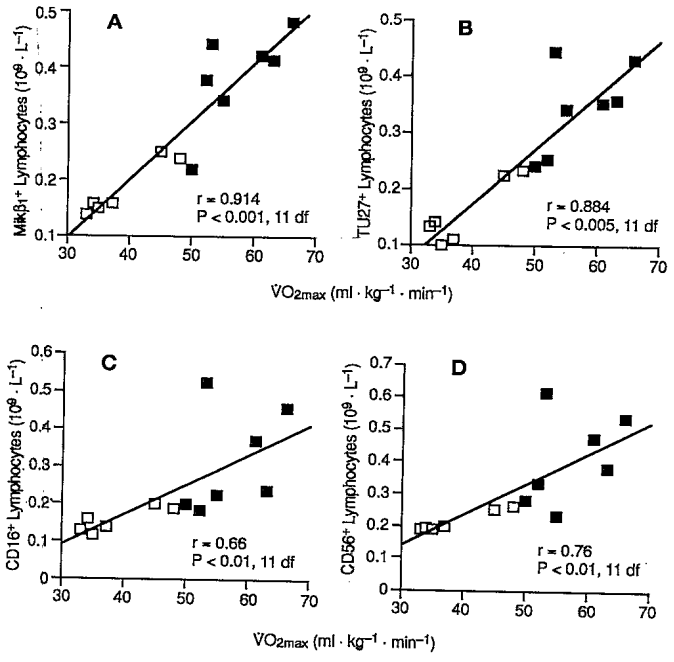

Fig. 2 Regression analysis of pooled data (all subjects) showing the correlation between V̇Omax and IL-2Rβ chain markers (**A** and **B**) and NK cell markers (**C** and **D**) on lymphocytes of trained (■) and untrained (□) resting subjects.

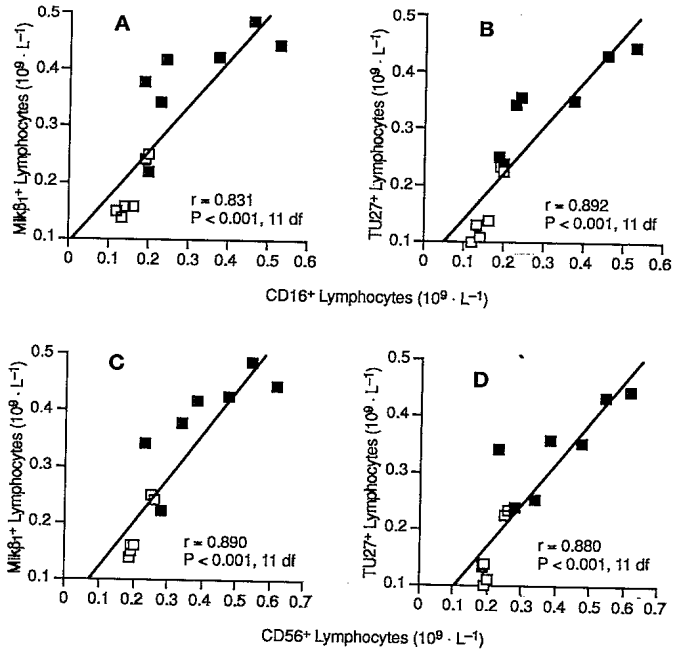

Fig. 3 Regression analysis of pooled data (all subjects) showing the correlation between IL-2Rβ chain markers and NK cell markers (CD16: **A** and **B**; CD56: **C** and **D**) for trained (■) and untrained (□) resting subjects.

Table 3 Two-colour analysis of IL-2R alpha and beta chain expression on lymphocyte subsets in untrained and trained subjects[a].

Lymphocyte subsets	IL-2Rα+ (CD25)		IL-2Rβ+ (p70-Mik-β1)		IL-2Rβ+ (p75-TU27)	
	% of each lymphocyte subset ± S.D.					
	Untrained	Trained	Untrained	Trained	Untrained	Trained
CD4	45.7 ± 2.2	54.1 ± 9.4*	1.1 ± 0.9	2.1 ± 0.6	< 1.0	1.9 ± 0.2
$CD8^{bright}$	11.5 ± 5.2	14.9 ± 6.3	< 1.0	< 1.0	< 1.0	< 1.0
$CD8^{dim}$	< 1.0	< 1.0	19.6 ± 4.4	37.3 ± 5.3**	14.9 ± 6.3	34.2 ± 8.2**
CD16	8.2 ± 8.9	10.9 ± 8.5	81.5 ± 6.3	90.2 ± 8.8**	79.6 ± 1.1	87.6 ± 9.0**
CD56	11.6 ± 6.5	12.3 ± 7.6	68.2 ± 1.5	86.3 ± 8.2**	65.1 ± 9.2	82.5 ± 6.7**

[a] Untrained subjects (n = 6); trained subjects (n = 7)
* $p < 0.05$, **$p < 0.01$ (comparison between the mean values of trained and untrained subjects)

Expression of IL-2Rβ on T lymphocytes and NK cells

Not suprisingly, the major lymphocyte subsets we identified as co-expressing the β receptor were of the NK cell lineage. Indeed, a strong association was observed between both markers for the IL-2Rβ chain and NK cell subsets (Fig. **3 A–D**). However, it was intriguing that trained subjects showed significantly more expression of IL-2Rβ within their NK cell populations, than did sedentary controls (Table **3**). A very pronounced peak of IL-2Rβ cells was always detectable among the circulating lymphocytes of trained subjects, as exemplified by anti-Mik-β_1 staining (Fig. **1B**). Typical contour plots of NK cells ($CD56^+$) co-stained for IL-2Rβ (Mik-β_1) are shown in Fig. **1C** and **D**; here the $CD56^{bright}$ and $CD56^{dim}$ phenotypes can be identified. Trained individuals showed significantly greater co-expression of CD56 and Mik-β_1 than untrained subjects (Table **3**) and it appears that the enhanced IL-2Rβ expression in trained subjects is because such individuals have a greater proportion of $CD56^{bright}$ NK cells (Fig. **1**, Panel **D**, Region **a**).

In addition to CD16 and CD56 cell populations, $CD8^{dim}$ cells also showed substantial β chain expression (Table **3**). Since $CD8^{dim}$ cells are a subset of NK cells (16), quantifying Mik-β_1 or TU27 expression on $CD8^{dim}$ cells provides data on another subpopulation of NK cells co-expressing the IL-2Rβ. The $CD8^{bright}$ lymphocytes (Fig. **4**, Region **a**) did not co-express the IL-2Rβ, but the $CD8^{dim}$ population (Fig. **4**, Region **b**) were almost entirely (>90%) Mik-β_1^+. Out of the

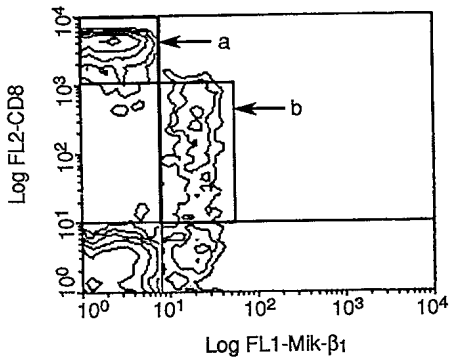

Fig. 4 Typical contour plot showing the co-expression of Mik-β_1 (IL-2Rβ chain) by CD8$^+$ lymphocytes in a trained subject. Region **a** indicates the CD8^{bright} population which is negative for the β receptor and region **b** shows the CD8^{dim} (CD8$^+$/CD16$^+$/CD3$^-$ phenotype) non-T non-B, NK cells which co-express the β chain. In this case bright cells refer to cells with fluorescence intensity greater than channel number 140 using a 4 decade log amplifier.

total population of CD8$^+$ cells, the proportion of CD8^{dim} cells co-expressing Mik-β_1 or TU27 in trained subjects was about twice that in untrained individuals (Table 3). The anti-Mik-β_1 mAb, however, provided better resolution between positive and negative supopulations than did anti-TU27.

In contrast to the expression of CD25 on their cell surface, very few (~1–2%) CD4$^+$ cells co-expessed Mik-β_1 or TU27 in either group of subjects (Table 3).

Discussion

Our results show an association between endurance training and circulating counts (percentage and number) of peripheral lymphocytes expressing markers for the p70–p75-IL-2Rβ and for NK cell subsets. In contrast, p55-IL-2Rα expression is apparently unrelated to aerobic fitness. These findings could have important practical implications particularly with respect to resistance against viral diseases and the IL-2 treatment of neoplasm.

The majority of cross-sectional comparisons between resting endurance trained athletes (TR) and sedentary controls (SC) have shown no significant difference in baseline immunocompetence as a result of regular training (2,3,11,12, 20,27). Further, because most immunologic responses to acute exercise are transient in nature (39), several authors have concluded that long-term physiologic adaptations may not occur in the immune system as a consequence of physical training (6, 40). Nevertheless, several investigators have reported an association between endurance training and a lasting enhancement of resting NK cell proportion and activity. Pedersen et al. (29) compared NK cell activity and percentages between 27 elite male cyclists and an age-matched sedentary control group. The trained group showed a greater resting NK cell activity (38% TR vs. 30% SC) and a higher median percentage of circulating CD16$^+$ NK cells (17% TR vs. 11% SC); moreover, NK cell activity was significantly correlated ($p < 0.05$, $r = 0.389$) with $\dot{V}O_2max$ ($l \cdot min^{-1}$). Similarly, Crist et al. (8) found a higher resting NK cell activity in 7 aerobically trained elderly women than in age-matched sedentary controls (38% TR vs. 29% SC). More recently, Nakachi et al. (23) reported an association between 'several good health practices' including moderate physical exercise and increased NK cell activity. Thus, there is a growing consensus that participation in regular physical activity, of moderate intensity and duration, can promote positive adaptations in natural immunity.

Comparing trained and control subjects, we found the basal NK cell count to be twice as high in trained individuals and the absolute number of granulocytes to be 32% higher in trained individuals. Furthermore, we observed a strong association between maximal aerobic power and both IL-2Rβ expression and circulating counts of NK cell subsets. In contrast, we found only an insignificant trend towards higher IL-2Rα expression in trained subjects compared with sedentary age-matched controls. This observation is in keeping with the few published reports which have compared IL-2Rα expression in physically active versus sedentary individuals (7,10,28). Taken together, these findings emphasize the diverse pattern of immune responsiveness to the modulating influences of chronic exercise. Thus, while we have demonstrated a very strong correlation between physical fitness and selected immune parameters, we cannot rule out other possible factors which may also contribute to the observed immune differences. Several soluble factors including other cytokines, prostaglandins, endorphins, and various classical neuroendocrine hormones have been suggested as possible mediators of the training-induced augmentation of natural immunity, but as yet no clear-cut mechanism exists (18,30,41).

In contrast to the above studies, two investigations have reported a reduction in resting NK cell percentages (31) and activity (53) following endurance training. The inconsistencies between studies may reflect among other things the amount of recovery time allowed following the last training session, intensity differences in training regimes, the use of animal versus human models, and/or failure to allow for circadian variations in natural immunity (13).

The cell-mediated immune response is considered an important host defense against cancer. Although limited data from epidemiological and experimental animal studies have shown inverse associations between cancer risk (36) and physical activity and between experimentally-induced tumors and exercise treatment (13), human experimental studies indicating a direct link between exercise and the modulation of natural immunity are lacking (50). Nieman et al. (24) found that the duration of upper respiratory tract episodes was significantly shortened in sedentary women who had completed a moderate exercise training programme, and they showed an associated increase (57%) in NK cell activity. MacNeil and Hoffman-Goetz et al. (14,19), noted that in mice physical conditioning increased NK cell cytotoxicity by up to 50% and that this enhancement of natural immunity was associated with a reduction in retention of experimental pulmonary tumors. We have shown that physically active individuals exhibit a phenotypically distinct pattern of NK cell surface receptor expression. Specifically, trained subjects showed enhanced levels of CD56^{bright} NK cells. Very low concentrations of IL-2 enhance the cytotoxicity and proliferation of these cells, with the release of other pleiotrophic cytokines such as IFNγ; they thus

serve as the sentries of the immune system, functioning to alert other cells of invasion of foreign material (42). It is possible that such cells could account for at least part of the augmented *in vitro* NK cell activity seen in trained subjects. However, the full physiological significance of NK cell modulation via exercise training in terms of primary tumor growth, secondary metastases, and host survival remains to be determined.

The systemic administration of recombinant human IL-2 has some therapeutic efficacy (10–20% response rate) in the treatment of patients with metastatic renal cell carcinomas and melanomas (17). However, when administered in high doses, IL-2 is accompanied by life-threatening, multi-system toxicity (43). The present study suggests that endurance training can increase IL-2Rβ expression. Presumably, it should therefore increase sensitivity to IL-2 stimulation, although *in vitro* measures of NK cytotoxicity and LAK activity will be required to confirm this assumption. Nevertheless, our findings seemingly imply that physical conditioning could be exploited as a tactic to enhance lymphocyte responsiveness to IL-2 when this cytokine is used in cancer treatment.

We conclude that a high level of physical fitness is associated with a persistent elevation of natural immunity, as measured by the proportion of circulating IL-2Rβ positive NK cells. Longitudinal studies employing a repeated-measures design may be useful to demonstrate whether a causal relationship exists between the effects of endurance training and enhancement of the IL-2/IL-2R system and to explore the possible role of other cytokines in this complex network.

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