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Association of Low Job Control with a Decrease in Memory (CD4+CD45RO+) T Lymphocytes in Japanese Middle-Aged Male Workers in an Electric Power Plant

Akinori NAKATA[1], Takeshi TANIGAWA[2]*, Yosei FUJIOKA[3], Fumihiko KITAMURA[1], Hiroyasu ISO[2], and Takashi SHIMAMOTO[2]

[1] National Institute of Industrial Health, 6–21–1, Nagao, Tama-ku, Kawasaki 214-8585, Japan
[2] Institute of Community Medicine, University of Tsukuba, 1–1–1, Tennoudai, Tsukuba 305-8575, Ibaraki, Japan
[3] Department of Public Health and Occupational Medicine, Graduate School of Medicine, University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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Abstract: To clarify the relationship between perceived job stress and lymphocyte subpopulations, a cross-sectional study was conducted in 231 male electric power plant workers (aged 40 to 60, mean 46 years). Job stress, i.e., job control, job demands, and social support at work, was assessed by means of the Japanese version of the Job Content Questionnaire. Blood samples were taken from all the workers, and numbers of CD4+ T lymphocyte subpopulations, total CD4+ T, T (CD3+) lymphocytes, CD16CD56+ natural killer (NK) cells, total lymphocytes, and white blood cells were determined. After controlling for age, number of cigarettes smoked per day, alcohol drinking, frequency of regular exercise, job demands, and social support at work by the partial correlation coefficients, numbers of memory (CD4+CD45RO+) T, total CD4+ T, and total T (CD3+) lymphocytes were positively correlated with job control ($p<0.05$). Neither job demands nor social support at work showed significant correlations with lymphocyte subpopulations. It is suggested that lower job control is associated with a decrease in the number of CD4+CD45RO+ T lymphocytes in male middle-aged workers.

Key words: Job control, Memory (CD4+CD45RO+) T lymphocyte, Natural killer cells, Job Content Questionnaire, Middle-aged worker

Introduction

Considerable evidence demonstrating a relationship between stress and immune function is accumulating in the past decade, and suppression of immune function is found in many of these studies[1–4]. Nowadays there is increasing interest in the relationship between job stress and the immune parameters[5–14] since the immune system is thought to link between job stress and ill health.

The relationship between job control and CD4+ T lymphocytes or its subpopulations remains controversial[5–7], although a few studies have shown that certain types and amounts of job stress reduce the numbers of T lymphocytes[5, 7] or natural killer (NK) cells[7]. Meijiman *et al.*[5], indicated an inverse relationship between job control and CD4+ T lymphocytes in male shift workers. In contrast, no significant relationship was found between job control and CD4+ T lymphocytes in male blue-collar workers[6], whereas a significant positive correlation was found between job control and subpopulation of CD4+ T lymphocytes, namely memory (CD4+CD29+) T lymphocytes. In our recent study[7], no significant association was observed between job control and CD4+ T lymphocytes or CD4+ T lymphocyte subpopulations, i.e., memory (CD4+CD45RO+) T and naive

*To whom correspondence should be addressed.

(CD4+CD45RA+) T lymphocytes in young male workers, but a significant decrease in naive (CD4+CD45RA+) T lymphocytes was observed in workers with high job strain, as defined as the combination of greater job demands and lower job control in the job-demands control model[15].

Inconsistency in the results could be explained, at least partly, by the following reasons. First, some potential and strong confounding factors such as age, smoking, alcohol drinking, and physical exercise had not always been statistically controlled[5–7]. Age has been reported to be associated with a decrease in naive (CD4+CD45R+) T lymphocytes[16]. Smoking is known as a strong factor that increases CD4+ T, CD4+CD45RO+ T, CD4+CD29+ T, and CD4+CD45RA+ T lymphocytes[17–21]; numbers of CD4+CD45RO+ T and CD4+CD29+ T lymphocytes were significantly correlated to daily cigarette consumption[17]. Moreover, alcohol drinking enhances the numbers of CD3+ T and CD4+ T lymphocytes[22]. Physical exercises such as an aerobic exercise for ten weeks[23] or short-term exercise for few minutes to few hours[24] also have an increasing effect on CD4+ T lymphocytes. Secondly, two out of three studies were done in relatively small samples[5, 6] (37 and 65 workers, respectively), which may not have been large enough to detect the effect.

The purpose of this study was therefore to know the relationship between job control, job demands, or social support at work and lymphocyte subpopulations in middle-aged male workers in an electric power plant. Perceived job stress was assessed by the Japanese version of the Job Content Questionnaire (JCQ)[25]; lifestyle factors such as smoking, alcohol drinking, and exercise were also assessed. Subpopulations of CD4+ T, total CD4+ T, T (CD3+) lymphocytes, and NK (CD16CD56+) cells were analyzed by flow cytometry.

Subjects and Methods

Subjects

A total of 231 healthy male workers aged 40 to 60 (mean 46) years underwent an interview and health check-up for this study in an electric power plant, in which approximately 861 male and 25 female workers had been employed and their age ranged from 20 to 60 years. The power plant generated and distributed electricity for domestic and industrial uses. The subjects were not exposed to hazardous chemicals that could affect immunological analysis nor had signs or symptoms of infection at the time of the study. The present study was carried out with the informed consent of all workers.

Job stress questionnaire

The Japanese version of the Job Content Questionnaire (JCQ)[25] developed by Karasek was used to assess job stress according to the job-demands control model[15, 26] and the demand-control-support model[27]. "Job control" evaluated the possibility to make decisions, to be creative on the job, and to use and develop one's abilities. "Job demands" evaluated the quantity of work, intellectual requirements, and time constraints of the job. "Social support at work" evaluated support from supervisors and colleagues in the workplace. The previous study by Kawakami *et al.*[28] showed that the Japanese version of the JCQ has acceptable levels of reliability and validity as follows: Cronbach's alpha reliability coefficients for the scales ranged from 0.61 to 0.89 for males and from 0.65 to 0.87 for females; the scales showed factor-based validity; and age and occupational distributions of the scale scores were in the expected direction.

Sociodemographic and lifestyle factors

Sociodemographic and lifestyle variables were age, smoking (number of cigarettes smoked per day), alcohol drinking (1 = no drinker, 2 = not regularly [less than once a week], 3 = regular intake of less than 69 g ethanol not every day, 4 = regular intake of 69 g or more ethanol not every day, 5 = less than 69g every day, and 6 = 69 g or more ethanol every day), and frequency of regular exercise (1 = no regular exercise, 2 = once or less a week, and 3 = more than twice in a week).

Measurement of lymphocyte subpopulations

All blood samples were obtained between 9.00 and 12.00 a.m. from subjects, and immunological analysis was conducted within 12 hours of blood collection. Ethylenediaminetetraacetic acid dipotassium (2K-EDTA) was used as an anticoagulant to collect venous blood from subjects for measurement of leukocytes counts and for immunofluorescence staining. All samples were transported and handled at a room temperature (i.e., 15–20°C).

The following sets of monoclonal antibodies were used to perform two or three-color direct immunofluorescence surface-marker analysis: anti-Leu4 (CD3-FITC) / anti-Leu11c+Leu19 (CD16CD56-PE), anti-Leu18 (CD45RA-FITC) / anti-Leu45RO (CD45RO-PE) / anti-Leu3a (CD4-PerCP), and mouse IgG1/mouse IgG2a (negative control). All monoclonal antibodies were purchased from Becton Dickinson (San Jose, California, USA).

CD4+ T lymphocytes can be divided into two different functional populations based on the expression of distinct isoforms of the surface molecule CD45. By using the

monoclonal antibodies anti-CD45RA (Leu18) and anti-CD45RO (Leu45RO), CD4+ T lymphocytes are classified into memory and naïve CD4+ T lymphocyte, respectively. Naïve (CD4+CD45RA+) T lymphocytes provide poor assistance to B cells for pokeweed mitogen (PWM)-induced immunoglobulin (Ig) synthesis, but they induce CD8+ T lymphocytes to become suppressive[29]. Conversely, memory (CD4+CD45RO+) T cells provide a good helper signal for PWM-induced Ig synthesis by B cells[30]. Both CD4+CD45RO+ T and CD4+CD29+ T lymphocytes are designated as memory cells, and CD4+CD45RA+ T lymphocytes are designated as naïve cells[31].

The protocol of the measurement of lymphocyte subpopulations has been described in detail elsewhere[7, 17]. Briefly, one hundred microliters of blood was incubated with each set of monoclonal antibodies for 15 minutes in darkness. All antibodies were used at optimal dilutions. After incubation, 2 ml of 1 × FACS Lysing Solution (Becton Dickinson, San Jose, California, USA) diluted 10 times with distilled water was added to each sample and the samples were mixed gently and incubated for 10 minutes in the dark to lyse the erythrocytes. Samples were centrifuged at 1,000 rpm for 5 minutes and the supernatant was aspirated. The pelleted cells were resuspended in 2 ml of phosphate buffered saline (PBS). After re-centrifugation, the pelleted cells were resuspended in 0.5 ml of PBS, and then subjected to immunofluorescence analysis on a flowcytometer (FACScan, Becton Dickinson, San Jose, California, USA).

We calculated the number in each lymphocyte subpopulation by multiplying lymphocyte counts by the percentage of positive cells in each category, as determined by flow cytometer.

Statistical analysis

The partial correlation coefficients controlling for age, number of cigarettes smoked per day, alcohol drinking, frequency of regular exercise, and other job stress variables were calculated to examine the relationships between job stress indicators (i.e., job control, job demands, or social support at work) and lymphocyte subpopulations. The significance level for all statistical analyses was set at a probability of less than 0.05 (two-tailed test). All data in this study were analyzed with the Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, USA).

Results

Age, job stress scores, lymphocyte subpopulations, white blood cells, number of cigarettes smoked per day, drinking habit, and frequency of regular exercise of all the workers are shown in Table 1.

Relationships between job stress scores and lymphocyte subpopulations are shown in Table 2. Job control was significantly and positively correlated with the numbers of CD4+CD45RO+ T, total CD4+ T and T (CD3+) lymphocytes, after controlling for age, number of cigarettes smoked per day, alcohol drinking, frequency of regular exercise, job demands, and social support at work by the partial correlation (Fig. 1). No significant relationship was found between job demands or social support at work and lymphocyte subpopulations (Table 2).

Discussion

In the present study we demonstrated that perceived job control is significantly and positively associated with numbers of CD4+CD45RO+ T, total CD4+ T and T (CD3+) lymphocytes in workers in an electric power plant (Table 2, Fig. 1). The findings suggest that the CD4+CD45RO+ T lymphocytes are sensitive to job control, independent of age, smoking, alcohol drinking, exercise, job demands and social support at work in middle-aged males. The decrease in CD4+CD45RO+ T lymphocytes may account for decreases in the numbers of total CD4+ T and T (CD3+) lymphocytes.

The mechanisms for the effects of job control on CD4+CD45RO+ T lymphocytes have not been fully elucidated but, psychological stress stimulates the cerebral cortex and hypothalamic-pituitary-adrenal axis, which could induce changes in the cellular immune system[1, 32]. Corticotropin-releasing hormone and arginine vasopressin released from the hypothalamus during stress lead to the secretion of adrenocorticotropic hormone from the pituitary gland and glucocorticoid from the adrenal gland. Glucocorticoid can cause non-committed type 0 T-helper cells to transform into type 2 T-helper cells and can inhibit the development of type 1 T-helper cells which is related to the cellular immune system[33–36]. A decrease in CD4+CD45RO+ T lymphocytes due to low job control might therefore be attributable to the different actions of glucocorticoid on two different types of T-helper cells.

The association between job control and memory T lymphocytes was consistent with our previous study investigating healthy male middle-aged (median age 49 years) workers[6]; job control was positively associated with the number of memory (CD4+CD29+) T lymphocytes after controlling for confounding factors such as age, cigarettes smoked per day, and blood lead concentrations. In contrast, in our recent study[7], we found no significant association

Table 1. Age, job stress scores, lymphocyte subpopulations, white blood cells, number of cigarettes smoked per day, drinking habit and frequency of regular exercise in male power plant workers (n=231)

Variable	Mean (SD, range)	N (%)
Age, years	46.3 (5.4, 40-60)	
JCQ scale scores:		
Job control	65.8 (8.6, 38–92)	
Job demands	29.6 (4.9, 12–48)	
Social support at work	23.5 (2.5, 13–31)	
Lymphocyte subpopulation (cells/mm^3):		
T (CD3+) lymphocytes	1106 (627, 165–4012)	
Total CD4+ T lymphocytes	688 (458, 95–2820)	
CD4+CD45RO+	422 (272, 69–1685)	
CD4+CD45RA+	266 (221, 27–1440)	
NK (CD16CD56+) cells	269 (192, 32–1616)	
Total lymphocytes	1587 (874, 270–5496)	
White blood cells (cells/mm^3)	6290 (1719, 2600–13700)	
Smoking:		
Number of cigarettes smoked per day	12.0 (13.9, 0–50)	
Smokers		117 (50.6)
Non-smokers		114 (49.4)
Alcohol drinking:		
69g or more ethanol everyday		9 (3.9)
Less than 69g everyday		30 (13.0)
Regular intake of 69g or more ethanol not everyday		81 (35.1)
Regular intake of less than 69g ethanol not everyday		27 (11.7)
Not regularly (less than once a week)		80 (34.6)
No drinker		4 (1.4)
Frequency of regular exercise:		
More than twice in a week		97 (42.0)
Once or less in a week		77 (33.3)
No regular exercise		57 (24.7)

Note: SD=Standard Deviation.

Table 2. Partial correlations of job stress scales and lymphocyte subpopulations controlling for age, number of cigarettes smoked per day, alcohol drinking, frequency of regular exercise and other job stress variables in middle-aged power plant workers (n=231)

Job stress scale	CD4+CD45RO+ T lymphocytes	CD4+CD45RA+ T lymphocytes	CD4+ T lymphocytes	T (CD3+) lymphocytes	NK (CD16CD56+) cells
Job control	0.19**	0.09	0.16*	0.17**	0.06
Job demands	− 0.02	0.04	0.01	0.01	− 0.03
Social support at work	− 0.09	− 0.03	− 0.07	− 0.12	− 0.05

* $p<0.05$, ** $p<0.01$.

between job control and memory (CD4+CD45RO+) T lymphocytes in young male (mean age 31 years) workers. A possible explanation for this discrepancy is that control over decision-making on the job is more important than job demands or social support at work in middle-aged workers, which consequently leads to a significant association between job control and memory T lymphocytes.

No significant association between job demands and lymphocyte subpopulations was found in the current study (Table 2). The results are in accordance with our previous observation[6] showing no significant association between job demands and lymphocyte subpopulations in middle-aged workers. Nevertheless, in our recent report[7], we found an inverse relationship between the number of CD57+CD16+ NK cells and job demands after controlling for age and smoking by the partial correlation in young workers. In

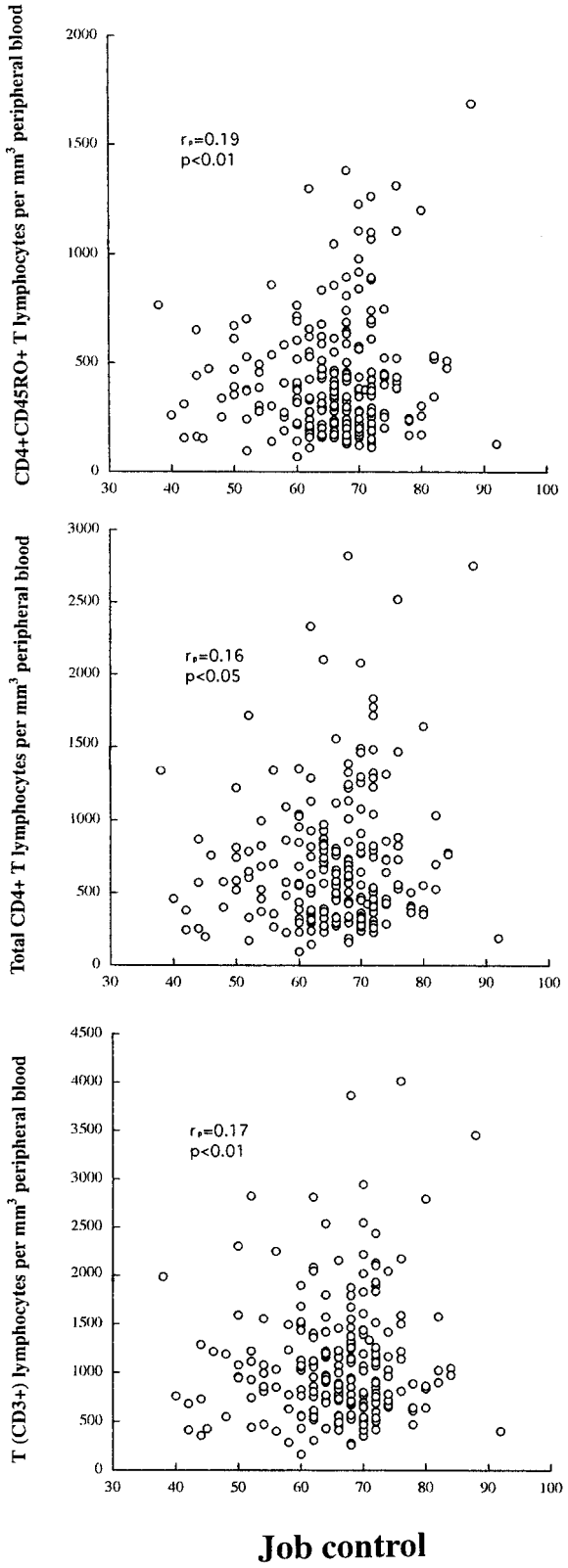

Fig. 1. Correlations of job control scores with numbers of CD4+CD45RO+ T, total CD4+ T and T (CD3+) lymphocytes. The r_p indicates the partial correlation coefficients controlling for age, number of cigarettes smoked per day, alcohol drinking, frequency of regular exercise, job demands, and social support at work.

addition, Meijiman *et al.*[5] observed an inverse relationship between job demands and the number of CD4+ T lymphocytes in young male shift workers. On the basis of these findings, we suggest that job control might be an important immunomodulating factor in middle-aged workers, whereas job demands are factors that modify the immune system in young-aged ones. Further research including workers with a wide age range is necessary to reach a valid conclusion.

Social support at work did not correlate with T lymphocyte subpopulations or NK cells (Table 2). The results did not support the buffering effect of social support on immune function as suggested by Theorell *et al.*[8] but, such finding is consistent with our previous report[6] that social support at work had no association with T lymphocyte subpopulations. On the other hand, in our recent report[7], the number of CD8+ T lymphocytes was positively correlated with social support at work. Since we did not measure CD8+ T lymphocytes, it might be considered that social support affects different cells from CD4+ T lymphocytes or NK cells. Alternatively, a limited distribution of social support scores as suggested by the mean ± standard deviation (i.e. 23.5 ± 2.5) in this study might not be enough to detect a statistical significance. Additional studies are needed to resolve this assumption.

In summary, we investigated the relationship between job stress and lymphocyte subpopulations. Although the study design was cross-sectional and thus could not establish causal relationships, the results showed that job control is associated independently with CD4+CD45RO+ T lymphocytes after controlling for relevant confounding factors. We also suggested that job control rather than job demands or social support at work is a determinant factor in modifying CD4+CD45RO+ T lymphocytes in middle-aged workers. But it remains uncertain whether a decrease in CD4+CD45RO+ T lymphocytes due to low job control is associated with any illness or pathologic conditions. Prospective studies are needed to examine this relationship.

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