Project Title: The Effect of High-Intensity Training on White Blood Cells, with a Special Focus on Anti-Tumoral Functions

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Research Plan

Background and Research Question

Epidemiological studies show that regular physical activity reduces the risk of developing several types of cancer (2,7). Furthermore, it has been suggested that physical activity can prevent tumor growth, as multiple studies indicate a reduced risk of recurrence in physically active cancer patients (1). These studies are observational and do not explain how physical activity reduces the risk of developing cancer. One proposed mechanism is the impact of physical activity on the immune system (3). Physical activity mobilizes leukocytes into the bloodstream and changes the levels of hormones and various metabolites in the blood, such as catecholamines, lactate, and intermediates in the TCA cycle (13,14). The latter is interesting because metabolism and metabolites in the local environment are directly linked to the activation and development of leukocytes (8,9).

The immune system inhibits cancer through continuous immunological surveillance, where different cells in the immune system identify and destroy malignant cells. High levels of CD8 T-cells, CD45RO+ T-memory cells, and NK cells in a tumor are linked to better prognoses for several cancers, while high levels of regulatory T-cells and myeloid cells are associated with tumor progression (4,12). CD8 T-cells prevent tumor growth by binding tumor-associated antigens, becoming activated effector cells, and eliminating cancer cells with their cytotoxic granules. The principal investigator recently published a study on a breast cancer model in mice, where exercising mice had slower cancer growth and lived longer than non-exercising mice (11). This effect was mediated by CD8 T-cells, which were activated and gained greater cytotoxic capacity from metabolites secreted into the bloodstream and secondary lymphoid organs during physical activity. Furthermore, lactate injections inhibited tumor growth in the mice, and lactate increased CD8 T-cell expansion in vitro. This is highly interesting because intense exercise can increase blood lactate levels more than tenfold from resting values (5). Thus, this study presents a potential mechanism by which physical activity can prevent tumor growth by potentiating the anti-tumor effects of CD8 T-cells and suggests a role for lactate in this process. Metabolites influence T-cell activation (10), and metabolic changes in activated T-cells are linked to epigenetic modifications such as histone and DNA acetylation and methylation (6). Whether epigenetic modifications also occur in T-cells after physical activity is crucial to study, as several metabolites are elevated both locally within tumors and in working muscles and blood after a workout.

The overarching question for this project is whether physical activity can alter the anti-tumor properties of T-cells. The goal is to study the functional effects of different types of exercise on T-cells, focusing on metabolism, epigenetic modification, activation, and functions related to cytotoxic properties.

Method

To describe how the immune system, specifically T-cells, reacts to exercise, we use high-intensity interval training: a high-intensity session consisting of three 30-second maximal cycling efforts with resistance equivalent to 7.5% of body weight. Training sessions and venous blood sampling, including blood lactate measurement, will be conducted in an existing research facility at the Division of Clinical Physiology.

Flow Cytometry: Immune cells will be analyzed using a flow cytometer at CIM, KI. Different antibody panels will be used to characterize the phenotype of leukocytes, particularly CD8 T-

cells, by using functional markers such as CD45RA, PD-1, CCR7, CTLA-4, Granzyme-B, CD62L, and ICOS.

Cell Culture: PBMCs will be isolated from blood using CPT tubes, and T-cells will be isolated via magnetic sorting and cultured in the presence of IL2 and CD3/CD28 Dynabeads to activate and proliferate them. To test the effect of intracellular lactate on T-cells, Rotenone (which increases lactate) or Dichloroacetate (which decreases lactate) will be added. To study the effect of extracellular lactate, sodium lactate will be added. Expansion and viability will be measured before and after each experiment, and flow cytometry will be conducted before and after culture.

Cytotoxicity: CD8 T-cells will be frozen after isolation and used within six months to examine the production of effector molecules in a 96-well plate, known as Elispot. Frozen CD8 T-cells will also be cultured with a cancer cell line to observe whether the CD8 T-cells affect the viability of cancer cells.

Cell Metabolism: Characterization of T-cell metabolism before and after physical activity will be performed using Seahorse. RNA-seq will analyze gene expression in CD8 cells after exercise. ATAC-seq will analyze accessible chromatin, which will be sequenced and mapped to genes and possible transcription factors. Samples will be sent to SciLife lab, Solna, for ATAC-seq and bioinformatic analysis.

Work Plan Including Preliminary Data

We have preliminary data showing that a high-intensity exercise session in humans leads to a threefold increase in CD8 T-cells and a twofold increase in CD4 T-cells in the blood, and blood lactate levels increase from about 1 mmol/L at rest to almost 20 mmol/L after high-intensity exercise (n=3). Preliminary data from SeaHorse show that CD8 T-cells taken after physical activity reduce their glucose metabolism compared to CD8 T-cells taken before physical activity.

Study Plan: Young, healthy subjects (n=20, men and women) will perform a high-intensity exercise session consisting of three 30-second maximal cycling efforts, which constitutes significant metabolic stress and results in high blood lactate levels. Venous blood samples will be taken before, immediately after, and 60 minutes after exercise and analyzed by flow cytometry, focusing on T-cell characterization. A venous blood gas will be taken before, immediately after, 30 minutes after, and 60 minutes after exercise. T-cells will be isolated from blood and cultured in the cell lab. Cell count and viability will be measured after culture to study the effect of a preceding high-intensity exercise session on T-cell expansion. Since T-cell metabolism affects their function, Seahorse will be used to study the metabolism of CD8 T-cells isolated in connection with high-intensity exercise. Cytotoxicity will be measured by culture with cancer cell lines and by cytokine secretion upon activation. RNA-seq will be performed on CD8 T-cells isolated before and immediately after exercise.

Significance

With this project, we aim to increase understanding of the mechanism by which physical activity can inhibit tumor growth. Active CD8 T-cells and ongoing immunological surveillance have a strong connection to prognosis, and in the proposed project, we want to study how different exercise modalities affect the number and function of circulating T-cells. These different modalities vary in duration and intensity, involving different stress levels, such as varying

catecholamine levels and exercise-induced metabolites like lactate. The strength of this project lies in studying cytotoxic T-cells within the same population, exposed to different types of exercise, and using relevant methods to examine expansion, metabolism, and expression of functional markers. By studying the effect of different exercise modalities, we hope to obtain information on what aspects of exercise are beneficial, which in turn can support the formulation of recommendations for affected individuals and the general public.

References

1. Ballard-Barbash R et al. Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. J Natl Cancer Inst. 2012 Jun 6;104(11):815-40.
2. Brown JC et al. Cancer, physical activity, and exercise. Compr Physiol. 2012 Oct;2(4):2775-809.
3. Christensen JF et al. Exercise Training in Cancer Control and Treatment. Compr Physiol. 2018 Dec 13;9(1):165-205.
4. Fridman WH et al. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012 Mar 15;12(4):298-306.
5. Goodwin ML et al. Blood lactate measurements and analysis during exercise: a guide for clinicians. J Diabetes Sci Technol. 2007 Jul;1(4):558-69.
6. Lu C and Thompson CB. Metabolic regulation of epigenetics. Cell Metab. 2012 Jul 3;16(1):9-17.
7. Moore SC et al. Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million Adults. JAMA Intern Med. 2016 Jun 1;176(6):816-25.
8. O'Neill LA et al. A guide to immunometabolism for immunologists. Nat Rev Immunol. 2016 Sep;16(9):553-65.
9. Pearce EL et al. Fueling immunity: insights into metabolism and lymphocyte function. Science. 2013 Oct 11; 342(6155):1242454.
10. Ramsay G and Cantrell D. Environmental and metabolic sensors that control T cell biology. Front Immunol. 2015 Mar 17;6:99.
11. Rundqvist H et al. Cytotoxic T-cells mediate exercise-induced reductions in tumor growth. Elife. 2020 Oct 23;9
12. Savas P et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat Rev Clin Oncol. 2016 Apr;13(4):228-41.
13. Schranner D et al. Metabolite Concentration Changes in Humans After a Bout of Exercise: a Systematic Review of Exercise Metabolomics Studies. Sports Med Open. 2020 Feb 10;6(1):11.
14. Strömberg A et al. Exercise-induced upregulation of endothelial adhesion molecules in human skeletal muscle and number of circulating cells with remodeling properties. J Appl Physiol (1985). 2017 May 1;122(5):1145-1154.