Constitution Oriented Regulation for Granulocyte/Lymphocyte Ratio within 24 hrs by Hot-Spring Hydrotherapy

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Abstract

Background: A recent excessive commercialization are particularly confusing for patients and doctors who seek remedies for heretofore undefined symptoms.

Introduction: Two type of leukocyte subsets were identified as granulocyte and lymphocyte. Further, two sort of personalities were found granulocyte rich and lymphocyte rich type. Both types were typical for the expressing mode of action and the character itself. However, extrapolating character have to be down-regulated to rule out the each risk of failures.

Volunteer and Method: Informed consented volunteers were divided as granulocyte-rich type and lymphocyte-rich type according to the reports by Abo. Their blood were collected and counted for their effect on regal hot spring hydrotherapy, in order to adjust a circadian rhythm, with regard to both physical and mental health.

Results: The results revealed that the granulocyte-rich persons, down-regulated their granulocyte and upregulated for the lymphocytes, being a neutral type. On the contrary to this, lymphocyte–rich type individuals down regulated their lymphocyte number, contrary to up regulation of granulocyte. Both regulations were in a dose-dependent manner on the value before the hydrotherapy.

Conclusion:
1) Volunteers were divides as granulocyte-rich type and lymphocyte-rich type.
2) Both type were regulated as neutral within 24 hrs.
3) The intensity of regulation was in a dose dependent manner upon the value before the trial.
4) These regulations were confirmed even 24 hrs after the event.
5) The regulation was confirmed from young aged to older one.

Keywords: Leucocyte Subset; Granulocyte; Lymphocyte; Subset Ratio Regulation; Hot-Spring Hydrotherapy

Abbreviations

CD: Cluster of differentiation. Each lymphocyte has name that expressed CD number, for example CD2, CD4, etc; FCM: Flow Cytometry; G-rich type: The individual that exhibit over 60% of granulocyte in peripheral blood, finding many in young gentleman; L-rich type: The individual that exhibit over 40% of lymphocyte in peripheral blood, finding lot in ladies and senile

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Introduction

A human, with other complex vertebrates, and even many invertebrates have evolved a system of internal transport for communicating components of the immune system. It is the blood, within the circulatory system, that executes these tasks. The blood contains two major types of cells: erythrocytes, or red blood cells, and leukocytes, or white blood cells. Leukocytes play important roles in the immune system. There are two basic types of leukocytes: the non-granular and the granular. The non-granular (agranular) leukocytes are further divided into two types: granulocyte and lymphocytes. Lymphocytes possess antibody receptors for antigens on their surfaces and are thus vital to specific immune response throughout the entire body, where they freely move about. Monocytes are produced in the bone marrow, but like other blood cells they are eventually found in the blood. They frequently exhibit amoeboid movement, and they are voracious phagocytes when they enter connective tissues as macrophages from the blood. Both are important for quick, on the spot, phagocytosis.

Granular leukocytes include neutrophils, eosinophils and basophils. The number of neutrophils increases in infections; neutrophils are not neutral at the sidelines but provide the first line of defense against invading foreign bodies and organisms. In allergies and parasitic infestations, the number of eosinophils increases. Basophils are important for the production of histamine, the primary cause of skin reactions in allergic responses. The “most phagocytic” of white blood cells are neutrophils and monocytes. This phagocytic property is largely manifest in the connective tissue. Both cell types ingest foreign particles, bacteria and degenerating cells and fragments, and are thus crucial to the body’s non-specific immune reactions. Such responses involve rapid elimination of foreign material and antibody is not necessarily involved. This is one feature of man’s immune system that is traceable in evolution to single-cell amoebae.

The immune system shares with the nervous system at least two characteristics. The young individual is born with a certain potential to learn and to react to numerous and varied environmental stimuli both systems can learn. Once information is learned by the immune and nervous systems, it becomes in a sense imprinted, and each system retains the information in varying degrees, a process defined as immunological memory. Despite the intense learning that young systems must do subsequent to birth and will continue to do throughout their life time, infants are born into the world with certain innate behavior patterns controlled by the nervous system, and certain innate or characteristic natural immunities. These immunological categories had been defined best by digital assessment of the constitution [1,2] which had been recognized by analog expression, face scale.

However, the immune system are not the same in an individual level. Abo reported that at least two type of individuals were found in a sense, leukocyte subset ratio. As illustrated in figure 1, granulocyte-rich type and lymphocyte-rich type individuals were identified in the different sexuality, even in same aged persons and the age difference in the same individual [3-5].

Figure 1

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Each type expressed merit and demerit, knowing to the situation. The purpose of this report was a trial to neutralize each demerit for regulating the inotropic style of leukocyte ratio.

Subjects and Methods

Subjects

32 healthy volunteers (Mean age 19.5 ± 10.2 years, 9 ~ 45 years for both sexes) were recruited and concurred with the Kanazawa Medical University Ethics Committee. Gender was 45.3% for women and 54.7% for men. They were pre-graduates of medical schools, medical faculty staff and their descendants. The attendant was divided into 2 groups, and each group was started with the agreement. Blood samples were prepared at the same time period as the initial sampling. 14 healthy volunteers (Age: 19.5 ± 10.2 years) participated in each report. For the adjustment of the circadian rhythm of leukocytes and affective hormones [5], peripheral blood was collected from the forearm vein at the same time period before and after hot spring hydrotherapy. Estimation of total leukocyte cell numbers were performed twice in peripheral blood at the same time period, and the circadian rhythm was adjusted. The laboratory of the Ishikawa Prefect, Preventive medicine association was asked for the accurate and reliable evaluation recognized. Total leukocyte count and leukocyte differential count were measured with an automated hematology analyzer, XE-2100 (Sysmex Kobe Co., Ltd. Japan).

Analysis of the β2-AR positive lymphocyte subsets by FCM

Blood was collected from the subject in a blood collection tube containing the anticoagulant EDTA -2 K, and the anti-β2-AR antibody (Santa Cruz Biotechnology, Inc., USA.) of the primary antibody was added to 100 μl of whole blood, which was reacted at 4°C. for 30 minutes, and in order to estimate CD + cells, blood was collected in a blood collection tube containing the anticoagulant EDTA -2 K, and the antibodies corresponding to 100 μl of whole blood were mixed. To estimate CD + cells, whole blood was collected from the attendant by blood collection tube containing the anticoagulant EDTA -2 K and 100 μl of whole blood was mixed with the corresponding antibodies. After washing away excess antibodies with PBS, the suspension was mixed with phycoerythrin (PE) -conjugated streptavidin (Beckman Coulter S.A., France) and fluorescence-activated monoclonal AB: peridine chlorophyll protein-cyanine 5.5 (PerCP-Cy 5.5) + CD2, fluorescence-in isothiocyanate (FIT) + CD4, FITC + CD8, FITC + CD 16, FITC + CD 19, FITC + CD 57 (Becton Dickinson (United States)), allo-phycocyanin (Asia Pacific Regional Telecommunications) + CD8, and APC + CD 57 (Beckman Coulter). After washing with phosphate buffered saline, cell suspensions were fixed with X 10 diluted cell FIX (Becton Dickinson) and analyzed by the flow cytometer system FACS Caliber (Becton Dickinson). Negative controls prepared isotype control antibodies against PE + streptavidin and CD antibodies. After incubation at 4°C for 0.5 h, these samples were hemolyzed with 10 fold diluted FACS lysates (Becton Dickinson) with large amounts of RBCs.

Statistical analysis

Statistical analysis was calculated by paired t-test and Wilcoxon signed-rank test together with a group for significance test (Before and after the test). In the examination of the correlation, Spearman’s correlation coefficient was found by the rank test. Data are expressed as mean ± mean standard error (s). A P value of < 0.05 was recorded as statistically significant. Kendall τRank correlations and bilateral p-values were also analyzed.

Results

Hot spring hydrotherapy within a short period

Identifying granulocyte-rich type or lymphocyte-rich type

32 healthy volunteers (Mean age 19.5 ± 10.2 years, 9 ~ 45 years for both sexes) were recruited and concurred with the Ethics Committee of Kanazawa Medical University. Gender was 45.3% for women and 54.7% for men. They were pre-graduates of medical schools, medical faculty staff and their descendants. The attendants were divided into 2 groups, and each group was started with the agreement. Blood samples were prepared at the same time period as the initial sampling. 16 healthy volunteers (Age: 19.5 ± 10.2 years) participated...
in each report. For the adjustment of the circadian rhythm of leukocytes and affective hormones [3-5], peripheral blood was collected from the forearm vein at the same time period before and after hot spring hydrotherapy. So as to confirm the regulatory effect for leukocyte subset in simultaneous report, we tried to check the number of leukocyte subset together with CD Positive lymphocyte in the following assay. The time schedule of test schedule was within 24 hrs in order to assess the effect on the short period, 24 hrs. The leukocytes in the peripheral blood of 16 subjects tended to be down-regulated after hot-spring hydrotherapy especially in younger individuals, on the contrary to the up-regulation in older counterparts, showing negative co-efficiency (p < 0.05) (Figure 2a and 2b). With this reproducible result were obtained in the figure, the turning point of vector was 40 years old, both female and male. We did not direct precisely how they enjoy the hydrotherapy but left participants free as they were. Summarizing from the interview, they tried to hydrotherapy 2 - 3 times for hot spring hydrotherapy and about 30 - 40 minutes per one hydrotherapy within 24 hrs. On the other hand, the older group were significantly up-regulated after hot-spring hydrotherapy (p < 0.05) [10-14]. The results indicate that the hot-spring hydrotherapy may influence the type-dependent manner. The manner of regulation was correlated with value day before for controversial vector according to each constitution.

Figure 2a: Granulocyte rich type.

Figure 2b: Lymphocyte rich type.
Type dependent regulation of leukocyte subset within 24 hrs

The attendants were recruited with informed and consented according to the Ethic Committee of Kanazawa Medical University and they were pre-graduates of medical schools, medical faculty staff and their descendants. They were divided into 2 groups, G-type and L-type, each group was started with the agreement. Blood samples were prepared at the same time period as the initial sampling. 16 healthy volunteers (Age: 19.5 ± 10.2 years) participated in each report. For the adjustment of the circadian rhythm of leukocytes and affective hormones [3-5], peripheral blood was collected from the forearm vein at the same time period before and after hot spring hydrotherapy. In order to confirm the change in leukocyte subset was systematic regulation for each constitution or not, we set up to access receptor positive cell. As shown in figure 2a and 2b, two different type of leukocyte were identified. The purpose of this report was to try to regulate each constitution base on the leukocyte subset in number and function. In figure 2a and 2b, we tried to confirm the regulation based on the constitution before trial. The lymphocyte were regulated for the number \( r = 0.671 \) rather than granulocyte \( r = 0.277 \). On the contrary to this finding, granulocyte rich type was clearly regulated by granulocyte number \( r = 0.475 \) rather than lymphocyte \( r = 0.371 \).

Dose dependent regulation of leukocyte subset within 24 hrs

The test members were pre-graduates of medical schools, medical faculty staff and their descendants. Blood samples were prepared at the same time period as the initial sampling. 16 healthy volunteers (Age: 19.5 ± 10.2 years) participated in each report. For the adjustment of the circadian rhythm of leukocytes and affective hormones [3-5], peripheral blood was collected from the forearm vein at the same time period before and after hot spring hydrotherapy. Both in figure 2a and 2b, all the issues listed in the figures were plotted before and after the hot-spring hydrotherapy. The horizontal axis was plotted the number before the test and vertical axis showed the relative value after the hydrotherapy after 24 hrs. As shown in the figure, all the functions controlled by the regulation for the normal level.

Age dependent quantitative regulation of CD+ cell within 24 hrs

About the turning point of age around hydrotherapy which were up or down regulation of the total leukocyte and subset were around 35 years old [1,7]. In this section we tried to confirm the change in leukocyte subset, CD+ cell as shown in figure 3. As shown in figure 3 age different type of CD+ cells were identified. CD+ cells were nominated from CD2, CD4, CD8, CD16, CD19 and CD56. They were counted and sorted by according to their ages From figure 3, both ages almost had the same amount of CD positive cell except for CD19 one. This result informed that antibody production might be poor in old ager. The purpose of this report was to try to regulate each constitution base on the leukocyte subset in number and function. In figure 2a and 2b, we tried to confirm the regulation based on the constitution before trial. The lymphocyte were regulated for the number \( r = 0.671 \) rather than granulocyte \( r = 0.277 \). On the contrary to this finding, granulocyte rich type was clearly regulated by granulocyte number \( r = 0.475 \) rather than lymphocyte \( r = 0.371 \).

Figure 3: The variation of lymphocyte subsets after hot spring bathing.

The vertical axis present the ratio of lymphocyte subsets percentage after hot spring bathing vs before hot spring hydrotherapy.

Qualitative aspect of regulation

Age-dependent discussion

So as to confirm the change in leukocyte subset was systematic regulation for each constitution or not, we set up to access receptor positive cell. As shown in figure 4a, two different type of constitution were identified. The purpose of this section was to try to identify each constitution based on the leukocyte subset function via CD+ cell ratio. The constitution was different in the two type of qualitative assessment as shown in figure 4a. Immune competence by both humoral and cellular immunity was good for L-type as compared to G-type. However, NK cell activity was eminent by G-type rather than L-type. CD + cells that were nominated from CD4/CD8, CD16/CD56, so as to suggest the functional condition of each groups.

Constitution dependent discussion

In order to confirm the functional change in leukocyte subset was systematic regulation for each age difference or not, we set up to access receptor positive cell. As shown in figure 4b, two different type of leukocyte were identified. The purpose of this report was to try to regulate each constitution base on the leukocyte subset in number and function. As shown in figure 4a, age different type of CD+ cells were identified. CD + cells were nominated from CD4/CD8, CD16/CD56, so as to suggest the functional condition of each groups, young and old aged individuals. They were counted and sorted by according to their ages. From figure 4b, both ages had almost the same amount of CD positive cell except for CD19 one.

Discussion

Cellular immunity is functionally related to the immune activity of cells of the immune system, particularly T-cells, whereas humoral immunity emphasizes the products of B cells, or antibodies. For cells of the immune system to recognize not-self they must bear recognition units. Such units, or receptors, are antibody, found on the surface of B-lymphocytes. The nature of T-cell receptors is a matter of much

**Figure 4a and 4b**: The variation of CD4/CD8 and CD16/56 ratio in G and L type, younger and older group after hot spring hydrotherapy.

So as to confirm the change in leukocyte subset was systematic regulation for each constitution or not, we set up to access receptor positive cell. As shown in figure 4b, two different type of constitution were identified. The purpose of this section was to try to identify each constitution base on the leukocyte subset function via CD+ cell ratio. The constitution was different in the two type of qualitative assessment as shown in figure 4b. Immune competence by both humoral and cellular immunity was good for L-type as compared to G-type. However, NK cell activity was eminent by G-type rather than L-type. CD + cells that were nominated from CD4/CD8, CD16/CD56, so as to suggest the functional condition of each groups.

debate. These surface patches of antibody are the "eyes and ears" of lymphocytes, rendering them capable of detecting antigen. After sensitive lymphocytes either select or are selected by antigen they are triggered/stimulated, to be able to reproduce their kind, leaving descendants ready for a second, faster reaction to the same antigen should it be encountered. This states in very simple terms the clonal selection theory of antibody synthesis. In other words, the fittest lymphocytes, due to the presence of receptors, will survive. It is believed that the immune system, acting as an immunologic surveillance system, evolved to police the surfaces of self cells and to readily dispose of those which maybe antigenically changed and are therefore not-self. According to this view, such cells could become cancerous and thereby would interfere with effective survival. In this way the immune system functions as the body’s guardian against internal threats; its role in external threats is clear [8-17].

As one undergoes senescence, there are major components of the entire process that reflect a general decline in immunologic vigor. When this occurs, individuals become more vulnerable to certain infections, malignancy and autoimmunity.

The mechanism of autoimmunity and its relation to immunologic imbalance is extremely interesting. Under normal circumstances individuals are tolerant to self components. If and when this tolerance breaks down, individuals may develop autoimmunity. Clearly autoimmunity occurs more frequently in patients with primary immunodeficiencies than in most individuals. Auto-immunity in immunodeficiency disease, or in individuals whose immune apparatus is intact, may be related to the entry of forbidden antigens rather than the entry of forbidden (non-self) clones of cells. Differentiated lymphocytes must first originate from some primitive source or stem cell which resides in the bone marrow (Figure 2). Further differentiation leads to the development of lymphoid stem cells that are destined for "education" in two sites. It is believed that, through migration, they enter the thymus in mammals and birds and end up as T-cells (lymphocytes) that effect cell-mediated responses [18-20]. Another population develops in situ into B-cells in mammals, or in birds they pass through the avian bursa of Fabricius to become B-cells, differentiate into plasma cells, and thus are capable of effecting humoral immune responses. Speculation in the past assigned the mammalian equivalent of the avian bursa of Fabricius to gut-associated lymphoid tissue, including the appendix and Peyer’s patches. Although not completely resolved, the generally accepted view is that the bone marrow possesses the greatest concentration of B-lymphocytes. Plasma cells which develop from stimulated B-cells are most active in antibody synthesis. In the serum, immunoglobulin levels generally correlate well with increased numbers of plasma cells in germinal centers of lymphoid nodules (lymph nodes, spleen). The thymus has no B-cells, no germinal centers, and therefore produces no antibody [21,22]. Recently, alternative and complementary medicines together with oriental and traditional medicines have attracted much attention. This new interest includes aromatherapy and herbal medications, acupuncture, moxibustion and yoga. However, these therapies have not been well defined. Some are simply based on legend or belief while others are traditionally applied but without scientific evidence. Then the accessibility of each therapy and remedy as per the need for their capacity through a scientific method especially in developed world by Western Medicine might question this [20,23-25].

Although the word Alternative and Complementary Medicine is not popular enough in Japan than US and European countries, The immune system is a totally integrative system. Professor Tomio TADA express this system as SUPER SYSTEM. It include brain, endocrine and immune system. For example, an immune system contains various cells, tissues and organs which protect organisms against potentially harmful pathogens from the external environment.

The present concept of the word immunity has proposed considerably from its original definition to the standard scale for CAM (complementary and alternative medicine).

Conclusion:

1) Volunteers were divides as granulocyte-rich type and lymphocyte-rich type.
2) Both type were regulated as neutral within 24 hrs.
3) The intensity of regulation were dose dependent manner upon the value before the trial.

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Disclosure Statement
The authors affirm that there are no conflict of interest and had no financial interest to the issue of this report.

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