The implication of alterations in leukocyte subset counts on immune function

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Short running head: Leukocyte counts and immune function

Abstract

Changes elicited by physical exercise in the numbers or the activity of blood T lymphocytes, NK cells and neutrophils are sometimes considered as indicators of altered immunocompetence. By comparing the pathological conditions, in which the changes in the numbers of cells resemble observations in the exercise studies, however, we notice large discrepancies in the clinical manifestations. Supplementary information regarding the differences in cytokines and mediators responsible for altered distributions helps to explain this difference and the significance of altered distribution of the cells for immunocompetence. Alterations in the numbers and activities of T lymphocytes, NK cells and neutrophils may serve as markers of sympathetic and glucocorticoid activities, which per se may influence immunocompetence not necessarily deterioration.
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Introduction

The immune system has various cellular components in different compartments of the body: blood and lymph, primary and secondary lymphoid organs, regional lymph nodes, gut associated lymphoid tissue, and most of the organs such as brain, liver, kidney, lung and skin. Among these compartments, cells in the blood compartment are the major targets of both clinical and physiological investigation because of their availability. Leukocyte and neutrophil numbers are commonly used clinical blood markers of acute inflammatory status. The number of blood leukocytes, however, is known also to alter in response to non-pathologic situations such as emotional or physical stressors including exercise. The kinetics of leukocyte fluctuation driven by both the duration and intensity of exercise are extensively documented in the review article by McCarthy (1).

Non-pathologic fluctuations of leukocytes as observed during and after exercise are often assumed to have certain significance for the individual's

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immunocompetence. These assumptions have often been made from the supposed role of each subset of leukocytes based on fundamental studies. However, such interpretations may not always be true. The aim of this article is to discuss the differences in the significance of increased or decreased blood leukocyte numbers in host defence mechanisms between pathological and physiological conditions.

A. Neutrophils

Clinical significance of neutropenia

Neutrophils are the major component of granulocytes in the blood. They differentiate in the bone marrow to highly potent bactericidal and fungicidal cells. Decreases in the number of blood neutrophils, namely, neutropenia, could be due to a variety of causes: auto-immune diseases such as systemic lupus erythematodes, hereditary diseases such as Chediak Higashi syndrome or glycogen storage disease type 1b, or non-hereditary drug toxicity (2). Various drugs, not only anti-neoplastic agents which directly affect myeloid precursor cells, may incidentally induce agranulocytosis or neutropenia because of myeloid suppression (3, 4). Various types of leukemia also cause dysregulation of myelopoiesis resulting in neutropenia.

Clinical manifestations of acute neutropenic patients depend on the underlying cause of neutropenia, but the common feature is the susceptibility to bacterial and fungal infections. Since neutropenia induced by non-anti neoplastic agents in various medical treatments is mostly specific to myeloid cells, it is in turn a good demonstration of the role of neutrophils in the host defence against bacterial and fungal infections (5). Elderly people are likely to present with decreased neutrophil count, but so-called benign idiopathic neutropenia subjects have been shown to respond properly to the granulocyte mobilization test induced by administration of hydrocortisone and adrenaline intravenously (6).

Clinical significance of neutrophilia and oxidative stress

While neutropenia is mostly caused in pathologic situations, neutrophilia is caused both in pathologic and non-pathologic situations. The major difference in the pathologic and non-pathologic neutrophilia is that the former mostly involves increased myelopoiesis induced by granulocyte colony-stimulating factor (G-CSF) (7, 8). Various factors contribute to non-pathologic neutrophilia. Neutrophilia observed during and after exercise mostly involves adrenaline and glucocorticoids (1). In contrast to neutropenia caused by myelosuppression, physiological neutrophilia per se does not lead to clinical manifestations.

Neutrophils play significant and essential roles in ischaemia -reperfusion injury of vascular endothelial cells (9). Blockade of neutrophil elastases or activation is effective in attenuating the clinical and pathological manifestations (10-13). A variety of pro-inflammatory cytokines, such as TNF-α, IL-1-β, and IL-6 are induced in ischaemia-reperfusion (I/R) injury. Pro-inflammatory cytokines promote expression of adhesion molecules such as integrins on

the vascular endothelial surface resulting in increased attachment of neutrophils (14, 15). Endothelial cells also produce IL-8 and macrophage inflammatory proteins (MIPs; CC chemokine) in responses to ischaemia, which effectively attract neutrophils and monocytes to the site of tissue injury/inflammation. Since the initial site of I/R injury is supposed to be at the endothelial surface, the responses reported are mostly common in various organs and tissues: lung (12, 16-18), liver (9, 19-21), heart (22-25), kidney (10, 11), brain (13, 26), and skeletal muscles (18, 27, 28).

Exercise and neutrophils

Exercise may involve certain oxidative damage as detected by carbonylated proteins in tissues such as skeletal muscles and lungs (29-31).

A series of pro- and anti- inflammatory cytokines is known to be induced during and after competitive endurance exercise (32). It is, however, unlikely that I/R injury, in which the neutrophil-endothelial interaction plays a significant role, causes the oxidative damage observed in such high-intensity endurance exercise unless complications such as severe dehydration or heat stroke are involved. In I/R injury, TNF-α plays a significant role in the promotion of endothelial damage (18, 20, 22, 27), but the increase in TNF-α production during or after exercise is rather modest or absent (33-36). Under inflammatory conditions, lysosomal enzymes such as elastases and myeloperoxidase are released in an explosive manner by degranulation. These enzymes also increase after prolonged endurance exercise, but the increase in the enzyme activity in the plasma was proportional to the increase in the number of neutrophils (37). The absence of amplification implies that the increase after exercise is not due to increased degranulation. Therefore, again it is not likely that neutrophil activation is involved in the oxidative stress of exercise, even when the number of neutrophils may rise several fold compared with the resting state. Claudicants with ischaemic vascular damage showed elevated plasma neutrophil elastase and up-regulated CD11b expression on neutrophils after exhaustive treadmill exercise. They also exhibited a neutrophilia, but the extent of the neutrophilia was similar to the control subjects (38). Thus, neutrophilia during and after exercise may not necessarily be related to oxidative tissue or endothelial damage due to neutrophil activation and degranulation.

B. Lymphocytes

Clinical significance of lymphopenia

Lymphopenia is caused by various pathological and physiological conditions. Viral infection is one of the frequent causes of lymphopenia. In one form of lymphopenia -inducing viral infection, human immunodeficiency syndrome virus (HIV) infects via CD4 molecules and chemokine receptor CXCR4 molecules. The host immune response targets and destroys infected CD4+ T cells, and an irreversible CD4 lymphopenia gradually develops (39-42). The manifestations of such immunodeficiency syndromes usually develop when the CD4+ T cell count drops below 200 per μL blood. Patients then usually suffer

	Pathological situation	Etiology	Immunological & clinical outcome	Non-pathological situation	Etiology	Immunological & clinical outcome
Neutrophil						
Neutropenia	drug-induced agranulocytosis	myeloid suppression	susceptible to bacterial and fungal infection			
Neutrophilia	acute infection/ inflammation	accelerated myelopoiesis (G-CSF)	–	exercise/stress	corticosteroid, catecholamines	healthy/fatigued
Lymphocyte						
Lymphopenia	HIV infection	selective CD4 destruction	susceptibility to fungal and viral infection	exercise/stress	chemokine receptor mediated	healthy/fatigued
	drug -induced (steroid)	chemokine receptor mediated	–			
Lymphocytosis	lymphopro-liferative diseases	neoplastic transformaton	disease progression			
NK cell						
NK lymphocytosis	–	genetic?	hypersesitivity to mosquito bites	exercise/stress/etc	catechola-mines	healthy/fatigued
NK deficiency	–	genetic?	recurrent HSV, CMV infection	intense exercise/stress	?	healthy/fatigued

Table Contrasting clinical outcome of pathological and non-pathological blood cell shifts

opportunistic infections such as pneumocystis carinii pneumonia, toxoplasmosis, mycobacterium infections, cytomegalovirus infection, mycosis, bacterial respiratory tract infection, hepatitis C virus infection, Kaposi's sarcoma and others. The profiles of the variety of complications in HIV positive patients suggest that the major impairment in the host defence caused by the absence of CD4+ T cells is the defective elimination of chronically infected intracellular microorganisms. Lederberger et al. (43) reported in their Swiss HIV Cohort Study with more than two thousand HIV infected subjects that the risk of opportunistic infection rose up to 2.5 fold when CD4+ T cell counts dropped below 200 per μL blood, and 5.8 fold when it dropped to below 50 per μL blood.

The decrease in circulating CD4+T cells from non-HIV causes such as common viral infections rarely leads to compromised immune functions as in HIV patients unless the host is elderly or infant, or under severe hyponutrition or dehydration. The difference in the clinical outcome is likely to be attributed to the nature of the lymphopenia. While loss of CD4+ T cells is the major cause of lymphopenia in HIV patients (39, 40, 42), lymphopenia or decreased circulating lymphocyte count of non-HIV causes is mostly due to the altered distribution of CD4+ T cells.

Exercise and lymphopenia

Circulating lymphocyte counts could be reduced by variety of factors that alter lymphocyte trafficking or distribution. Administration of G-CSF or pro-inflammatory cytokines such as IL-1 leads to a reduction in circulating lymphocyte counts (8). Exogenous glucocorticoid administration also elicits a fall in circulating lymphocyte counts (44). Endogenous glucocorticoid is suggested to be the major factor involved in transient lymphopenia after exercise, because post-exercise lymphopenia was only observed in the subjects whose plasma cortisol concentration was elevated during exercise (45). Elaborate regulation of chemokine receptor CXCR4 by endogenous cortisol was shown to be involved in post-exercise lymphopenia (46). In this study, we showed that incubation of lymphocytes with post-exercise plasma with varying concentrations of cortisol augmented CXCR4 on CD4+ T cells in a dose-dependent manner. Together with the dose-response of CXCR4 expression on CD4+ T cells to exogenous cortisol, the fluctuation of cortisol concentration in the physiological range is suggested to finely regulate the level of CXCR4 expression. The circadian fluctuation in the number of CD4+ T cells may correspond to the circadian fluctuation in the cortisol concentration due to the regulation of CXCR4 expression. Augmented expression of CXCR4 would drive T cells to tissues expressing CXCL12, the ligand for CXCR4, on endothelial cells. The lung, bone marrow, and liver are the potential candidates of the site of lymphocyte destination, because they express abundant transcripts of CXCL12 (47). The fact that administration of the CXCR4 antagonist AMD3100 to healthy subjects induces marked leukocytosis and lymphocytosis supports this assumption (48). Therefore, the major cause of glucocorticoid-induced lymphopenia is the altered distribution of lymphocytes among different compartments in the body.

Because glucocorticoids are generally accepted as immunosuppressive, such reduction in the number of lymphocytes is often considered to be an indicator of immunosuppression in general. In animal studies, however, Dhabhar et al (49, 50) showed endogenous glucocorticoid induced by acute restraint stress deploys lymphocytes to the skin, which resulted in an enhancement of the delayed type hypersensitivity (DTH) skin reaction. Interestingly, bronchial asthma patients who manifested acute exacerbation immediately after the withdrawal of steroid treatment were shown to have increased recruitment of T cells in the airways (51). Direct suppression of T cell activation by glucocorticoid administration might have prevented asthmatic symptoms during the therapy despite the accumulation of T cells in the airways. However, acute withdrawal of glucocorticoid administration might have activated the airway recruited T cells, which lead to acute asthmatic exacerbation.

In animal experiments, exercise training induced substantial thymic involution due to apoptosis of thymic cells by endogenous glucocorticoid (52). This result is likely to be interpreted as immunosuppressive. But in another study, steroid treatment only affected the naïve T cell population in the thymus without affecting peripheral memory resident T cells (53). Thymopoiesis recovered quickly after the cessation of steroid administration. Therefore, although pharmacological administration of glucocorticoid is generally immunosuppressive during the treatment, transient induction of endogenous glucocorticoid of a shorter duration as such in exercise or acute stress may not always be immunosuppressive.

It must be noted that true immunosuppression is similar to an anergy to certain pathogens. Hepatitis B virus carriers are anergy to hepatitis B virus (54). They don't have illness because their immune system neither reacts to the virus nor virus infected cells. Getting ill often means that the immune system is in action instead of suppression. Microbes are not always required to initiate an immune response. Cytokines may independently elicit a series of immune reactions. For example, interferon alpha/beta treatment for otherwise asymptomatic and apparently healthy hepatitis C patients elicits flu-like symptoms characterized by high fever, malaise and anorexia (55).

Milder decreases of unknown aetiology in circulating CD4+ T cell counts in the elderly may have certain clinical significance. Nakayama et al. (56) found that non-responders to the tuberculin PPD skin test had smaller numbers of circulating CD4+ T cells and Th1 cells compared with responders in immobile elderly subjects. They followed the subjects for 2 years and found that the odds of developing pneumonia was 2.57 fold greater in the tuberculin non-responders compared with the responders. This prospective study is a good example showing the significance of altered blood cell count in the actual host defence, no matter whether attenuated IFN-γ response is directly involved in the increased establishment of bacterial pneumonia.

The same group suggested, however, without denying the importance of immunocompetence, that older adults are at higher risk of pneumonia when their cough and swallowing reflexes are impaired (57). They found patients with basal ganglia infarction have higher incidence of pneumonia due to impairment in cough and swallowing reflexes (58). They found the impaired reflexes resulted in silent aspiration of oral contents including bacteria.

Accordingly, oral care to reduce the amount of oral bacteria was shown successfully to reduce the incidence of pneumonia (59). Amantadine or levodopa administration also improves the impaired swallowing reflex. Because both swallowing and cough reflexes are mediated by endogenous substance P (SP) released from vagal sensory nerves in the pharynx and upper airways, the addition of a low dose capsaicin to a liquid or food, which stimulates the release of SP, may help prevent aspiration pneumonia. Angiotensin-converting enzyme inhibitor decreases SP catabolism resulting in improvements in both reflexes (57, 60, 61). These results and facts clearly show the greater importance of defence mechanisms independent of the immune system.

C. Natural killer cells

Natural killer (NK) cells are a distinct subset of lymphocytes. They were one of the immune components that attracted the attention of exercise scientists, because their number and activity in the blood fluctuated greatly under the influence of exercise (62). Many have assumed that an increase in the numbers or the activity of blood NK cells is somewhat beneficial based on the experimental evidence that the mononuclear cell fraction containing NK cells lysed certain cancer cell lines or virus infected cells. But, if we carefully look at the clinical outcomes of NK deficient patients with variety of genetic defects, we realise that NK cells are not super-weapons. They only have a relatively narrow range of targets.

Target recognition of NK cells

To date, although the mechanism responsible for target recognition of NK cells is still a hot topic of research, we already have a good outline of the principles regarding which or what type of cells are targeted by NK cells. The key molecules in the recognition of target cells are both classical and non-classical major histocompatibility complex class I (MHC I) molecules on target cells and killer activating receptors such as NKG2D, and inhibiting receptors such as NKG2A on NK cells (63). Normal cells express classical MHC I molecules which are recognized by NK receptors with immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The inhibitory recognition does not trigger NK cells to attack the recognized cells. On the other hand, stressed cells that express variant MHC I molecules such as MICA and MICB bind to killer activating receptors such as NKG2D and trigger the killing sequence (64, 65).

Murine paired immunoglubulin-like receptors (PIRs) are a pair of structurally similar receptors expressed concomitantly on NK cells, B cells and macrophages. The pair recognizes specific viral structures and transduces activating or inhibitory signals. PIR-A requires homodimeric Fc receptor common γ chain, which harbours an immunoreceptor tyrosine-based activation motif (ITAMs), for its efficient cell surface expression and for the delivery of activation signaling. PIR-B contains (ITIMs) in its cytoplasmic portion and inhibits receptor-mediated activation signaling *in vitro* (66).

Roles of NK cells in the host defence system

The ability of NK cells to lyse viral- or bacterial-infected cells is apparently limited. NK cells lyse herpes simplex virus (HSV), cytomegalovirus (CMV), EB virus, and papilloma virus infected cells (67-69), but not adenovirus infected cells (68, 70). Mycobacterium infected cells are also targeted by NK cells (71).

A more practical way to look at the role of NK cells *in vivo* is to see the clinical manifestations of NK deficient patients. A common feature of immunodeficiency manifested in NK cell deficient patients is the severe course of HSV or CMV infection, both viruses known to infect immunocompromised patients (72-75). NK cells, however, do not only lyse virus-infected cells but also to initiate adaptive T cell responses through a costimulatory pathway, OX40 and OX40 ligand interaction (76, 77). Therefore, clinical pictures observed in NK deficient patients may be affected by the absence of adaptive T cell responses resulting in a wider spectrum of viral infection.

Human NK cells destroy various human cancer cells of various origins such as melanoma, sarcoma, ovarian carcinoma, colon carcinoma, or leukemic cells *in vitro* (78-82). *Ex vivo* NK sensitivity of cancer cells or cancer cell-lines does not necessarily correspond to clinical outcomes. Although NK cells of a sarcoma patient can lyse freshly isolated sarcoma cells, cancer cells *in vivo* are not eliminated (79).

Contrary to our expectations based on *in vivo* studies, tumour associated findings are not reported in NK deficient patients except a case lacking CD3-CD16+ NKH+ NK cells but not NKT cells suffering recurrent condylomata, vulvar and cervical carcinoma *in situ*, pulmonary infiltrates of unknown significance, and a hypercoagulable state (83). Recurrent viral infection as in the other types of NK deficiency is not documented in this case. It is possible that NKT cells have a specialized role in tumour protection. Interestingly, a case, who has a profound deficiency of NKT cells and NKT cell activity, is susceptible to otherwise non-pathogenic attenuated vaccine strain of varicella virus (84).

Because tumour development needs a longer time for incubation, cohort studies better illustrate the role of NK activity. Natural cytotoxic activity of peripheral-blood mononuclear cells was assessed by isotope-release assay in 3625 residents of a Japanese population mostly older than 40 years of age, between 1986 and 1990 (85). The members of the cohort underwent an 11-year follow-up survey looking at cancer incidence and death from all causes, and the association between NK activity assessed at baseline and cancer incidence found in the subsequent follow-up was analyzed. They found 154 cancer cases in the follow-up. The relative risks of cancer incidence in those with NK activity in the highest and medium tertile compared with the lowest tertile were 0.72 and 0.62 respectively for men, 0.52 and 0.56 for women. They found, however, no association of questionnaire-based life style factors including physical activities. This study is a clear demonstration of NK activity in tumour surveillance. The fact that a result of a single baseline measurement is associated with cancer risks in an 11-year period has a more striking implication. The increase and decrease in the numbers and the cytotoxic activity of NK cells of a rather transient or acute manner observed in the majority of exer-

cise or stressor studies may not have significance in tumour surveillance. Future studies will reveal the cause of the varying ability of NK cells, which may arise from the genetic polymorphisms of NK function associated molecules such as killer cell immunoglobulin-like receptors (KIRs) (86-88). Association between the polymorphism and NK function will be elucidated in future studies.

Higher NK cell activity or larger number of circulating NK cells does not seem to be always favorable. Elevated activity and numbers of blood NK cells were detected in cases of patients with dermatological problems (89-91). They manifest skin lesions such as severe hypersensitivity to mosquito bites. Immunohistochemical study of the biopsy specimens taken from the lesional skin demonstrated an infiltrate of the cells bearing the natural killer cell phenotype, indicating a role of these cells in the development of the abnormal skin reactions to mosquito bites (90). Large granular lymphocytosis, namely increased number of circulating NK cells, in these patients does not seem to reflect acute reactive immune responses, because large granular lymphocytosis and enhanced NK activity remain even after the hypersensitivity skin reaction was cured. One of the case studies demonstrated that the increased NK cell activity was due to an abnormal expansion of large granular lymphocytes (89). They showed that the enhanced NK cell activity in this patient could be a compensatory adaptation to a primary T cell defect, because blood mononuclear cells from the patient showed a remarkable decrease in T cell mitogenic response, which was not restored by depletion of the NK cell subpopulation from the mononuclear cells (89). Therefore, an excess expansion of normal NK cells or an enhancement of NK cells activity may not always be beneficial.

Exercise-induced changes in NK cells

Both the number and the activity of circulating NK cells fluctuate greatly during and after exercise as well as in response to other stressors. There are several factors involved. The role of sympathetic activity and catecholamines in the distribution of NK cells in humans is reviewed in the previous article (92). Briefly, experimental results from quadriplegic and paraplegic spinal cord injury patients, splenectomized patients and finally a noradrenaline injection study clearly show that NK cells are mainly driven from the spleen into the circulation depending on the sympathetic outflow and catecholamines. The increase in blood NK cells after electrically stimulated exercise was lower in the quadriplegic spinal injury patients, who have spinal injuries at a higher level of the spinal cord affecting the sympathetic outflow compared with the paraplegic patients (93). Because the increase in circulating spleen cells is largely attenuated in the splenectomized patients compared with the healthy subjects, the spleen is assumed to be the major source of NK cells (94). Noradrenaline administration that raised plasma concentration to that observed at 75 % of maximal oxygen uptake induced a NK cell increase comparable to that elicited by the exercise load (95). β-endorphin is also involved in the regulation of NK activity. A series of animal studies by Hori et al. (96) showed intracerebroventricular IFNα or β-endorphin stimulates hypothalamic opioid receptors resulting in analgesia and decreased NK activity in the spleen.

Because surgical ablation of sympathetic outflow prevents this decrease, it is likely that β-endorphin through splenic sympathetic activation drives NK cells from the spleen into the circulation.

NK cells attached to the vascular endothelial cells *in vitro* are known to detach with only short incubations (as short as 5 minutes) with catecholamines in a dose dependent manner (97). Addition of a selective β-2 adrenoreceptor antagonist blocked the catecholamine dependent detachment of NK cells (98). Adrenaline as compared to noradrenaline was more effective in inducing the detachment, which is in line with the higher affinity of β-2 adrenoreceptors for adrenaline. We demonstrated *in vivo* that acute restraint stress expelled intraparenchymal NK cells from murine lung via β-adrenergic stimulation (99). The relationship between the amount of β-adrenergic receptors on the cell surface corresponds well with the circadian pattern of blood leukocytes including NK cells (100). NK cells, γδ-T cells, CD8+ T cells and neutrophils increase in the daytime and express higher amount of β-adrenergic receptors compared with CD4+ T cells and B cells.

The changes in NK activity independent of NK cell number can also be the result of altered NK cell distribution. Suzui et al. (101) showed that after high-intensity training sessions, the increase in CD56 (bright) NK cells was greater than the increase in CD56 (dim) NK cells. Since CD56 (bright) NK cells have lower cytoxicity, the overall NK cell activity appeared to be reduced. Therefore, blood NK cell activity may be a reflection of altered distribution in the different compartments of the body. The level of catecholamines was lower throughout the training session in the high-intensity group, although statistically not significant. If the density of β-adrenergic receptors expression on CD56 bright NK cells is higher than that of CD56 dim NK cells, such a difference may explain the altered distribution.

Recently "mirthful laughter" is suggested to be beneficial for the immune system because NK activity is enhanced (102-104). The enhanced blood NK activity in the results of these studies can be explained as a reflection of enhanced sympathetic activity by laughter, because Bennett et al. (103) reported a concomitant increase in circulating granulocytes with an increase in the NK cells in response to mirthful laughter. Laughter per se maybe beneficial in other ways, but possibly not through the "activation" of NK cell immunity.

Thus, experimental evidence supports the idea that NK cell distribution and activity are largely dependent upon sympathetic activity. In other words, the observed alterations of NK cell numbers and activities may reflect sympathetic activity independent of NK cell immunity. In addition, central opioid action may also be mediated by changes in sympathetic activity (96).

We have recently reported that there was no change in the serum level of granulysin, a cytotoxic protein produced and secreted by NK cells, during and after exhaustive treadmill exercise when there was a marked increase in the number of blood NK cells (105). Granulysin is a potential marker for the size of the whole-body NK cell population as suggested by the absence of granulysin in immunodeficiency patients lacking NK cells and the gradual increase in granulysin after bone marrow transplantation concomitant with the recovery of NK cells (106). Granulysin can be produced upon activation of NK cells and cytotoxic T cells in pathological conditions such as in viral infections, but are

produced constitutively mainly by NK cells in healthy humans (105, 106). Therefore, our observation supports the idea that fluctuation in the numbers of NK cells during and after exercise may reflect altered distribution of NK cells without substantial changes in the whole-body NK cell pool. Fluctuation of NK cells observed in other physical or psychological conditions may resemble the fluctuation observed in exhaustive physical exercise.

Conclusion

The fluctuations in the number of blood neutrophils, T lymphocytes, and NK cells during and after exercise are largely dependent on changes in either sympathetic activity or circulating glucocorticoids. However, the changes in the numbers of cells per se reflecting altered distribution in the body may not instantly affect the immune responses in which they become involved. The clinical or physiological significance of such changes may indirectly be involved in the host defence against microbes, but we still lack convincing epidemiological evidence. Alternatively, the changes may serve as useful measures of glucocorticoid and sympathetic activities.

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