Immunological analysis into KI-disorder based on traditional Japanese Oriental diagnostic system

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Abstract

Although traditional medicine is an all-around medical service, it has been pointed out that traditional medical procedures are not objective, and are independent of reductionism. The aim of this study was to determine the immune status of the patients with KI-disorder. We assessed the association between KI-disorder and the population of peripheral lymphocytes (CD3, CD19, CD4, CD8, CD16, CD56, CD158a/b:killer cells immunoglobulin-like receptors) in 22 patients with rheumatoid arthritis. The results showed a negative correlation between the score of KI-deficiency and the population of the CD16+CD158b+ cells, and also showed that patients with KI-deficiency have a tendency to have a reduced population of CD16+CD158b+ cells. The inverse relationship between these was also observed in a 3-month follow-up study. Thus, it is possible, in part, to assess the condition of KI-deficiency objectively using the population of CD16+CD158b+ cells. However, the diagnostic utility of CD158b+expressing cells is expected to be limited from the viewpoints of complexity.

Key words KI-deficiency, killer cell immunoglobulin-like receptors, complexity, Kampo diagnosis, immune status.

Introduction

The system of diagnosis and therapy in the field of Japanese Oriental Medicine is constructed based on traditional procedures, which is different from the corresponding system of Western Medicine. Therefore, it is probably most objective to examine the basic theories of Japanese Oriental Medicine such as the six stage of disease or KI (elemental energy), blood and body fluids, from clinical viewpoints. However, it has been pointed out that traditional procedures are not objective and are independent of reductionism, although traditional medicine is an all-around medical service. Therefore, it is very important to assess traditional diagnoses objectively using the methods of Western Medicine. Regarding the effects of Japanese Oriental medical treatment, it has been reported recently that a Kampo formula increases the natural killing cytolytic activity (NK activity). It is also known that KI-disorders improve in patients treated with the Kampo formula. However, KI-disorder has not been quantified in the methods of Western Medicine.

Here, we assessed the association between the population of peripheral lymphocytes and KI-disorders in order to clarify the immune status in KI-disorder.

Patients and Methods

Patients : Because we needed to analyze their immunological status, patients with rheumatoid arthritis (RA) were selected as the subjects of this study, to compensate for the effects of basic diseases, because patients

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with RA possess uniform immune status. The subjects of this study were 22 patients (m/f: 6/16; age: 52.6±18.1; lansbury activity Index: 37.6±29.5) with RA who visited Toyama Medical and Pharmaceutical University. All patients met the American College of Rheumatology 1987 revised criteria[1] for the diagnosis of RA.

Evaluation of KI-disorder : The diagnoses of KI-deficiency, KI-stasis and imbalance of KI-distribution were made according to the criteria set forth by Terasawa.[2] Table I lists the symptoms and the scoring system for the KI-deficiency. KI-stasis and imbalance of KI-distribution were also diagnosed according each diagnostic score system.[2] A total score of 30 points or higher indicates the presence of KI-deficiency, KI-stasis or imbalance of KI-distribution according to each criterion.

Reagents : Fluorescein isothiocyanate (FITC) - conjugated anti-human CD3, phycoerythrin (PE)-conjugated anti-human CD3, PE-conjugated anti-human CD19, PE-conjugated anti-human CD4, PE-conjugated anti-human CD8, FITC-conjugated anti-human CD16, PE - conjugated anti-human CD56, PE-conjugated anti-human CD158a (EB6) and PE-conjugated anti-human CD158b (GL183) were purchased from Immunotech, Marseille, France.

Cells : Peripheral blood was obtained from the patients after fasting in the early morning. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood by Lymphoprep (Nyegaard, Oslo, Norway) gradient centrifugation.[3] Each PBMC sample was incubated in a culture dish in a humidified 5% CO_2/95% air atmosphere at 37℃ for 60 minutes. After the incubation, non-adherent cells were collected. These cell suspensions were washed twice in phosphate-buffered saline (PBS).

Table I Diagnostic score of KI-deficiency

	points		points
general physical fatique	10	dull eyes, feeble voice	6
lack of will power	10	tongue faint red, edema	8
easy fatigability	10	pulse of deficiency-type	8
sleepiness during the day	8	markedly diminished abdominal tension	8
loss of appetite	4	organ ptosis	10
easily acquiring diseases	8	reduced tension of the lower abdomen	6
being easily frightened	4	tendency of diarrhea	4

A total count of more than 30 points indicates KI-deficiency.
Mild symptoms are assigned half their point value.

Cell phenotype : Surface phenotyping was carried out using a two-color immunofluorescence staining technique, with isotype-specific mouse anti-human antibody conjugated with either FITC or PE.[4] Each sample of stained cells was suspended in 0.5 ml of PBS and analyzed by flow cytometry. Lymphocyte subsets were identified by gating analysis, and fluorescence profiles were obtained for 10000 cells of each sample. Negative controls for each experiment were performed with FITC- and PE-labeled mouse immunoglobulin-G (Ig-G).

Statistical analysis : Data are expressed as mean (SD) values. All data were collected in a computer database and analyzed using the StatView-J 4.02 program (Abacus Concepts, Berkeley, CA, USA). Spearman rank correlation analysis was performed between each part of each KI-disorder and each lymphocyte subpopulation. For all statistical tests, correlations were regarded as statistically significant at $p<0.05$.

Follow-up study : Four weak, elderly patients treated with Kampo formula were followed-up by repeatedly determining the KI-deficiency score and CD158a/b molecule expression during a 3-month period. The changes of these parameters were assessed.

Results

The association between KI-disorder and lymphocyte subpopulations

The results of the evaluation of KI-stasis and imbalance of KI-distribution are shown in Tables II and III, respectively. Neither KI-stasis nor imbalance of KI-distribution was related to the CD4/CD8 ratio, or to the population of T (CD3, CD4, CD8), B (CD19) or NK (CD16, CD56) cells. There was no relationship with either KI-stasis or imbalance of KI-distribution and the expression of killer-cell immnoglobulin-like receptors (KIRs): CD158a/b, which are related to NK cell function.

Table IV shows the results of the study concerning KI-deficiency. No particular population of peripheral lymphocytes was correlated with the KI-deficiency

Table II The relationship between KI stasis score and the subpopulation of peripheral lymphocytes

KI stasis score versus	Probability	Spearman's rank correlation coefficient
CD4/CD8 ratio	0.311	0.305
CD3	0.109	0.466
CD19	0.240	0.351
CD3CD4	0.836	0.064
CD3CD8	0.630	0.148
CD3CD16	0.086	0.516
CD16 CD56	0.226	0.361
CD16CD158a	0.612	0.173
CD16CD158b	0.546	0.205

Table IV The relationship between KI-deficiency score and subpopulation of peripheral lymphocytes

KI-deficiency score versus	Probability	Spearman's rank correlation coefficient
CD4/CD8 ratio	0.790	0.082
CD3	0.265	0.350
CD19	0.591	0.173
CD3CD4	0.284	0.337
CD3CD8	0.291	0.332
CD3CD16	0.246	0.382
CD16 CD56	0.390	0.273
CD16CD158a	0.459	0.250
CD16CD158b	0.048	0.456

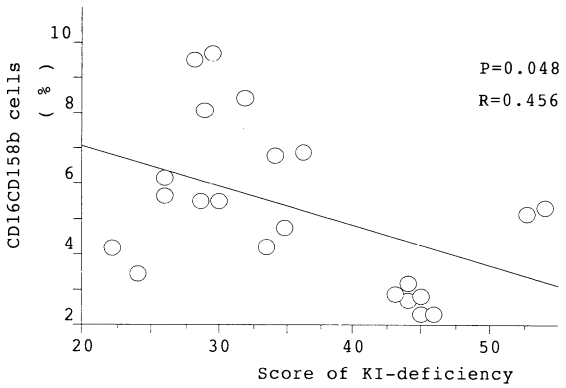

Figure 1 The relationship between the score of KI-deficiency and the population of CD16+CD158b+ cells.

score. In contrast, a significant relationship was found between the population of CD16+CD158b+ cells and the KI-deficiency score (Table IV). These two factors were negatively correlated (Figure 1).

We have further analyzed the relationship between the lymphocyte subpopulation and deficiency of blood, which is a reduction of blood volume, or stasis of body fluids, which are disturbances of all non-red fluids of an organ. However, there were no relationships between those (data not shown).

Table III The relationship between imbalance of KI score and the subpopulation of peripheral lymphocytes

Imbalance KI score versus	Probability	Spearman's rank correlation coefficient
CD4/CD8 ratio	0.872	0.050
CD3	0.485	0.213
CD19	0.758	0.095
CD3CD4	0.332	0.293
CD3CD8	0.584	0.168
CD3CD16	0.571	0.182
CD16 CD56	0.486	0.213
CD16CD158a	0.491	0.233
CD16CD158b	0.265	0.368

Table V The comparison of KIRs expression between the patients with and without KI-deficiency

	CD16CD158a cell	CD16CD158b cell %
Ki-deficiency N=14	0.403±0.425	4.734±2.279
no-Ki-deficiency N=8	1.825±1.609	6.300±2.578

Comparison between two groups: patients with or without KI-deficiency

The population of KIR-expressing cells had a tendency to be reduced in patients with KI-deficiency compared to those without KI-deficiency, although the difference was not significant (Table V).

Follow-up study

We observed a negative correlation between the KI-deficiency score and the population of CD16+CD158b+ cells.

Therefore, we performed a follow-up study on the time-course of changes in the population of CD16+ CD158b+ cells and the score of KI-deficiency in 4 patients, which had been selected in a randomized procedure. The results are shown in Figure 2. Two patients were treated with Hochu-ekki-to and other two patients were treated with Ogi-kenchu-to or Juzen-taiho-to, respectively. As KI-deficiency improved in the patients who were treated with Kampo formula, the population of CD158b expressing-cells in their peripheral lymphocytes simultaneously decreased.

Discussion

The concepts of KI, blood and body fluid are important when patients are diagnosed by Japanese Oriental

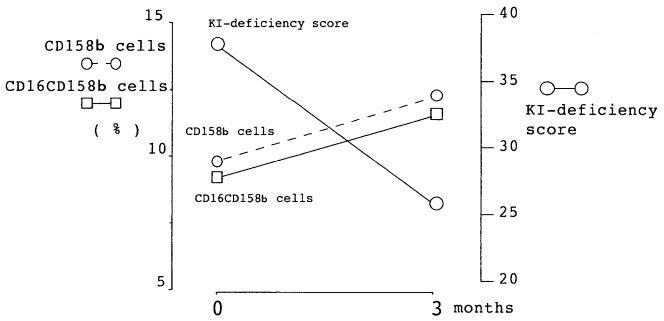

Figure 2 The average changes in score of KI-deficiency, the population of CD16+CD158b+ cells and CD158b+ cells during the follow-up period are shown. The score of KI-deficiency was decreased while the population of CD16+CD158b+ cells was increased.

Table VI Classification of studies to assess traditional concepts objectively

Subject of the study	Example
1 target group of a formulae	analysis of target group of Kakkon-to, Sho-saiko-to, Ninjin-to, *etc.*
2 traditional pathological concepts	analysis of KI-deficiency, Oketsu syndrome, Stasis of body fluids, *etc.*
3 diagnostic methods	quantative analysis of pulse diagnosis, inspection of tongue, abdominal diagnosis

*It is considered that the results from subjects no. 2 and 3 should be integrated

(Kampo) Medicine. These diagnoses have been determined using the traditional system, which is different from the system of diagnosis in Western Medicine. It is difficult for doctors in the field of Western Medicine to use Kampo treatment, and also to recognize the condition of patients from the point of view of the traditional concepts. Therefore, several investigators have attempted to objectively assess these traditional concepts. Studies aiming to assess Kampo diagnosis objectively can be categorized into 3 groups (Table VI). The first tries to determine the condition of patients to whom a Kampo formula, for example, Kakkon-to should be administered. The second attempts to analyse the traditional pathological concepts such as KI, blood or body fluid. The third one tries to quantitatively analyze traditional aspects of the patients examination such as pulse, tongue and abdomen. The present study is classified into the second group, and the majority of recent studies belong to the second or third group. The Oketsu syndrome, which is generally associated with a state of insufficient blood-circulation and blood stasis, has been especially well analyzed in this respects. Patients with Oketsu syndrome have been demonstrated to have high blood viscosity[5] and Keishi-bukuryou-gan has been shown to improve this condition.[6] Furthermore, erythrocyte deformity was observed in patients with Oketsu syndrome, and this deformity was shown to be related to erythrocyte viscoelacity.[7] Recently, the association between atherosclerosis and Oketsu syndrome has been studied.[8,9] Pharmacological effects of the diuretic action of Kampo formulae have been analyzed by several investigators.[10,11] However, the concept of KI-disorder has not been adequately studied. This is the first report of the analysis of KI-disorder using the assessment of immune status.

Alternatively, it has been reported that one kind of disease in Western Medicine is related to the condition of KI-deficiency. Egashira *et al.* demonstrated that patients with chronic pulmonary emphysema are mainly in the condition of KI-deficiency, and that their treatment with formulae with tonic effect: Ho-zai (ex: Hochu-ekki-to, Juzen-taiho-to, Ninjin-yoei-to, *etc.*), which are supplementary regimens, results in the improvement of their lung function as well as KI-deficiency.[12] It has been reported that the administration of ninjin-yoei-to expands the populations of CD8+CD11b/c+ cells and CD8+ CD11b- cells in peripheral blood lymphocytes of patients with multiple sclerosis.[13] In addition, Juzen-taiho-to increases NK activity in postoperative gastric cancer patients.[14] These findings suggest that Ho-zai might modulate the immune status in patients with the condition of KI-deficiency.

In the present study, patients with KI-deficiency were analayzed from the viewpoint of immune status. The results showed that the expression of KIRs on peripheral lymphocytes was lower in patients with KI-deficiency than in those without KI-deficiency. It is difficult to explain how the results of CD158a+ cells are different from those of CD158b+ cells in the relationship to KI-deficiency score. We consider it possible that the functions of CD158a and CD158b molecules are not always in accordance, which accounts for the discrepancy. KIRs were molecularly cloned in 1995,[15] and have been shown to transmit positive and/or negative signals.[16,17] It is now widely accepted that the expression of KIRs is involved in the cytotoxic function of NK cells, as demonstrated by a number of studies focusing on these receptors.[18] It has been considered that the upregulation of

KIRs by interleukin (IL) -2 or IL-15 results in enhanced ability to sort target cells such as virus-infected cells from uninfected cells according to major histocompatibility complex (MHC) class I expression.[19,20] Therefore, low expression of KIRs in patients with KI-deficiency might be associated with greater susceptibility to microbes. From the clinical viewpoint, it is possible, in part, to assess the condition of KI-deficiency objectively using the expression of KIRs, although we cannot fully diagnose patients thereby as having the condition of KI-deficiency from the viewpoints of complexity.[21]

In conclusion, an association between the population of peripheral lymphocytes and KI-deficiency was demonstrated, i.e. patients with KI-deficiency have a tendency to have low expression of KIRs, although the diagnostic usefulness of this expression is limited from clinical viewpoints.

和文抄録

漢方医学での病態を把握し有効方剤を選択するためには，伝統医学的手法を応用することが，現在でも最も客観的な方法であると考えられる。しかしながら，伝統医学的手法は心身一如を前提とし，きわめて全人的な指標を中心としているが故に，西洋医学的な客観性，すなわち要素還元性に乏しいと指摘されている。この研究では気の異常を臨床免疫学的手法で客観化することを目的とした。22名の関節リウマチ患者の気虚，気鬱，気逆の病態と末梢血リンパ球のサブセット（CD3, CD19, CD4, CD8, CD16, CD56, CD158a, CD158b）の関連を検討した。その結果，気虚と CD16+CD158b+cell の population が有意な負の相関を示し，気虚を伴う患者群では CD16+CD158b+cell の population が少ない傾向にあった。この逆相関の関係は4名の3ヶ月の追跡研究でも観察された。このことから活性化した NK 細胞の減少は，気虚の病態を呈した患者の一つの特徴と考えられる。このような検討から気虚など複雑な要素が関与する病態の一部を客観化して同一症例での経過観察などに応用可能と考えられる。ただし複雑性の科学の観点から診断への応用に関しては制限される必要がある。

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