**HIGHLIGHTED TOPIC | Exercise and Inflammation**

High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects

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Physical exercise elicits a mobilization of large numbers of T lymphocytes into the bloodstream from peripheral lymphoid compartments, but the frequency of senescent cells in the mobilized population is not known. Eight male runners (age: 29 ± 9 yr; maximal O2 uptake 62 ± 6 ml·kg−1·min−1) performed an intensive treadmill-running protocol at 80% maximal O2 uptake to volitional exhaustion. Blood lymphocytes isolated before, immediately after, and 1 h after exercise were assessed for cell surface expression of KLRG1, CD57, CD28, CD45RA, CD45RO, CD62L, and lymphocyte subset markers (CD3, CD4, CD8, CD56) by flow cytometry. The percentage of all CD3+ T lymphocytes expressing KLRG1 and CD57 increased with exercise (P < 0.01). The change in T-lymphocyte KLRG1 expression was attributed to both CD4+ and CD8 bright T cells, with the relative change being greater for the CD8 bright population (P < 0.01). Mobilized T-lymphocyte populations expressing KLRG1 and CD57 appeared to extravasate the peripheral blood compartment after 1 h of recovery. In conclusion, T lymphocytes with a senescent phenotype are mobilized and subsequently removed from the bloodstream in response to acute high-intensity exercise. This suggests that T lymphocytes contained within the peripheral lymphoid compartments that are mobilized by exercise are likely to be at a more advanced stage of biological aging and have a reduced capacity for clonal expansion than blood-resident T cells.

**IMMUNE CELL SENESCENCE OCCURS as a consequence of chromosome telomere shortening in response to repeated antigenic stimulation and/or excessive exposure to oxidative stress (5, 28).** The ability of T lymphocytes to clonally expand via cell division, mount an effective antiviral response, and form “memory” functions in response to an antigenic stimulus is fundamental to the adaptive immune response. T-lymphocyte “replicative” senescence occurs when a cell goes through excessive rounds of cell division, resulting in the progressive erosion of chromosome telomeres leading to proliferative arrest. Senescent T lymphocytes are functionally compromised, as they can no longer enter the cell division cycle and are associated with age-related dysfunctions of the immune system. Indeed, the increased risk of infectious diseases and autoimmune disorders in the elderly is believed to be the result of an accumulation of senescent T lymphocytes (5, 17).

The relationship between chronological aging and biological aging is not always synchronous. Identifying the cell surface expression of senescent T-lymphocyte markers is now considered to be useful indicators of biological aging (17, 29, 30). Lymphocytes with a memory phenotype (i.e., CD45RA−/CD45RO+/CD62L−) express inhibitory natural killer (NK) receptors capable of blocking the effector function of cytotoxic T lymphocytes (21). One such inhibitory NK receptor is the killer cell lectin-like receptor G1 (KLRG1) (29, 30). It has previously been shown that KLRG1 expression on the cell surface identifies a population of T lymphocytes unable to undergo further clonal expansion after mitogen stimulation (30), suggesting that T lymphocytes expressing KLRG1 are in a state of senescence and at an advanced stage of biological aging. The elderly have been shown to have a greater number of KLRG1 expressing T lymphocytes in blood than younger subjects (17). This is coupled with a reduced number of lymphocytes expressing the cell surface antigen CD28, an important costimulatory molecule for the activation and proliferation of naive T cells (12, 13, 17). In addition to KLRG1, the cell surface receptor CD57 has also been identified as a marker of replicative senescence on human T lymphocytes (1).

Acute bouts of aerobic exercise are known to elicit large changes in the numbers and phenotypes of blood lymphocytes. An initial increase in the blood lymphocyte count during and immediately after exercise is quickly followed by a lymphocytopenia during the recovery phase (23, 25, 26). This effect is more pronounced for the CD8+ and NK cell populations (8,
Although cell surface adhesion/activation molecules and adrenergic receptors appear to be important for the blood mobilization and extravasation of lymphocytes in response to exercise (7, 25), less is known about their functional status, history of cell division, and stage of biological aging. It has been shown previously that CD4+ and CD8+ lymphocyte subsets that do not express CD28 are mobilized into the peripheral blood compartment in response to an acute bout of exercise in both young and old subjects (2, 11). Mononuclear cells in the bloodstream after exercise have been found to have shorter chromosome telomere lengths than cells in the blood before exercise, with young and older subjects, respectively, showing a preferential mobilization of CD8+ and CD4+ lymphocytes with short telomeres (2).

The aim of this study was to examine the effects of acute high-intensity treadmill running on blood lymphocyte subsets expressing cell surface glycoproteins indicative of immune cell senescence (KLRG1+, CD28-, or CD57+) and naive (CD45RA+ or CD62L+) and memory (CD45RO+) phenotypes. A young subject cohort was chosen to establish an indication of the functional status, the history of cell division, and the biological age of lymphocyte subset populations mobilized by exercise, independently of chronological age. It was hypothesized that the exercise bout would mobilize the mobilization of lymphocyte subset populations expressing KLRG1 (a novel cell surface marker of T-lymphocyte senescence) into the peripheral blood compartment.

METHODS

Subjects. Eight healthy, aerobically trained, male club level runners volunteered to participate in this study [mean ± SD age: 29 ± 9 yr, height: 176 ± 6 cm, mass: 71 ± 7 kg, maximal O2 uptake (VO2max): 62 ± 6 ml·kg⁻¹·min⁻¹]. Each subject gave his written, informed consent, and the institution provided ethical approval. The subjects were not taking medication, had no infectious illnesses 6 wk before their participation in the study, and refrained from strenuous physical activity 48 h before each exercise testing protocol.

Experimental design. The VO2max of each subject was assessed at 0900 in the morning using a motorized treadmill (Woodway, ergo ELG 55, Weil am Rhein, Germany) and the incremental running protocol described by Simpson et al. (25). Oxygen uptake (breath by breath) was measured during the test using online gas analysis (CPX MedGraphics, Oldham, UK). One week later and at the same time of day, subjects completed an intensive treadmill running protocol at a speed corresponding to 80% of the predetermined VO2max (mean running speed: 15.8 ± 1.3 km/h). The subject was asked to maintain this pace until volitional exhaustion (mean running time: 29.6 ± 9 min). Subjects consumed water ad libitum during the treadmill test to maintain hydration status. Intravenous blood samples were collected before, immediately after exercise, and 1 h later.

Blood lymphocyte phenotype analysis. The methods used in the present study to determine total and differential leukocyte counts and isolate lymphocytes from whole blood have been described elsewhere (25). Lymphocyte surface expression of KLRG1, CD57, CD28, CD62L, CD45RA, or CD45RO on CD3+, CD4+, or CD8+ lymphocytes was determined using two-color direct immuno-fluorescence assays. Isolated lymphocytes (0.5 × 10⁶) were incubated for 45 min at room temperature with 100 µl (working dilution) of each monoclonal antibody (MAb) using appropriate combinations of one FITC or Alexa Fluor 488 conjugated MAb (CD3, KLRG1, CD57, CD28, CD45RA, or CD45RO) and one phycoerythrin-conjugated MAb (CD3, CD4, CD8, or CD56). All MAbs used were previously titrated to determine optimal conditions for analysis by flow cytometry and were purchased from Immunotools (Friesoythe, Germany), except the anti-KLRG1 (provided by Hanspeter Pircher, University of Freiburg, Germany) and anti-CD62L (R&D Systems) MAbs. Appropriate isotype controls were used in each assay to account for background binding of immunoglobulin.

After incubation, lymphocytes were resuspended in 0.5 ml PBS-BSA and analyzed on a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA) equipped with a 15-mW argon ion laser emitting light at a fixed wavelength of 488 nm. Fluorescent signals were collected in logarithmic mode (4 decade logarithmic amplifier), and cell numbers per channel in linear mode. An electronic gate was placed around the lymphocyte population in the flow cytometry forward and side scatter mode, and 10,000 gated events were acquired for analysis. FITC and Alexa Fluor 488 fluorescence were detected in the FL1 filter centered at 530 nm with a 30-nm half-peak band pass. Phycoerythrin fluorescence was detected in the FL2 filter centered at 578 nm with a 28-nm band pass. Any overlapping of the two emission spectra was adjusted using electronic color compensation. Electronic markers were placed at the limit of the negative controls to quantify the percentage of lymphocytes and lymphocyte subsets that were positive (+) and negative (−) for each cell surface antigen(s). Total numbers of lymphocytes expressing the measured cell surface antigens were determined by multiplying the percentage values obtained from the flow cytometer by the corresponding total lymphocyte count.

Statistical analysis. All results are presented as means ± SD, unless stated otherwise. Statistical analysis was conducted using SPSS for Windows version 12 (Chicago, IL). Changes across the three sampling time points were detected using one-way repeated-measures ANOVA. The assumption of sphericity for the ANOVA was tested by the Huynh-Feldt method. Where significant effects were found, pairwise t-tests with Bonferroni correction for multiple comparisons were used to detect differences among the pre- and postexercise measures. Statistical significance was accepted at P < 0.05.

RESULTS

During the analysis of CD8+ lymphocytes, two distinct populations with strikingly different fluorescent intensities were found (CD8 dim and CD8 bright). At resting levels, the CD8 bright population comprised 62 ± 2% of all CD8+ cells. When the coexpression of the T-cell marker CD3 and CD8 was assessed simultaneously, it was found that the CD8 bright population expressed CD3 (100% +), whereas the CD8 dim cells mostly lacked CD3 expression (<10% +). Therefore, only the CD8 bright cell population was analyzed to identify the expression of cell surface glycoproteins on CD8+ T cells. All CD4+ lymphocytes in the analytical gate expressed CD3.

All subjects successfully completed the intensive treadmill running protocol. The total numbers of blood lymphocyte subsets expressing the measured cell surface glycoproteins are presented in Table 1. The total number of lymphocytes, CD3+, CD4+, CD8 bright, and CD56+ lymphocytes increased immediately after the exercise protocol (P < 0.05). This was followed by a fall in the number of all lymphocyte subsets 1 h after cessation of exercise, resulting in a lymphocytopenia (P < 0.05).

The numbers of all T-lymphocyte subsets expressing KLRG1 or CD57 increased with exercise (P < 0.05) before returning to the preexercise values 1 h later. No change in the number of CD4+ or CD8 bright cells expressing CD28 occurred with exercise (P > 0.05). The numbers of all T-lymphocyte subsets expressing CD62L+, CD45RA, or CD45RO increased with exercise (P < 0.05), with the numbers of CD62L+ cells...
falling below the preexercise values 1 h later (P < 0.05). The numbers of all CD56+ cells expressing each of the measured cell surface glycoproteins increased after exercise before falling below the preexercise values 1 h later (P < 0.05), with the exception of the CD28+ and CD45RO+ subsets that did not change immediately after the exercise test (P > 0.05).

The percentages of all CD3+, CD4+, CD8 bright, and CD56+ cells expressing KLRG1 and CD57 in response to the exercise test are shown in Fig. 1. Compared with the preexercise values, a greater percentage of the CD3+ T lymphocytes in the bloodstream immediately after exercise expressed KLRG1 (P < 0.001) and CD57. The increase in KLRG1 expressing T lymphocytes after exercise was attributed to a change in both the CD4+ T-cell and the CD8 bright T-cell populations, with the relative change being greater for the CD8 bright population. This suggests that a.

**DISCUSSION**

This study examined the effects of intensive treadmill running on blood lymphocyte subset counts and analyzed cellular expression of glycoproteins indicative of immune cell senescence and naive and memory phenotypes in aerobically trained subjects. Supporting the hypothesis, it was found that, compared with the blood-resident cells, T lymphocytes mobilized into the bloodstream after exercise had greater levels of expression of the immune cell senescence markers KLRG1 and CD57. Lymphocytes with this phenotype also appear to extravasate the bloodstream within 1 h after cessation of exercise. Consistent with previous studies, it was found that KLRG1 expression was greater on CD8+ T cells and NK cells at rest compared with the CD4+ T-cell population (17, 30). The increase in KLRG1 expressing T lymphocytes after exercise was attributed to a change in both the CD4+ T-cell and the CD8 bright T-cell populations, with the relative change being greater for the CD8 bright population. This suggests that a.

Fig. 1. The percentage of all CD3+, CD4+, CD8 bright, and CD56+ blood lymphocytes expressing the immune cell senescence markers killer cell lectin-like receptor G1 (KLRG1) and CD57 on the cell surface, before and after an intensive treadmill running protocol to volitional exhaustion. Values are means ± SE. Statistically significant differences from the preexercise value (•P < 0.05, **P < 0.01).

J Appl Physiol • VOL 103 • JULY 2007 • www.jap.org
large proportion of T lymphocytes that enter the bloodstream from the peripheral tissues in response to exercise are incapable of cellular proliferation. Indeed, the proliferative response of blood lymphocytes is known to be reduced immediately after acute exercise when a fixed number of cells are stimulated with mitogen in vitro (9, 14, 15). Although this reduced mitogenic response appears to be influenced by a greater postexercise proportion of unresponsive cells (i.e., NK cells) in culture (9), other studies that have made numerical adjustments for the number of T cells in the sample have still found a reduction in lymphocyte proliferation after high-intensity exercise (14, 15). It is possible, therefore, that these observations might be explained by a greater proportion of KLRG1\(^+\) and CD57\(^+\) cells among the total T-cell population in the postexercise sample. Furthermore, cells lacking expression of the costimulatory molecule CD28 are known to have a reduced proliferative capacity (16), and, as shown in the present study, the proportion of T cells expressing CD28 was reduced after exercise.

The increased number of senescent T cells in the blood compartment after exercise could be due to a redistribution of previously activated lymphocytes that are at an advanced state of biological aging. It is also likely that the T cells expressing KLRG1 and CD57 (but lacking CD28 expression) that enter the bloodstream after exercise have shortened telomeres and a long history of cell division. In support of this, Bruunsgaard

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**Fig. 2.** Representative flow cytometry dot plots showing the expression of KLRG1 on CD3\(^+\), CD4\(^+\), CD8 bright, and CD56\(^+\) lymphocyte subsets in response to an acute bout of intensive exercise. PE, phycoerythrin. Values are reported as the percentage of each lymphocyte subset population expressing KLRG1.
et al. (2) previously showed in a group of young subjects that acute cycling exercise resulted in a blood-borne recruitment of CD8+, but not CD4+, lymphocytes with shortened telomeres. The increase in CD8+ cells with shorter telomeres occurred concomitantly with an increase in CD28− cells within the CD8+ population (2). It is not known, however, if this can be attributed to a population of CD8+ T cells, as Bruunsgaard et al. (2) analyzed mean telomere length in all blood CD8+ cells and not the isolated CD3+/CD8− T-cell population. Many CD8+ cells in the blood compartment do not express CD3 (about one-third of all CD8+ cells) and are considered to be a population of immature NK cells (8).

Both the origin and destination of blood lymphocytes mobilized in response to exercise remain elusive. As many of the T lymphocytes that entered the bloodstream after exercise had a senescent phenotype, it is likely that these are a mature T-cell population with a long history of cell replication. It is, therefore, unlikely that these cells were mobilized from the primary lymphoid organs such as the thymus. Rather, these lymphocyte populations may enter the peripheral circulation from the lymphoid organs such as the thymus, the spleen, Peyer’s patches, or other tertiary lymphoid organs. It is unlikely that these cells were mobilized from the primary lymphoid organs where maturation can occur (24). In the circulation and compartmentalization of lymphocytes between the blood and lymph nodes (3, 4). Immature lymphocytes expressing CD45RA+ (naive) and CD45RO+ (memory) T-cell subsets were mobilized after exercise, suggesting that many organs might be involved in the exercise-induced mobilization of lymphocytes into the peripheral blood.

Within 1 h after the exercise test, the mobilized lymphocytes with a senescent phenotype appeared to extravasate the peripheral blood compartment. In addition to its role in cell migration via tethering to endothelial ligands and rolling adhesion, CD62L acts as a cell surface homing receptor for the recirculation and compartmentalization of lymphocytes between the blood and lymph nodes (3, 4). Immature lymphocytes expressing CD62L are known to preferentially migrate to the secondary lymphoid organs where maturation can occur (24). In the present study, many of the lymphocyte populations that left the peripheral blood compartment during the recovery phase of exercise did not express CD62L or CD45RA. It could be postulated that these cells are destined for tissues where mature lymphocytes are known to sequester, such as the skin, the gut, the lungs, and/or the mucosal tissue (6, 19, 20, 22).

It has been reported recently that KLRG1 ligates with E- N-, and R-cadherins, which are ubiquitously expressed in various tissues, to inhibit NK cell cytotoxicity (10). Tumor cells tend to have a downregulation of E-cadherin (27), which is believed to leave them more prone to killing by NK cells expressing KLRG1 (10). Conversely, NK cells lacking expression of this inhibitory receptor could lead to nonspecific killing of viable cells, which could have important implications for athlete immune health, if KLRG1 expression is altered in response to exercise. Despite increased numbers of NK cells expressing KLRG1 after exercise, there was no change in the percentage of all NK cells expressing KLRG1. It is unlikely, therefore, that NK cell KLRG1 expression is being altered at the cellular level in response to exercise.

In conclusion, it was shown in this study that a population of T lymphocytes expressing KLRG1 (a novel cell surface marker of T-lymphocyte senescence) is mobilized and subsequently removed from the bloodstream in response to an acute bout of high-intensity exercise. As a result, T-lymphocyte populations contained within the peripheral lymphoid compartments that are responsible for the exercise-induced increase in blood T-cell population are likely to be at a more advanced stage of biological aging and have a reduced capacity for clonal expansion than blood-resident T cells. Future research should attempt to characterize the effects of regular intensive and moderate exercise training on the prevalence of senescent T lymphocytes in both athletic populations and the general exercising public.

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