Alteration in blood leukocyte profile due to exercise and its implication

Ryoichi Nagatomi

*Division of Biomedical Engineering for Health & Welfare, Tohoku University Graduate School of Biomedical Engineering,
2-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8575, Japan*

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Abstract Evaluation of peripheral blood leukocytes has been considered a popular window to the immune system and its response. The numeration of leukocytes has long been a practical clinical marker of acute inflammation. Yet, the numbers and function of circulating leukocytes may also change during and after exercise to an extent comparable to inflammatory responses. A considerable amount of effort to elucidate the underlying mechanism of such changes in circulating leukocytes, however, has revealed quite a different picture showing a major contribution of the neuroendocrine system independent of inflammatory processes. Interestingly, a good correlation of the blood leukocyte profile with athletic performance in endurance runners was reported. It is therefore important to understand and interpret observed changes depending on the context to avoid misinterpretation, especially regarding potential immunological risks unlikely to happen from exercise. A review of the literature, therefore, suggests a minor or negligible immune perturbation of exercise.

Keywords : exercise, inflammation, NK cell, leukocyte

Introduction

White blood cell (WBC) count is one of the most popular and practical clinical markers of inflammation. Upon inflammation commonly elicited by various types of bacterial infection, local production of myeloid growth factors such as granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) induces proliferation and differentiation of bone marrow myeloid progenitors. This, in turn, gives rise to the release of differentiated granulocytes mainly composed of neutrophils into circulation, resulting in an increase of WBCs, namely granulocytosis or leukocytosis¹⁾. While leukocytosis refers to an increase in any subset of WBCs, granulocytosis is a term referring to increases in the limited subsets of leukocytes including neutrophils, eosinophils and basophils, among which neutrophils outnumber the others. Inflammation due to infection does not always result in leukocytosis. Viral infection commonly induces cellular interferon (IFN) beta production, which limits the proliferation of bone marrow progenitor cells resulting in leukocytopenia, a decrease in the number of WBCs in circulation. Sepsis is commonly associated with leucopenia, possibly due to systemic inflammatory reaction syndrome (SIRS) in which massive production of chemokines removes leukocytes from circulation. Altogether, the critical changes in the number of WBC in circulation often reflect states of inflammation that need clinical attention.

Both increases and decreases in WBC or their subsets may also be induced by acute stress responses, among which exercise is the most common. The extent of changes may be comparable to changes elicited in pathological reactions, but exercise-induced changes in the number of circulating leukocytes often rely on physiological responses independent of inflammatory reactions. However, because the changes in WBC count are often accompanied by changes in the levels of circulating cytokines, the influence of exercise-induced changes on the immune system is often a matter of debate. The aim of this review is to get an overview of the current knowledge on exercise-induced alterations in subsets of leukocytes and to discuss the significance of such alterations.

Non-inflammatory mechanisms responsible for exercise-induced alterations of leukocytes

Exposure to stressors including acute bouts of exercise activates the hypothalamic-pituitary-adrenocortical (HPA) axis resulting in the production of glucocorticoids. Additional elevation of plasma glucocorticoid concentration that exceeds the diurnal variation of glucocorticoids may alter the distribution of leukocytes. Nakagawa et al. showed in an experimental study using rabbits that the administration of synthetic glucocorticoid dexamethasone mobilized granulocytes in the non-circulating marginated pool in bone marrow and lengthened their half-life in circulation from 4.95 hours to 9.45 hours²⁾. Shinkai et al. showed glucocorticoid responses to 60% $\dot{V}O_2$ max exercise of 1 hour varied among healthy young human

volunteers, and reported a marked difference in the blood leukocyte kinetics post-exercise³⁾. In those who exhibited an increase in plasma cortisol, significant lymphopenia lasted for several hours post-exercise, most prominent in CD4⁺ T cells. In non-responders of cortisol, no lymphopenia was observed. One of the mechanisms responsible for exercise-induced lymphopenia in cortisol responders may be the up-regulation of CXCR4 on CD4⁺ T lymphocytes by cortisol. CXCR4 up-regulated CD4⁺ T cells rapidly mobilize to tissues expressing high levels of CXCR4 ligand CXCL12/SDF-1, such as lymph nodes, spleen and bone marrow. Okutsu et al. demonstrated that short incubation of human T lymphocytes *in vitro*, with post exercise plasma with an elevated dose of cortisol, dependently up-regulated the expression of CXCR4⁴⁾. CXCR4 up-regulated CD4⁺ T cells could not be recovered from post-exercise blood samples; but it was likely that the up-regulated cells had already migrated to CXCL12/SDF-1 rich tissues.

Catecholamines play a significant role in exercise. Functional hyperemia of skeletal muscles is mediated by beta-adrenergic stimuli⁵⁾. Prolonged exercise involves lipolysis mediated by catecholamines^{6,7)}. Catecholamines, upon binding to beta-adrenergic receptors on leukocytes, suppress the function of adhesion molecules on granulocytes, lymphocytes and natural killer (NK) cells without affecting the expression levels of adhesion molecules⁸⁾. Cat-

echolamines, therefore, induce increases in all subsets of leukocytes during exercise releasing leukocytes from the marginal pools into circulation. In addition, lipopolysaccharide (LPS)-induced granulocytosis was demonstrated to be blocked by an alpha-adrenergic antagonist⁹⁾.

To conclude, exercise induced alterations in circulating WBC and its subset counts are therefore a result of the physiological re-distribution of circulating cells across different compartments of the body in response to activation of the HPA axis and sympathetic nervous system independent of inflammatory reactions. It must be noted, however, that even these non-inflammatory responses may affect inflammatory processes through modification of adhesion molecules or chemokine receptors.

Clinical relevance of exercise-induced alterations of leukocytes

Exercise-induced alterations of leukocytes do not rely on pathological responses, such as inflammation, and depend upon activation of the neuroendocrine system. There is a potential cross-talk between the neuroendocrine system and immune system. One of such cross-talks may be the regulation of chemokine receptor expression by glucocorticoids. Whether such cross-talks are clinically relevant or not is still a matter of debate. "Immunosuppression after vigorous or prolonged exercise" refers to

Fig. 1 Inflammatory and non-inflammatory mechanisms responsible for leukocyte mobilization

B: B lymphocyte, DC: dendritic cell, Mφ: monocyte, Nφ: neutrophil (granulocyte), NK: natural killer cell, γδ: gamma delta T cell, LN: lymphnodes, SN: sympathetic nervous system

blunted responses or dysregulated responses of measurable cytokines or *ex vivo* cellular responses, which often are surrogate measures of immune responses. In the latter half of this review, the clinical relevance of non-inflammatory alterations in blood leukocytes is looked at.

The above-mentioned chemokine receptor CXCR4 has a significant role in determining the distribution of most of the circulating leukocytes. In bone marrow, granulocyte progenitors express a high level of CXCR4, but during differentiation, terminally matured cells lose CXCR4 on the cell surface. Because of this mechanism, only myeloid progenitors respond to CXCL12 expressed by stromal cells, and therefore are retained in the bone marrow. Therefore, down-regulation of CXCR4 during maturation of hematopoietic cells leads to the release of mature cells into circulation^{10,11}.

A hereditary disease with heterozygous mutations of CXCR4, the WHIM syndrome is characterized by papillomavirus infection resulting in warts, hypo- γ -globulinemia, recurrent respiratory infections, and moderate/severe granulocytopenia¹²⁻¹⁴. Because granulocytes don't lose mutated CXCR4 even after maturation, they are retained in the bone marrow without being released in the circulation resulting in granulocytopenia. IgG production is significantly limited because B cells have less chance to encounter antigen presenting cells retained *in situ*. The cause of non-fatal recurrent infections in WHIM patients is considered to be a result of granulocytopenia and of hypo- γ -globulinemia¹⁵. In contrast to other inherited diseases characterized by granulocytopenia, patients with WHIM do not usually experience life-threatening infections, as inflammatory reactions induce rapid neutrophil mobilization from bone marrow and increase the granulocyte count in circulation¹⁶. Exercise-induced glucocorticoid does not induce granulocytopenia, because terminally differentiated granulocytes do not express CXCR4 even after exposure to glucocorticoids. The only affected cell types in response to glucocorticoids are T cells, but no T cell-associated immune dysregulation has been reported in WHIM patients.

Exercise is associated with remarkable alterations in the number of circulating Natural killer (NK) cells. Blood NK cell count largely increases during and after strenuous or prolonged exercise¹⁷⁻²¹. A significant decrease in NK cells, following such exercise and lasting for hours, is considered as a hypothetical "open window", a state vulnerable to infectious agents and pathogens²².

As for the clinical relevance of circulating NK cells, Imai et al. showed in their 11-year cohort study a significant association between baseline NK cell activity and cancer onset or mortality as an endpoint²³. This is the first and the only cohort study that has demonstrated the relevance of NK cell activity so far. They isolated NK rich mononuclear cell fraction to evaluate natural killer cell anti-tumor activity *in vitro* from blood samples obtained from 3625 community-dwelling adults over the age of

40. They tracked the population for 11 years and counted the number of cases of cancer onset and death in association with baseline NK cell activity tertiles. During the 9-year observation period, excluding the initial 2 years to avoid leading time bias, they found 154 cancer deaths. There was no significant difference in cancer incidence or mortality among the highest NK cell activity tertile and the mid tertile groups. The lowest tertile group had a significantly high mortality rate even after adjustment of age and other potential confounders. It appears NK cell activity is a modifiable risk factor by, for example, exercise. Considering there was no dose-dependency in the association between NK cell activity tertiles and cancer mortality, the important message is in the relationship between low NK cell activity at baseline with higher cancer risk. NK cell activity or cell numbers that easily fluctuate upon various stimuli, including exercise and even laughter²⁴. The low NK cell activity observed in this study at the baseline, however, is most likely to be irrelevant of various NK cell up-regulating or down-regulating activity, and may possibly be related to yet unknown disadvantageous polymorphism in targeting cancer progenitor cells.

The role of NK cells is clearly demonstrated in a case report of a family with NK cell deficiency²⁵. They are susceptible to severe cytomegalovirus (CMV) or herpes simplex virus (HSV) infection, but not to seasonal flu, including influenza, or bacterial infection. This report clearly shows the relatively narrow range of defense by NK cells targeting chronically infected cells with HSV family viruses, but not others. Therefore, mirthful laughter and exercise per se may be beneficial, but not good enough to benefit susceptibility to infection of viruses or bacteria other than HSVs. These patients undergo bone marrow transplantation to gain NK activity.

Moreover, the alteration in circulating number of NK cells in response to exercise, may not be the change in total pool of NK cells. In order to test this hypothesis, we focused on the plasma concentration of granzysin, a cytotoxic granule secreted mainly by NK cells. In NK-deficient patients the blood granzysin level is almost nil. A successful bone marrow transplant results in a gradual increase in the level of plasma granzysin parallel to the increase in NK cell activity²⁶. If the size of the NK cell pool is modulated by an acute bout of exercise, the granzysin level should change. An exhaustive bike exercise with the duration of 30 min resulted in more than a six-fold increase in the number of NK cells immediately after exercise, and a decrease beyond the baseline to almost 50%; but the level of plasma granzysin stayed at the same level even after 24 hours beyond the plasma half-life of granzysin²⁷. Our results clearly suggest that the alteration in the number or activity of NK cells in response to exercise is a shift in the distribution of NK cells, which has no direct clinical relevance in association with their promised targets. Clinical relevance of the alterations in NK cells needs to be further investigated.

Conclusion

Leukocyte count changes to an extent that resembles clinically relevant immune responses may be elicited mainly by the activation of the HPA axis and sympathetic nervous system. To date, there is little evidence that the alteration induced by exercise, when we examine the mechanism by which the changes are elicited, has any clinical significance. We have recently demonstrated that the serial examination of blood lymphocyte/neutrophil (L/N) ratio was associated with athletic performance of long distance runners²⁸⁾. Therefore alteration in the leukocytes may reflect the status of the HPA axis and sympathetic activity and not the immune system itself. A more in-depth clinical or population-based approach is necessary to establish the clinical relevance of these changes. We need to think differently.

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