

Lifestyle and T-helper 1 and 2 Related Cytokines in Healthy Volunteers

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ABSTRACT. Background: Allergic and immunological disorders are often caused by environmental substances.

Methods: To determine whether lifestyle affects the Th1/Th2 cytokine balance, a total of 318 healthy volunteers were subjected to a questionnaire for lifestyle grouping and serum analysis for levels of Th1/Th2-related cytokines. Interferon (IFN)- γ , interleukin (IL)-2, IL-4 and IL-10 were analyzed by flow-cytometry.

Results: Serum levels of the four cytokines studied were not measurable in approximately 60% of volunteers (IFN- γ 56.3%, IL-2 61.6%, IL-10 50.9%, IL-4 61.6%). Among volunteers for whom cytokine levels were measured, there were no correlations between cytokine levels and lifestyle. However, factor analysis demonstrated that a better lifestyle was strongly linked with higher levels of Th1 cytokines such as IFN- γ and IL-2.

Conclusions: These results suggest that a better lifestyle may prevent allergic diseases.

Key words ① lifestyle ② cytokine ③ Th1/2 ④ questionnaire
⑤ immunology

INTRODUCTION

It is widely acknowledged that the immune system is affected by environmental and lifestyle factors. Most allergic diseases such as bronchial asthma, atopic dermatitis and allergies to pollen and/or food are triggered and induced by numerous environmental factors^{1,2}. In addition, dysregulation of autoimmunity is thought to be caused in part by environmental and/or occupational exposure to certain substances^{3,4}. Systemic scleroderma (SSc) is known to occur in people exposed to silica and solvents^{5,6}. Rheumatoid arthritis is associated with exposure to silica, solvents and pesticides^{7,8}. T cells closely related to the onset of various allergies and autoimmune diseases are Th1 and Th2 cells. Both differentiate from naive T cells and are categorized according to the cytokines each type of cell produces. Th1 cells secrete interferon (IFN)- γ , interleukin (IL)-2 and tumor necrosis factor (TNF)- α , while Th2 cells produce IL-4, -5, -6 and -10^{9,10}, although another Th subpopulation (Th17) has been identified^{11,12}.

Lifestyle factors may also be important in determining sensitivity to foreign allergens or substances that affect self-tolerance¹³⁻¹⁶. Morimoto *et al.* reported that lifestyle and mental health status were associated with natural killer and lymphokine-activated killer cell activities²¹. Shirakawa *et al.* reported that maternal lifestyle affected immunoglobulin (Ig) levels of cord blood²². In addition, large-scale surveys of lifestyle have demonstrated that several comprehensive factors, and not a single factor such as smoking, drinking or subjective stress, played a significant role in controlling the contribution of lifestyle¹³⁻¹⁶.

In the present study, we examined the relationship between lifestyle and serum levels of Th1/2-related cytokines in healthy volunteers. Th1 dominance is found in patients with generalized autoimmune diseases such as systemic lupus erythematosus (SLE) and SSc, while Th2 dominance is present in patients with allergies and organ-specific autoimmune diseases such as Hashimoto disease and type I diabetes mellitus^{9,10}. Thus, it may be better that the Th1/2 balance is not affected in any particular way. However, good lifestyle may lead to a shift in the Th1/2 balance that tends to prevent frequent or severe illness.

MATERIALS AND METHODS

Subjects

The subjects were 318 Japanese volunteers (age 50.3 ± 9.13 ; male, 151; female, 169) working at Kawasaki Gakuen, an educational institution for medicine and welfare chartered by the Ministry of Education and the Kawasaki Medical School Hospital. The questionnaire was taken when participants visited the hospital for a general annual health examination for workers, and serum samples for the measurement of cytokines were only collected from individuals who had given informed consent. Volunteers having a pollen allergy, bronchial asthma, allergic rhinitis or atopic dermatitis were omitted from the study. This study was approved by the Institutional Committee for Ethics of the Kawasaki Medical School.

Lifestyle questionnaire

The questionnaire used to assess lifestyle consisted of the following questions: 1) Do you have breakfast every morning? 2) Do you sleep 7 to 8 hours per night? 3) Do you eat a nutritionally balanced diet? 4) Are you a non-smoker? 5) Do you play certain sports or do physical exercises at least once a week? 6) Is your

daily intake of alcohol less than 630 ml of beer or 180 ml of Sake? 7) Do you work less than 9 hours (per day)? and 8) Do you maintain moderate mental stress levels?

Originally developed by Morimoto *et al.*, the questionnaire has been used in various investigations²¹⁻²⁴ and has undergone slight modification. The questionnaire elicited simple "Yes" or "No" answers, and the number of questions yielding a "Yes" response (referred to here as the "score") was used to divide the subjects into the following three categories: Good ("Yes" answers ranged from 7 to 8), Medium (5 to 6), and Bad (0 to 4) lifestyle.

Measurement of cytokines

The levels of IFN- γ and IL-2 in serum samples obtained from volunteers were measured as the cytokines produced by Th1 cells. Levels of IL-4 and IL-10 as Th2 cytokines were also measured. The individual samples were applied to Cytometric Bead Array of Human Th1/Th2 cytokine Kit II (CBA, BD Bioscience, San Jose, CA, USA), and measurements were made using FACSCalibur flow-cytometry (BD Bioscience) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were carried out using StatView-J 5.0 software (SAS Institute Inc., Cary, NC, USA). Examinations were made of the correlation between serum levels of individual cytokines and lifestyle score and group (category "Bad" as 1, "Medium" as 2, and "Good" as 3), differences in levels of each cytokine among lifestyle scores and groups, and factor analysis using numbered lifestyle groups (as mentioned above, 1, 2 and 3) and serum levels of all four cytokines (IFN- γ , IL-2, IL-4 and IL-10). Since all volunteers needed to possess numerical values for the factor analysis, those in which one of the four cytokines was not present in the measurable range were omitted. As a consequence, the factor analysis was performed using data from 28 volunteers.

RESULTS

Results from the lifestyle questionnaire

The results for each question (number of subjects answered "yes" and percentage) were as follows: Q-1) 279 (87.7%), Q-2) 119 (37.4%), Q-3) 231 (72.6%), Q-4) 254 (79.9%), Q-5) 97 (30.5%), Q-6) 270 (84.9%), Q-7) 198 (62.3%), and Q-8) 201 (63.2%). The question that elicited the fewest "Yes" responses concerned participation in regular sports or physical exercise (97 out of 318, 30.5%). The next lowest score was obtained for the question regarding sleeping time (119/318, 37.4%). The highest score was obtained for the question concerning breakfast (279/318, 87.7%), followed by the question regarding the drinking of alcohol (270/318, 84.9%). Scores for the other four questions ranged between these values: smoking (254/318, 79.9%), nutrition (231/318, 72.6%), subjective stress (201/318, 63.2%), and working hours (198/318, 62.3%).

The number of volunteers with scores of 0 to 8 was 0, 2, 9, 33, 54, 82, 81, 40 and 17, respectively. Numbers with Bad, Medium, and Good lifestyles were 98 (30.1%), 163 (51.3%) and 57 (17.9%) out of 318 volunteers, respectively (Table 1). These results were slightly better than those of other reports using the same questionnaire¹³⁻¹⁶. The reason why the level of lifestyle in this study tended to be better may be that volunteers in this investigation pay more attention to their health because they are working in institutes of

Table 1. Assessment of the lifestyle questionnaire.

Number of questions answered "Yes"	Assessed lifestyle	Number	(%)
Score	number		
0-4	Bad	98	(30.8)
0		0	
1		2	
2		9	
3		33	
4		54	
5-6	Medium	163	(51.3)
5		82	
6		81	
7-8	Good	57	(17.9)
7		40	
8		17	

Table 2

A) The distribution of volunteers for measurable and unmeasurable serum levels of each cytokine.

Cytokine	Measurable samples (%)	Unmeasurable samples (%)
IFN- γ	139 (43.7)	179 (56.3)
IL-2	122 (38.4)	196 (61.6)
IL-4	156 (49.1)	162 (50.9)
IL-10	122 (38.4)	196 (61.6)

B) Number of volunteers whose serum levels of Th1/2 cytokines shifted to Th1 or Th2.

Th1/2 balance	Number of volunteers
Th1 dominant	144
Th2 dominant	126

The Th1/2 balance was calculated as follows:

[serum levels of IFN- γ and IL-2] minus [serum levels of IL-10 and IL4]

Th1 dominant : > 1.0, Th2 dominant : < 1.0

(48 volunteers were omitted since no cytokines were measurable)

medicine and welfare, and the age of volunteers was also slightly higher in this study than in other studies²¹⁻²⁴.

Serum levels of cytokines

As shown in Table 2-A, serum levels of the four cytokines studied were not measurable in approximately 50 to 60% of volunteers (IFN- γ 56.3%, IL-2 61.6%, IL-10 50.9%, IL-4 61.6%). The averages and standard deviations of each cytokine among volunteers in whom the target cytokine was measurable are shown in Figure 1. IFN- γ had the highest levels among the four cytokines, followed by IL-4, IL-10, and finally IL-2. There were several volunteers whose levels of IFN- γ , IL-10 and IL-4 were 2 times higher than the average (Fig. 1).

As shown in Table 2-B, calculations of cytokine balance ([serum levels of IFN- γ and IL-2] minus [serum levels of IL-10 and IL4]) revealed that 45.3% (144/318) and 39.6% (126/318) of volunteers exhibited Th1 and Th2 dominance, respectively.

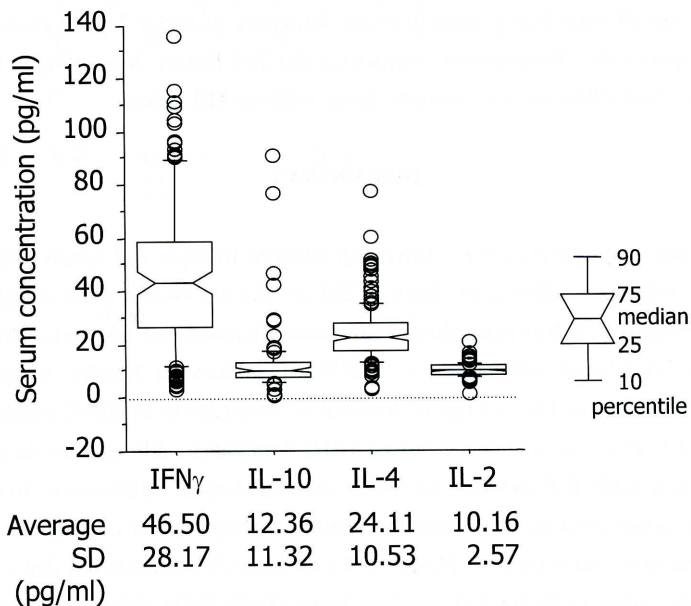


Fig. 1. Percentile graphs of measured cytokines in healthy volunteers. Values beyond the 10 to 90 percentile region are plotted. The average and standard deviation (SD) are shown below the graphs.

Table 3. Factor analysis of subjects in whom all four cytokines were measured (n = 28).

Parameter	Factor 1	Factor 2
IFN γ		.759
IL-10	.868	
IL-4	.900	
IL-2		.805
Numbered Lifestyle Group		.634
1: Bad		
2: Medium		
3: Good		
Contribution	.359	.307

Statistical analyses

There were no significant differences in individual cytokine levels among the groups (Bad, Medium and Good), scores (1 to 8), and answers (Yes or No) to each question. In addition, there were no significant positive or negative correlations between the serum levels of cytokines or the Th1/2 balance calculated with the above formula and answers, scores and groups (data not shown). The only interesting finding from the statistical analyses concerned the results of the factor analysis. As shown in Table 3, factor analysis performed using serum levels of cytokines and lifestyle group as parameters extracted two factors. The first factor (factor 1) indicated that IL-10 and IL-4 showed a strong link (0.868 and 0.900 were the individual loading values, respectively). In factor analysis, a loading value close to 1.0 indicates a strong contribution to the formation of the factor and a value greater than ± 0.4 means the parameter contributed significantly to the extraction of factors positively and negatively. The first factor is considered the Th2 factor. IFN- γ , IL-4

and the Lifestyle group all contributed strongly to the formation of factor 2, with loading values of 0.759, 0.805 and 0.634, respectively. This factor is considered the Th1 factor. Interestingly, the results of factor analysis showed that a better lifestyle was strongly linked with the Th1 factor.

DISCUSSION

In the present study, we examined the relationship between lifestyle and serum levels of Th1/2-related cytokines in healthy volunteers. However, shifts to Th1 or Th2 are known to be closely related to certain immunological abnormalities such as generalized autoimmune diseases and allergies, respectively^(9),10). Thus, there should be no significant imbalance of Th1/Th2 cytokines in healthy volunteers free of any immunological abnormalities, and no linkage of lifestyle to serum levels of Th1/2 cytokines. As expected, approximately similar numbers of volunteers showed Th1/2 dominance, although this dominance was defined by the formula shown in Table II-B and may not reflect any pathological significance. In addition, there were no differences in individual cytokine levels among lifestyle scores/groups or correlations between cytokines, Th1/2 balance and lifestyle scores/groups. However, factor analysis demonstrated that a better lifestyle was strongly and positively linked to higher Th1 cytokine levels (Table II-B), although the number of volunteers examined was relatively small ($n = 28$) because the analysis required all parameters to have numerical values and the number of volunteers in which all four cytokines were measurable was limited.

Lifestyle factors may be important in determining sensitivity to foreign allergens or substances that affect self-tolerance²¹⁻³⁰⁾. Previous reports showed that lifestyle and mental health status were associated with natural killer and lymphokine-activated killer cell activities, and that maternal lifestyle affected Ig levels of cord blood^(13),15). In addition, large-scale surveys of lifestyle have demonstrated that several comprehensive lifestyle factors, and not a single factor such as smoking, drinking or subjective stress, played a significant role in controlling the immune contribution of lifestyle⁽¹³⁾⁻¹⁶⁾.

Based on these results, it is hypothesized that (i) these cytokines are measurable when people undergo slight but perceptible immunological changes without exhibiting any significant pathological symptoms of immunological abnormality, (ii) a better lifestyle is associated with a Th1 shift in the cytokines produced, and (iii) a better lifestyle does not seem to directly affect the Th1/2 balance. Regarding the Th1 and Th2 shifts linked to generalized autoimmune disorders such as SLE and SSc and allergic diseases such as asthma, pollen/food-based allergies and atopic dermatitis, it appears that a better lifestyle, which seemed to induce a slight Th1 dominance according to the factor analysis, prevents allergic reactions.

Although the factor analysis was performed with a relatively small number of volunteers, it may support the recommendation of an improved lifestyle in order to prevent allergic diseases, the incidence of which is increasing drastically⁽¹⁷⁾. More analyses are required to determine the effects of lifestyle on the various aspects of immunological alteration.

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