workplace (19). The workers with a past history of PTSD seem to stand alone and unaided in the workplace. Glover H suggested that many Vietnam veterans and survivors of Nazi persecution suffer from 'survivors guilt' and are inclined to avoid intimacy with other people (20). He observed that their psychological properties stemmed from loss of colleagues and loss of sense of control. In the present study, almost half of the patients with a past history of PTSD were exposed to traumatic experiences relevant to loss. Therefore, interpersonal conflict may be partially attributable to loss. Further investigation remains to be explored. van der Kolk proposed that intrusion symptoms are the key to understanding the mechanisms of PTSD pathology (21). Avoidance is considered to be a consequence of intrusion. Since intrusion is often provoked by a personal contact with other persons, PTSD patients tend to avoid interpersonal relationships that will give rise to intrusion and stressful conditions. Previous studies showed that psychological property of PTSD could impair their ability to communicate with other people (22). This is also evident in the fact that 5 men disagreed to go through the interview. It is suggested that interpersonal conflict may be a manifestation of avoidance as defined by criteria C (avoidance) in the diagnosis for PTSD.

These data and derived viewpoints confirm how important it is to understand and manage any adjustment problems of patients with a past history of PTSD using the central concepts of PTSD, or criteria B, C, and D, as basic guidelines.

In conclusion, PTSD has long-term implications for immunity, health, and social life.

References

1. Lee KA, Vaillant GE, Torrey WC, Elder GH: A 50-year prospective study of the psychological sequellae of World War II combat. Am.J.Psychiatry 1995; 152:516-522.

2. Boscarino JA: Diseases among men 20 years after exposure to severe stress: implications for clinical research and medical care. Psychosom Med 1997;59: 605-614.

3. Yehuda R, Kahana B, Binder-Brynes K, Southwick SM, Mason JW, Giller EL: Low urinary cortisol excretion in Holocaust survivors with posttraumatic stress disorder. Am J Psychiatry 1995;152:982-986.

4. Laudenslager ML. Aasal R, Adler L, Berger CL, Montgomery PT, Sandberg E, Wahlberg LJ. Wilkins RT. Zweig L, Reite ML: Elevated cytotoxicity in combat veterans with long-term post-traumatic stress disorder: preliminary observations. Brain Behav Immun 1998;12:74-79.

5. Boscarino JA, Chang J: Higher abnormal leukocyte and lymphocyte counts 20 years after exposure to severe stress: research and clinical implications. Psychosom Med 1999;61:378-386.

6.Wilson SN, van der Kolk B, Burbridge J, Fisler R, Kradin R: Phenotype of blood lymphocytes in PTSD suggests chronic immune activation. Psychosomatics 1999;40:222-225.

7. Robinson S, Rapaport-Bar-Sever M,

Rapaport J: The present state of people who survived the holocaust as children. Acta Psychiatr Scand 1994;89:242-245.
8. Robinson S, Rapaport J, Durst R, Rapaport M, Rosca P, Metzer S, Zilberman L: The late effects of Nazi persecution among elderly Holocaust survivors. Acta Psychiatr Scand 1990;82:311-315.
9. Bramsen I, van der Ploeg HM: Fifty years later: the long-term psychological adjustment of ageing World War II survivors. Acta Psychiatr Scand 1999;100;350-358.
10. Weiss D: Psychometric review of the Impact of Event Scale-Revised. In BH Stamm Ed. Measurement of stress, trauma, and adaptation. Lutherville, MD; Sidran Press 1996;186-188.
11. Asukai N: Validation of Japanese version of the Impact of Event Scale-Revised. In Annual reports of the research council of PTSD; the Ministry of Health and Welfare. 2000.
12. Robins LN, Cottler L, Bucholz K, Compton W: Diagnostic Interview Schedule for DSM-IV. St Louis Washington University 1995.
13. Kawamura N, Kim Y, Asukai N: Suppression of cellular immunity of male workers with a past history of posttraumatic stress disorder. Am J Psychiatry 2001;158:484-486.
14. Born J, Lange T, Hansen K, Molle M, Fehm HL: Effects of sleep and circadian rhythm on human circulating immune cells. J Immunol 1997;158;4454-4464.
15. Hurrell JJ, McLaney MA: Exposure to job stress: A new psychometric instrument. J work Environ Health 1988;14:27-28.
16. Haratani T: Psychometric properties of the Japanese version of Job Content Questionnaire and NIOSH generic job stress questionnaire in Annual Reports of job stress and its effects on human health. Ministry of Labor, Tokyo, 1997.
17. Johnson JV, Hall EM: Job strain, work place social support, and cardiovascular disease: a cross-sectional study of a random sample of the Swedish working population. Am J Public Health 1988;78:1336-1342.
18. Rosen J, Reynolds CF 3d, Yeager AL, Houck PR, Hurwitz LF: Sleep disturbances in survivors of the Nazi Holocaust. Am J Psychiatry 1991;148:62-66.
19. Appelberg K, Romanov K, Honkasalo ML, Koskenvuo M: Interpersonal conflicts at work and psychosocial characteristics of employees. Soc Sci Med 1991;32:1051-1056.
20. Glover H: Survival guilt and the Vietnam veteran. J Nerv Ment Dis 1984;172:393-397.
21. van der Kolk B, Friedman M, Friedman M, Mcfarlane, Higson-Smith C: Resilience in the face of exposure to trauma. 16th Annual Meeting of The International Society for Traumatic Stress Studies, 2000.
22. MacDonald C, Chamberlain K, Long N, Flett R: Posttraumatic stress disorder and interpersonal functioning in Vietnam War veterans: a mediational model. J Trauma Stress 1999;12:701-707.

ストレス学雑誌に掲載され、学会優秀論文賞を受賞した。

Coemergence of insomnia and a shift in Th1/Th2 balance toward Th2 dominance.

Shotaro Sakami[a,b], Toshio Ishikawa[a], Norito Kawakami[c], Takashi Haratani[d], Akira Fukui[e], Fumio Kobayashi[f], Osamu Fujita[g], Shunichi Araki[d], and Noriyuki Kawamura[a,1]

[a] Division of Psychosomatic Research, National Institute of Mental Health, Chiba, Japan;
[b] Department of Public Health, University of Tokyo, Tokyo, Japan;
[c] Okayama University Graduate School of Medicine and Dentistry, Japan;
[d] National Institute of Industrial Health, Kanagawa, Japan;
[e] Department of Internal Medicine, University of Nagoya, Aichi, Japan;
[f] Department of Psychosocial Medicine, Aichi Medical University, Aichi, Japan; and
[g] Aichi University of Education, Health Care Center, Aichi, Japan

Abstract

Objectives: Insomnia is associated with physical and mental disorders. We examined the effect of insomnia on immune functions, focusing on T helper1/T helper2 (Th1/Th2) balance by a cross sectional design.

Methods: We provided a self-administered questionnaire for evaluating sleep habits, smoking, and medical disorders to 578 men without any toxic exposure (20-64 years old) and measured natural killer (NK) cell activity in 324 men and production of interferon-gamma (IFN-γ) and interleukin-4 (IL-4) after stimulation with phytohemagglutinin (PHA) in 254 men. According to the criteria of DSM-IV, in which insomnia is classified into primary and secondary insomnia, we assessed the effect of insomnia on immune functions controlling for age and smoking in groups with and without medical disorders respectively.

Results: The prevalence of insomnia in the present study was 9.2%. In the absence of medical disorders, insomniac men had a significantly lower IFN-γ and IFN-γ/IL-4 than non-insomniac men. Men with insufficient sleep or difficulty initiating sleep (DIS) had a significantly lower IFN-γ/IL-4 than those without insufficient sleep or DIS. In the presence of medical disorders, insomniac men had a significantly higher IL-4 than non-insomniac men. Men with difficulty maintaining sleep (DMS) had a significantly lower IFN-γ/IL-4 than men without DMS. NK cell activity was independent of insomnia.

Conclusions: The present results showed a link between insomnia unrelated to medical disorders and a shift in Th1/Th2 balance toward Th2 dominance, indicating that sleep quality should be reconsidered in terms of etiology of immune-related diseases.

Key Words: sleep, insomnia, life style, immune functions, Th1 and Th2, NK cell activity

Introduction

Sleep plays a role in host defense, exerting an influence on immune system. Disrupted sleep and circadian rhythm alter immune functions [1-3].

Peripheral blood mononuclear cell (PBMC) counts and in vitro PHA-stimulated IL-2 production are significantly higher during sleep deprivation than nocturnal sleep [1]. After partial sleep deprivation, reduction of NK cell activity was observed [2]. A recent study showed that total sleep deprivation elevated serum TNF- α recepter1 and plasma IL-6 levels [4]. Almost all the findings from the studies on depression, asthma, HIV, and bacterial infections supported the existence of the association between sleep and immune functions [5-9].

Most investigations on the relationship between sleep and immunity have been conducted in sleep-deprived humans and animals. However, there have been scarce findings on immune functions associated with sleep quality in real life. Insomnia is a highly prevalent complaint [10,11] and the subjects with insomnia have an increased risk of heart diseases, diabetes, stroke, anxiety, and depression [12]. Insomnia also results in impaired performance leading to absenteeism from work, reduced productivity, and a higher rate of accidents [12,13]. Thus it is worthwhile elucidating the relationship between insomnia and immunity in order to bring relief into those problems mentioned above and to enrich our knowledge about the effects of insomnia on human health.

Th1 and Th2 imbalance has an adverse effect on health and diseases. Th1 cells play an important role in eradicating intracellular pathogens and are also implicated in many autoimmune diseases. Th2 cells enhance IgE productions from B cells and mediate allergic processes. IFN- γ and IL-4 are representative cytokines secreted from Th1 and Th2 cells, respectively, and IFN- γ /IL-4 ratio, which reflects Th1/Th2 balance, is of critical importance in the pathogenesis of immune-related diseases. In addition, NK cell functions is partly regulated by Th1/Th2 balance, since NK cells and their cytotoxicity are promoted by IFN- γ and inhibited by IL-4. Recently a longitudinal study showed that those subjects with a lower level of NK cell activity have an increased risk of cancer [14]. A rapid progress has been made in understanding the immune mechanisms involved in depression, asthma, HIV, and bacterial infections, and showed the contribution of Th1 and Th2 cells are significantly important to these mechanisms. An elevated proinflammatory cytokine level in sleep loss suggests that Th1/Th2 balance may be altered in insomnia [4].

The objective of the present study is to examine a possible link between chronic insomnia and Th1/Th2 balance by a cross sectional design. We provided a self-administered questionnaire to male subjects for evaluating the subjective sleep quality and problems and medical disorders according to DSM-IV criteria [15], in which insomnia is subdivided into primary insomnia and insomnia related to specific etiologies, and measured PHA-stimulated IFN- γ and IL-4 productions and NK cell activity. In addition to medical disorders, age [16,17] and smoking behaviors [17-19] have been proposed as factors regulating immune functions. Therefore, these factors were also included in the questionnaire and were analyzed as confounding factors.

Methods

Subjects

At first, 578 adult men without exposure to any toxic substances (20-64 years old) were recruited from an electric equipment manufacturing company in Japan. They were administered a self-check questionnaire for evaluating sleep habits, smoking behaviors, and medical disorders. Based on their self-reported illness, the subjects were divided into 263 men who did not have any medical disorders (20-64 years old) and 315 men

who had any medical disorders (20-64 years old) (Table 1). We obtained heparinized blood samples from the subjects and measured NK cell activity in 324 men (21-64 years old) and cytokine productions in 254 men (20-64 years old) with written informed consent.

Table 1. Frequensies of Medical Disorders in 578 Male Workers

Disease categories	n
Psychiatric disorders	7
Hypertension	38
Ischemic heart diseases	5
Other heart diseases	22
Cerebral infarction and hemorrhage	4
Diabetes mellitus	18
Malignancies	2
Gastrointestinal diseases	88
Liver diseases	16
Renal diseases	15
Musculoskeletal diseases	176
Other disorders	46

Sleep questionnaire

Insomniac individuals were identified using a sleep questionnaire that was developed for the present study. The most central feature of insomnia is the subjective perception of inadequate sleep duration or quality [20]. Based on criteria in the previous studies [21], insomnia was categorized as the perception of insufficient sleep along with having experienced one or more of the following symptoms during the past year: difficulty initiating sleep (DIS); difficulty maintaining sleep (DMS); early morning awaking (EMA) in order to eliminate sleep mal recognition syndrome of ICSD (The International Classification of Sleep Disorders) [22].

Four questionnaire items provided information about sleep-associated problems during the past year to see the chronic effects of sleep habits on immune functions:

Q1. Do you sleep well? Possible ratings: 1=very well, 2=well, 3=normal, 4=poor, 5=fairly poor.

Q2. How long does it take you to fall asleep? Opened-question.

Q3. Do you tend to wake up frequently during the night? Possible ratings: 1=no, 2=yes.

Q4. Do you tend to wake up too early in the morning? Possible ratings: 1=very often, 2=often, 3=occasional, 4=never.

For a definition of insufficient sleep, DIS, DMS, and EMA, the following criteria were required following the previous study [23]: poor sleep; sleep latency exceeding 30 minutes; troubled by nocturnal awakening; often wake up too early.

The prevalence of insomnia in the current study was 9.2 % (Table 2). The prevalence of insomnia with and without any medical disorders was 5.9 % and 3.3 %, respectively. Table 3 showed the prevalence of insomniac symptoms in men without any medical disorders. This table was

divided into two parts by the immunological assessments.

Table 2. The Prevalence of Insomnia and the Characteristics of Sleep Problems

		+[1]	-[2]	Total
Insomnia[3]	n	53	524	577
	%	9.2	90.8	100
Insufficient sleep	n	58	519	577
	%	10.1	89.9	100
DIS[4]	n	145	430	575
	%	25.2	74.8	100
DMS[5]	n	277	299	576
	%	48.1	51.9	100
EMA[6]	n	162	415	577
	%	28.1	71.9	100

[1] The subjects with insomnia and relevant sleep problems.

[2] The subjects without insomnia and relevant sleep problems.

[3] Insomnia = the perception of insufficient sleep along with having experienced one or more of DIS, DMS, and EMA during the past year.

[4] DIS = difficulty initiating sleep.

[5] DMS = difficulty maintaining sleep.

[6] EMA = early morning awaking.

Table 3. The distribution of Insomniac Symptoms in Insomniac Men without Any Medical Disorders

	Immunological assessment			
	NK cell activity (n=10)		IFN-γ and IL-4 (n=8)	
	n	%	n	%
Insufficient sleep & DIS	1	10.00	0	0.00
Insufficient sleep & DMS	4	40.00	3	37.50
Insufficient sleep & EMA	0	0.00	0	0.00
Insufficient sleep & DIS & DMS	2	20.00	2	25.00
Insufficient sleep & DIS & EMA	0	0.00	0	0.00
Insufficient sleep & DMS & EMA	1	10.00	0	0.00
Insufficient sleep & DIS & DMS & EMA	2	20.00	3	37.50

Cigarette smoking questionnaire

The number of cigarettes smoked per day was assessed by a questionnaire.

Immunological assessments

Preparation of peripheral blood mononuclear cell (PBMC)

Heparinized blood samples were collected at 10 am from consenting male subjects. The cells were stored at room temperature and processed within 4 h. PBMC were isolated by density-gradient centrifugation on a Lymphoprep (Nycomed, Oslo), according to the manufacturer's instructions. After isolation, the PBMC were washed twice and resuspended at 2×10^6/ml in RPMI 1640 medium containing 10% FCS, 2 mM glutamine, 100 U/ml penicillin and 100 U/ml streptomycin (Dainippon, Tokyo).

Cytotoxicity assay

NK cell activity was measured against K562 using a standard 4h -^{51}Cr release assay. Target cells were labeled with [^{51}Cr] sodium chromate (New England Nuclear, Boston, MA) at 37°C for 1h, washed, and resuspended at 2×10^5/ml in RPMI 1640 medium containing 10% FCS, 2 mM glutamine, 100 U/ml penicillin and 100 U/ml streptomycin. Labeled target cells were incubated with effector cells at E: T=20: 1 in U-bottom 96-well plates at 37°C for 4h. Radioactivity in the supernatant was determined by a gamma counter. The assay was performed in quadruplicate. The percentage of specific lysis as cytotoxicity was determined according to the formula: % specific lysis = [(mean experimental cpm release - mean spontaneous cpm release)/(mean maximal cpm release - mean spontaneous cpm release)].

Cytokine assay

For determination of IFN-γ and IL-4, a whole blood assay was applied [1]. Blood was drawn into syringes pretreated with heparin (Beckton-Dickson, NJ, USA) at 10 am and stored at room temperature for no longer than 4h before the assays. Aliquots of 50 μl of blood were resuspended under laminar airflow in 400 μl of RPMI 1640 medium containing 2 mM glutamine, 100 U/ml penicillin and 100 U/ml streptomycin. For stimulation of IFN-γ and IL-4, 2.5 μg of PHA (Sigma-Aldrich Japan, Tokyo) was added, dissolved in 50 μl of a medium containing 50% RPMI and 50% sterile water (final concentration, 5 μg/ml). At the end and in the beginning of each measurement, an unstimulated control was included to exclude contaminations of blood and reagents. The samples were incubated for 48 h at 37°C with 5% carbon dioxide in humidified air. The supernatants were harvested and stored at -80°C until assay. The samples were thawed only once and all cytokine levels were measured in duplicate by ELISA kits (Human Immunoassay ELISA kit, BioSource International, Camarillo, CA), according to the manufacturer's instructions.

Statistical Analysis

For statistical analysis, data on IFN-γ, IL-4, IFN-γ/IL-4 were logarithmically transformed because of their skewed distributions (Table 4). Pearson's correlation analysis was performed to evaluate the associations among age, smoking behaviors, and immune functions. ANCOVA (analysis of covariance) was applied to assess the effect of insomnia and medical disorders on immune functions controlling for age and smoking. A p value < 0.05 was considered to indicate statistical significance. All tests were two-tailed.

Table 4. Descriptive Analysis of Immunological and Lifestyle Variables

Variable	n	Mean (SD)	Median	(Range)
NK cell activity (%)	324	50.96 (12.92)	52	(7 - 73)
IFN-γ (pg/ml)	251	95.18 (105.53)	50	(1 - 522)
IL-4 (pg/ml)	254	12.10 (13.86)	7.6	(0 - 83)
IFN-γ / IL-4	251	13.35 (14.25)	8.28	(0 - 87.50)
Age (y)	578	37.93 (9.13)	37	(20 - 64)
Insufficient sleep[1]	577	2.23 (0.92)	2	(1 - 5)
DIS (min)	575	16.21 (13.84)	10	(0 - 100)
DMS[2]	576	1.48 (0.50)	1	(1 - 2)
EMA[3]	577	2.98 (0.88)	3	(1 - 4)
Smoking (cigarettes/d)	571	12.39 (12.19)	15	(0 - 60)

[1] sufficient sleep: 1 = very well, 2 = well, 3 = normal, 4 = poor, 5 = fairly poor.

[2] wake up frequently: 1 = no, 2 = yes.

[3] wake up too early in the morning: 1 = very often, 2 = often, 3 = occasionally, 4 = never.

Results

Age, smoking, and immune functions

Age was inversely correlated with NK cell activity, IFN-γ, and IFN-γ/IL-4 ratio (NK cell activity: r = -0.171, p = 0.002; IFN-γ : r = -0.181, p = 0.004; IFN-γ/IL-4 ratio: r = -0.181, p = 0.004). The number of cigarettes smoked per day was inversely correlated with NK cell activity and positively correlated with IFN-γ and IL-4 production (NK cell activity: r = -0.113, p = 0.042; IFN-γ : 0.249, p < 0.001; IL-4: r = 0.229, p < 0.001). The results suggest that both age and smoking should be adjusted in evaluating the relationship between insomnia and immune functions.

Correlations among immunological variables

To evaluate the influence of IFN-γ and IL4 productions on the IFN-γ/IL-4 ratio among the subjects without any medical diseases, we constructed Table 5. There is a strong positive association between IFN-γ and IL-4 levels. IFN-γ/IL-4 ratio was more correlated with IL-4 than with IFN-γ.

Insomnia and immune functions

The subjects were divided into 4 groups according to the presence or absence of insomnia and medical disorders. One-way ANCOVA with LSD post hoc was conducted to assess the effect of insomnia on immune functions controlling for age and smoking. As shown in Figure 1, NK cell activity was independent of insomnia. In the absence of medical disorders, IFN-γ was significantly lower in insomniac men than non-insomniac men. In the presence of medical disorders, IL-4 was significantly higher in insomniac men than non-insomniac men. In the absence of medical disorders, IFN-γ/IL-4 ratio was significantly lower in insomniac men than non-insomniac men. This comparison was very meaningful since the statistical power was

relatively high (power, 0.765) and the observed difference in Th1/Th2 ratio was relatively large (effect size, 0.99).

Insomniac symptoms and IFN- γ /IL-4 ratio

The subjects were divided into 4 groups according to the presence or absence of insomniac symptoms and medical disorders. ANCOVA with LSD post hoc was applied to assess the effect of insomniac symptoms on IFN- γ /IL-4 ratio. Figure 2 shows that in the absence of medical disorders those men who complained of insufficient sleep had a lower IFN- γ /IL-4 ratio compared with those who did not complain of insufficient sleep and men with DIS had a lower IFN- γ /IL-4 ratio than those without DIS. In the presence of medical disorders, IFN- γ /IL-4 ratio in men with DMS was lower than those without DMS. IFN- γ /IL-4 ratio was not associated with EMA.

Table 5. Correlation Matrix between Immune Functions in Men without Insomnia nor Any Medical Disorders (n=106)

	IFN-γ		IL-4		IFN-γ / IL-4	
Variable	r[1]	p	r	p	r	p
IFN-γ (pg/ml)	1.00	———				
IL-4 (pg/ml)	0.73	<0.001***	1.00	———		
IFN-γ / IL-4	0.19	0.060	-0.51	<0.001***	1.00	———

[1] Partial correlation coefficients controlling for age and smoking. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

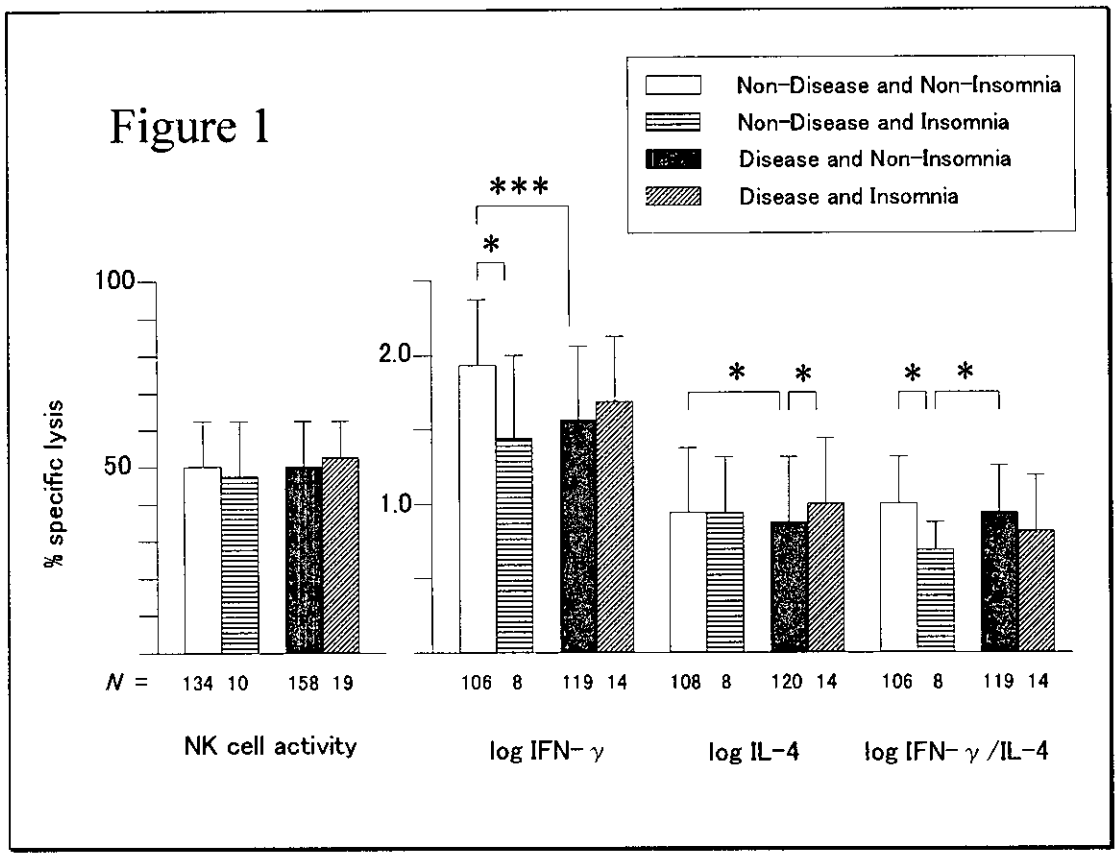

Fig. 1.

Means (SD) of immunological variables in the presence or absence of insomnia and medical disorders. The means of immune functions among 4 categories were compared by ANCOVA (analysis of covariance) with LSD post hoc controlling for age and smoking.

(NK cell activity) ANCOVA: $F(3, 315) = 1.954$, $p = 0.121$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.481$; with medical disorders: $p = 0.335$.

(IFN-γ) ANCOVA: $F(3, 241) = 4.829$, $p = 0.003$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.034$; with medical disorders: $p = 0.371$.

(IL-4) ANCOVA: $F(3, 244) = 3.165$, $p = 0.025$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.949$; with medical disorders: $p = 0.025$.

(IFN-γ/IL-4) ANCOVA: $F(3, 241) = 3.051$, $p = 0.029$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.013$; with medical disorders: $p = 0.205$.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

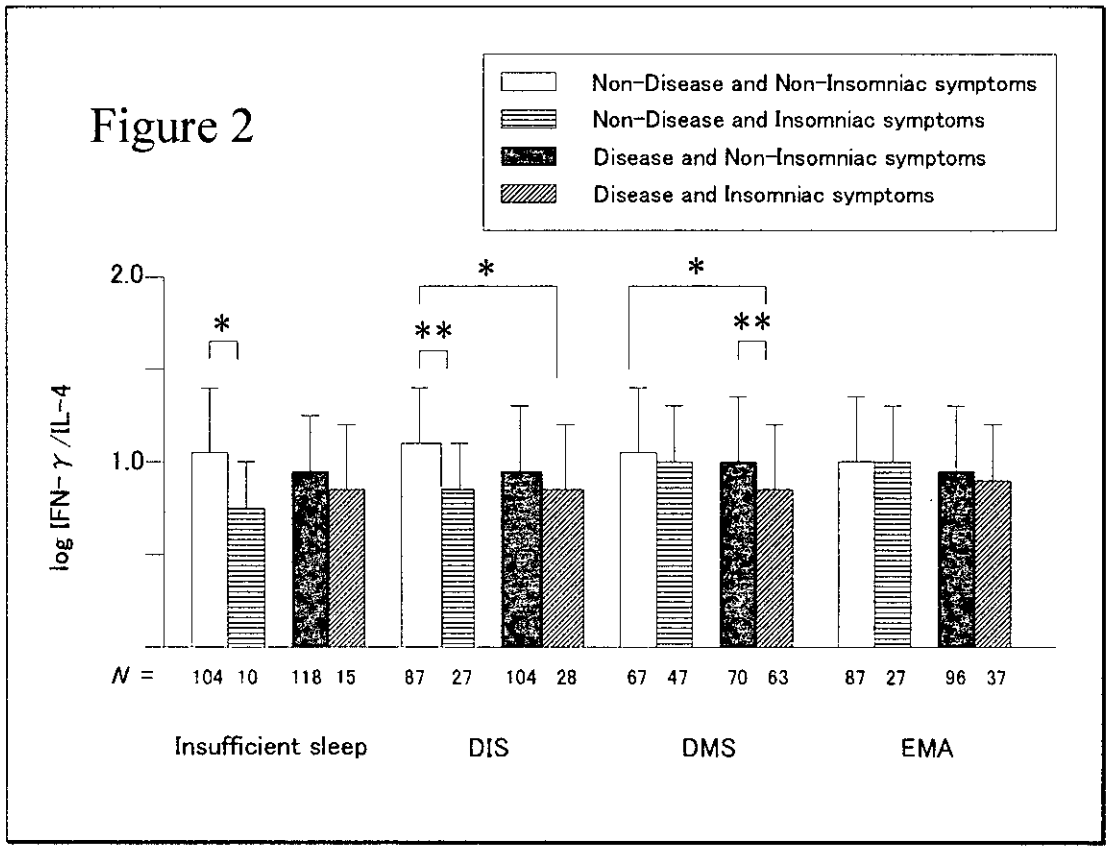

Fig. 2.

Means (SD) of IFN-γ/IL-4 ratio in the presence or absence of insomniac symptoms and medical disorders. The means of IFN-γ/IL-4 among 4 categories were compared by ANCOVA (analysis of covariance) with LSD post hoc controlling for age and smoking.

(Insufficient sleep) ANCOVA: $F(3, 241) = 2.696$, $p = 0.047$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.014$; with medical disorders: $p = 0.415$.

(DIS) ANCOVA: $F(3, 240) = 3.155$, $p = 0.026$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.009$; with medical disorders: $p = 0.324$.

(DMS) ANCOVA: $F(3, 241) = 3.062$, $p = 0.029$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.606$; with medical disorders: $p = 0.006$.

(EMA) ANCOVA: $F(3, 241) = 0.637$, $p = 0.592$. Multiple comparisons (non-insomnia vs. insomnia): without any medical disorders: $p = 0.796$; with medical disorders: $p = 0.436$.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Discussion

In the present study, the subjects were divided into men with and without medical disorders according to the criteria of DSM-IV, in which insomnia is classified into primary insomnia and insomnia related to specific etiologies, and then, the effects of insomnia on immune functions were differentially evaluated. Primary insomnia lacks in any medical disorders and substance exposures, and men with this relevant type of chronic insomnia had a lower IFN-γ level and IFN-γ /IL-4 ratio than non-insomniac men with high statistical power and high effect size. Secondary insomnia is the case in which underlying medical conditions should be involved as a cause. Men with relevant insomnia had a higher IL-4 level than non-insomniac men, whereas there is no significant difference in IFN-γ and IFN-γ /IL-4 ratio between them. In the case with secondary insomnia, however, it is likely that the results are not absolutely reliable considering the effects of medication and the variety of pathophysiology of respective disorders. Because of a small number of concurrent insomnia in men with respective medical disorders, the underlying mechanisms cannot be reasonably inferred. In this article, consideration is to be focused on the results relevant to primary insomnia.

With respect to insomniac symptoms, DIS and insufficient sleep in the absence of medical disorders were associated with a shift in Th1/Th2 balance in favor of type 2. From the results of **Table 3** and Fig2, Th2 shift in insomniac subjects without medical diseases can be supposed to be mainly due to insufficient sleep and secondly due to DIS. Further studies should address these two associations. As for DMS and Th1/Th2 balance, in the medically ill subjects, the persons with DMS showed significant Th2 shift as compared with those without DMS. The causes of DMS in the present study might include nocturnal urination and others rather than psychological problems. Our present study cannot completely eliminate the possibility of association between DMS and Th2 shift among the population without diseases.

It is well known that DIS and DMS are sleep problems typical in anxiety and underlying conditions such as depression. Because psychiatric disorders were excluded from the case without medical disorders, it is possible to say the results may suggest that perceived stress, subclinical anxiety and depression was associated with a Th2-shift. Compared with studies of depression, only a handful of studies examined immune alterations in anxiety [24]. This implication remains to be explored.

IFN-γ was lower in insomniac men without medical disorders than non-insomniac men. IFN-γ /IL-4 ratio was contributed by IL-4 rather than by IFN-γ. The correlations of IFN-γ and IL-4 with IFN-γ /IL-4 ratio was considerably smaller compared with that between IFN-γ and IL-4, which indicates that IFN-γ /IL-4 ratio is relatively independent of IFN-γ and IL-4 levels. It may be inappropriate to discuss Th1/Th2 balance using the data on IFN-γ or IL-4 as alternatives.

However, our present results clearly indicate a link between insomnia and Th1/Th2 balance and can be interpreted as insomnia causing a shift in Th1/Th2 balance toward Th2 dominance. Among the possible mechanisms that mediate the effect of sleep on cytokine productions, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis may be involved in the causality.

ANS is considered to help organize immune responses sequentially and spatially [25]. Sympathetic nerve stimulations by hyperarousal suppress immunocompetent cells in the blood stream [25]. Norepinephrine and epinephrine

inhibit the production of IL-12, tumor necrosis factor-alpha (TNF- α), and IFN- γ by antigen-presenting cells and Th1 cells, whereas they stimulate the production of IL-10 and transforming growth factor-beta (TGF- β) [26]. Other neurotransmitters (eg, neuropeptide Y, vasoactive intestinal peptide) produced within the lymphoid microenvironment modulate several immunological functions including cytokine productions [27,28]. Together these studies suggest that sympathetic activation may contribute to a Th2-shift in Th1/Th2 balance observed in insomniacs.

Glucocorticoids favor Th2 development [29,30]. Many studies have reported that sleep loss and disordered sleep increase plasma cortisol concentration [1,31]. Because, however, the effect of sleep loss on cortisol release is generally small, it is unlikely that cortisol mediates the effect of disturbed sleep on immune functions. Anyway, as we did not assess either ANS function or glucocorticoid level, further investigation should be undertaken.

On the other hand, insomnia may be the result of a Th2-shift in Th1/Th2 balance. Clinical and experimental evidences can endorse this hypothesis. Some anti-allergic drugs evoke sleepiness. Human histocompatibility leukocyte antigens (HLA) association observed in narcolepsy suggests that autoimmunity plays an important role in the disorder [32]. Sleep quality in infants with milk allergy suffering from chronic insomnia became normalized after cow's milk was excluded from the diet [33]. In the present study, insufficient sleep and DIS unrelated to medical disorders was significantly associated with a Th2-shift in Th1/Th2 balance. This agrees with the previous report showing that spontaneous sleep is inhibited by IL-4 injection in rabbits [34].

Furthermore, empirical studies have revealed a very high prevalence of concurrent atopic disorder in people with depression, who almost always complain about insomniac symptoms [35]. Individuals with allergy substantially have cholinergic hyperresponsiveness and β -adrenergic hyporesponsiveness in ANS. Similar autonomic imbalances play a causal role in depressive behaviors as well [36]. It is hypothesized that imbalance in ANS underlies the insomnia-related Th2-shift in Th1/Th2 balance.

The present results suggest that, when the effect of a lifestyle factor on human immunity is to be elucidated, other factors also should be considered. Most studies to date have compared the effect of sleep and sleep deprivation on immune functions. Because we assessed sleep quality non-intrusively, other factors were controlled. Age-related decline in NK cell activity and IFN- γ /IL-4 ratio observed in the present study coincide with the findings from the previous studies [16,17]. Although smoking-induced alteration in Th1/Th2 balance was not found in the present study, smoking was entered into a general linear model since many studies showed Th2 dominance in smokers [18,19]. In addition, psychological stress does not simply act as a sleep-disturbing factor [37], but suppresses cellular immunity and induces a shift in Th1/Th2 balance toward a predominant type 2 cytokine response [38]. Further studies controlling for socio-psychological factors are needed.

Despite the difficulty in interpretation of the data that is due to the nature of the cross-sectional design, the present results indicate that a shift in Th1/Th2 balance toward Th2 dominance in insomnia is worth considering not only from the perspectives of neuro-immune interactions but also from therapeutic and preventive applications for allergy, autoimmunity and other detrimental diseases such as cancer. Further studies including DSM-IV diagnosis of specific insomnia should be

undertaken.

References

1. Born J, Lange T, Hansen K, Molle M, Fehm HL: Effects of sleep and circadian rhythm on human circulating immune cells. J Immunol 1997;158:4454-4464.

2. Irwin M, McClintick J, Costlow C, Fortner M, White J, Gillin JC: Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. FASEB J 1996;10:643-653.

3. Dinges DF, Douglas SD, Zaugg L, Campbell DE, McMann JM, Whitehouse WG, Orne EC, Kapoor SC, Icaza E, Orne MT: Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. J Clin Invest 1994 ;93:1930-1939.

4. Shearer WT, Reuben JM, Mullington JM, Price NJ, Lee BN, Smith EO, Szuba MP, Van Dongen HP, Dinges DF: Soluble TNF-alpha receptor 1 and IL-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight. J Allergy Clin Immunol 2001;107:165-170.

5. Leonard BE: The immune system, depression and the action of antidepressants. Prog Neuropsychopharmacol Biol Psychiatry 2001;25:767-780.

6. van Keimpema AR, Ariaansz M, Nauta JJ, Postmus PE: Subjective sleep quality and mental fitness in asthmatic patients. J Asthma 1995;32:69-74.

7. Darko DF, Mitler MM, Henriksen SJ: Lentiviral infection, immune response peptides and sleep. Adv Neuroimmunol 1995;5:57-77.

8. Everson CA, Toth LA: Systemic bacterial invasion induced by sleep deprivation. Am J Physiol Regul Integr Comp Physiol 2000;278:905-916.

9. Pollmacher T, Schuld A, Kraus T, Haack M, Hinze-Selch D, Mullington J: Experimental immunomodulation, sleep, and sleepiness in humans. Ann N Y Acad Sci 2000;917:488-499.

10. Ancoli-Israel S, Roth T: Characteristics of insomnia in the United States: results of the National Sleep Foundation Survey. I . Sleep 1999;22:347-353.

11. Jansen C, Lindberg E, Gislason T, Elmasry A, Boman G: Insomnia in men- a 10-year prospective base study. Sleep 2001;24:425-430.

12. Stooler MK: The socio-ecomonics of insomnia: the materials and methods. Eur Psychiatry 1997;12 :41-48.

13. Leger D, Scheuermaier K, Philip P, Paillard M, Guilleminault C: SF-36: evaluation of quality of life in severe and mild insomniacs compared with good sleepers. Psychosom Med 2001;63:49-55.

14. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K: Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795-1799.

15. American Psychiatric Association: Diagnostic and statistical manual of mental disorders, 4th Edn (DSM- IV). Washington: The American Psychiatric Association, 1994.

16. Shearer GM: Th1/Th2 changes in aging. Mech Ageing Dev 1997;94:1-5.

17. Kusaka Y, Kondou H, Morimoto K: Healthy lifestyles are associated with higher natural killer cell activity. Prev Med 1992;21:602-615.

18. Hughes DA, Haslam PL, Towsend PJ, Turner-Warwick M: Numerical and functional alterations in circulatory lymphocytes in cigarette smokers. Clin Exp Immunol 1985;61:459-466.

19. Byron KA, Varigos GA, Woottton AM: IL-4 production is increased in cigarette smokers. Clin Exp Immunol 1994;95:333-336.

20. Morin CH: The nature of insomnia and the need to refine our diagnostic criteria. Psychosom

Med 2000;62:483-485.
21. Dodge R, Cline MG, Quan SF: The natural history of insomnia and its relationship to respiratory symptoms. Arch Intern Med 1995;155:1797-1800.
22. Diagnostic Classification Steering Committee, Thorphy MJ (Chairman): The International Classification of Sleep Disorders: Diagnostic and Coding Manual. Rochester, MN: American Sleep Disorders Association, 1990.
23. Kim K, Uchiyama M, Liu X, Shibui K, Ohida T, Ogihara R, Okawa M: Somatic and psychological complaints and their correlates with insomnia in the Japanese general population. Psychosom Med 2001;63:441-446.
24. Koh K, Bong MD, Bong K: Reduced lymphocyte proliferation and interleukin-2 production in anxiety disorders. Psychosom Med 1998;60:479-483.
25. Nagatomi R, Kaifu T, Okutsu M, Zhang X, Kanemi O, Ohmori H: Modulation of the immune system by the autonomic nervous system and its implication in immunological changes after training. Exerc Immunol Rev 2000;6:54-74.
26. Irwin M, Thompson J, Miller C, Gillin JC, Ziegler M: Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: clinical implications. J Clin Endocrinol Metab 1999;84:1979-1985.
27. Delgado M, Leceta J, Sun W, Gomariz RP, Ganea D: VIP and PACAP induce shift to a Th2 response by upregulating B7.2 expression. Ann N Y Acad Sci 2000;921:68-78.
28. Kawamura N, Tamura H, Obana S, Wenner M, Ishikawa T, Nakata A, Yamamoto H: Differential effects of neuropeptides on cytokine production by mouse helper T cell subsets. Neuroimmunomodulation 1998;5:9-15.
29. Petrovsky N, Harrison LC: Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? J Immunol 1997;158:5163-5168.
30. Visser J, van Boxel-Dezaire A, Methorst D, Brunt T, de Kloet ER, Nagelkerken L: Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. Blood 1998 ;91:4255-4264.
31. Vgontzas AN, Bixler EO, Lin HM, Prolo P, Mastorakos G, Vela-Bueno A, Kales A, Chrousos GP: Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: clinical implications. J Clin Endocrinol Metab 2001;86:3787-3794.
32. Dauvilliers Y, Neidhart E, Lecendreux M, Billiard M, Tafti M: MAO-A and COMT polymorphisms and gene effects in narcolepsy. Mol Psychiatry 2001;6:367-372.
33. Kahn A, Rebuffat E, Blum D, Casimir G, Duchateau J, Mozin MJ, Jost R: Difficulty in initiating and maintaining sleep associated with cow's milk allergy in infants. Sleep 1987;10:116-121.
34. Kushikata T, Fang J, Wang Y, Krueger JM: Interleukin-4 inhibits spontaneous sleep in rabbits. Am J Physiol 1998;275:1185-1191.
35. Wamboldt MZ, Hewitt JK, Schmitz S, Wamboldt FS, Rasanen M, Koskenvuo M, Romanov K, Varjonen J, Kaprio J: Familial association between allergic disorders and depression in adult Finnish twins. Am J Med Genet 2000;96:146-153.
36. Marshall PS: Allergy and depression: a neurochemical threshold model of the relation between the illnesses. Psychol Bull 1993 ;113:23-43.
37. Urponen H, Vuori I, Hasan J, Partinen M: Self-evaluations of factors promoting and disturbing sleep: An epidemiological survey in

Finland. Soc Sci Med 1988;26:443-450.
38. Marshall GD Jr, Agarwal SK: Stress, immune regulation, and immunity: applications for asthma. Allergy Asthma Proc 2000;21:241-246.

この論文は、Neuroimmunomodulation に受理され掲載された。

Development of the Overt-Covert Aggression Inventory

Takao Miyazaki [(1)] Takahiro Shimizu[(2)] Gen Komaki [(1)] Osamu Fujita [(3)] Hirohito Tsuboi[(4)] Fumio Kobayashi [(4)] Noriyuki Kawamura[(1)]

(1) Division of Psychosomatic Research National Institute of Mental Health National Center of Neurology and Psychiatry
(2) Department of Psychology, Tsukuba University
(3) Department of Psychiatry, Aichi Education University
(4) Department of Health and Psychosocial Medicine, Aichi Medical University School of Medicine

Abstract

The expression of anger in Japanese people is different from that of other races. We developed a new, brief, inventory; the Overt Covert Aggression Inventory (O-CAI) to assess aggressive behavior of Japanese people by focusing on their uniqueness, and examined its reliability and validity. The O-CAI, the Center for Epidemiological Studies Depression Scale, the Japanese version of the Buss-Perry Aggression Questionnaire and Picture-Frustration Study were administered. Internal consistency, test-retest reliability, concurrent validity, and construct validity of the scale were examined. We confirmed that O-CAI has adequate reliability and sufficient validity. However, further studies of the construct validity and discriminant validity are required.

Studies on the relationship between aggression and health have attracted considerable attention since Friedman and Rosenman (1959) proposed the concept of Type A Behavior Pattern (TABP) as a cause of Coronary Heart Disease (CHD). The results of these studies, however, have been inconsistent (Lahad, Heckbert, Koepsell, Psaty, & Patrick, 1997). Several researchers have focused on the relationship between Coronary Heart Disease and hostility. It is known that the latter is related to Type A Behavior Pattern (Dembroski, & Costa, 1987: Friedman, & Booth-Kewley, 1987). The factor structure of the Japanese version of the Jenkins Activity Survey (JAS), standardized on the Japanese population, was not fully congruent with the original Jenkins Activity Survey (Hayano, Takeuchi, Yoshida, Jozuka, Mishima, & Fujinami, 1989). Moreover, Fukunishi, Hattori, Hattori, Imai, Miyake, Miguchi, and Yoshimatsu (1992) demonstrated that Japanese Coronary Heart Disease patients demonstrating a high Type A Behavior Pattern had higher depression-proneness scores measure by the Depression-Related Personality Trait Scale (Yoshimatu, Miguchi, Miyake, Ozaki, Minagawa, Takeuchi, & Ito, 1989) than patients with other diseases. The depression-prone personality coincides with what Tellenbach (1961) has named "Typus Melancholicus", which is widely accepted as the premorbid personality of depression in Germany and Japan. This suggest that anger and the aggression may overlap with depression in the Japanese population. Moreno, Fuhriman, and Selby (1993) also examined the relationship between hostility, aggression, and depression. According to their findings, depression is linked to hostility/anger and most strongly related to attitudes such as resentment, suspicion, guilt and intropunitivenes (attitudinal forms of the hostility/anger), than to behaviors such as assault,

and verbal hostility (motoric forms of the hostility/anger).

Research has shown that there are differences between European and American expression of anger and aggression in comparison with Japanese. For example, a study of facial expressions demonstrated that the Japanese, in comparison with Indians and North Americans, were more sensitive to signs of aggression in others and that they easily recognized aggression from subtle changes in facial expressions (Mandal, Harizuka, Bhushan & Mishra, 2001). A preliminary clinical investigation related to the current study using the State-Trait Anger Scale indicated that the Japanese scored higher on anger-in (covert aggression). On the other hand, Europeans and Americans scored higher on anger-out (overt aggression). According to the Buss and Durkee (1957) aggression scale, the former is "covert and latent indirect aggression"—latent expression of aggression—and the later is "overt and obvious, direct aggression ", i.e., verbal and aggressive behavior.

In order to investigate the relationship between aggression and health in Japanese people, it is necessary to develop new assessment tools that take the unique characteristics of the Japanese into consideration. This is particularly the case in the assessment of anger, because in Japanese people the effect of anger and aggression must be measured after excluding the effects of depression. Therefore, we developed the Overt-Covert Aggression Inventory (O-CAI), which can assess Japanese expressions of anger in the motoric form. Each sub-scale of the O-CAI, consists of 5 items, each of which are scored on a 5-point Likert scale. The objective of this study was to clarify the psychometric characteristics of the O-CAI, and to evaluate its reliability and validity.

Method

Participants in Study 1 were 3420 participants from amongst factory workers (Male: 3,104, female: 316, mean age 38.28 years ± 10.17). Participants in Study 2 were 111 workers (male: 57, female: 54, mean age 41.71 years ± 12.45). Participants in Study 3 were 100 students (male: 79, female: 21, mean age 22.70 years ±3.00). Participants in Study 4 were 126 employees of hospitals and other organizations (65 males, 61 females, age range 19-64 years, mean age 37.4 years, SD=10.5). All participants voluntarily consented to the study after being informed of its purpose.

Psychological Assessment Instruments

We used the following instruments in the present study. (1) The O-CAI (Table 2). (2) The Center for Epidemiological Studies Depression Scale, Japanese version (CES-D): A 20-item depression scale, in which the clinical cut-off point for depression is 16 (Radolf, 1977). (3) The Picture-Frustration Study (P-F Study): The Picture-Frustration Study is a projective test for assessing thought content in response to frustration (Rosenzwieg, 1945). It consists of 24 sketches, each of which depicts a common, frustrating situation in interpersonal relationships. The figure at the left in each sketch is shown making statements that help to describe the frustration felt by the other individual. The person on the right in each picture is shown with a blank caption balloon over his head. Respondents fill the caption balloon with what they think the person on the right would say in each situation. Responses to each item can receive one of 67 different scores, including non-redundant pair combinations of 11 response types, as well as un-scorable responses. In this study, we used only the direction of aggression, which includes "Extra-aggression" (aggression out, directed at others or the environment),"Intra-aggression" (aggression directed in, on self) and "Im-aggression" (non-directed aggression, or evasion). The

Japanese version of the Picture-Frustration Study was developed by Ichiya, (1986). (4) The Japanese version of the Buss-Perry Aggression Questionnaire (BAQ): A 24-item scale measuring aggression that includes physical aggression, short temper, hostility, and verbal aggression (Ando, Soga, Yamasaki, Shimai, Shimada, Utsuki, Oashi, & Sakai, 1999).

Procedure

In Study 1, we examined the psychometric properties of the O-CAI and assessed the factor validity and construct validity of this scale. Participants completed the O-CAI, and the Center for Epidemiological Studies Depression Scale (recovery rate 89.9%). All questionnaires were administered to participants and collected with the cooperation of the company's health center. Participants decide it of their own free will. In Study 2, we assessed the test-retest reliability of the O-CAI, 2weeks later (recovery rate 100%). In Study 3, we assessed the construct validity of the O-CAI using the Picture-Frustration study. Because of the cost of administering the Picture-Frustration study, Study 3 was conducted on sample of students (recovery rate 100%). We trained 5 psychology students to rate the Picture-Frustration Study. Raters discussed the scores until inter-rater agreement was 80%, using criterion derived from a previous study (Graybill & Blackwood, 1996). In Study 4, to further examine the concurrent validity of the O-CAI, we administered the Buss-Perry Aggression Questionnaire and the O-CAI to a sample of working people.

Data Analysis

Data were analyzed using SPSS for Windows, ver.10 and AMOS, ver.4.0.

Results

Statistical Characteristics and Item Analysis of O-CAI; (Study1).

Table 1 shows the mean score and standard deviation of each item. The range of the mean scores for all items in the O-CAI was 1.8-3.0, indicative of no extreme bias on any item, or a ceiling effect. We also calculated the item-total correlation coefficients to examine the item discriminant validity of the O-CAI (Table 1), which indicated that it was acceptable (0.42-0.53). These results suggested that the O-CAI has good psychometric properties.

Reliability of the O-CAI (Study 1 & Study 2)

We examined the Internal consistency of the subscales of O-CAI using Cronbach's coefficient α. The coefficients of overt and obvious direct aggression scale and that of covert and latent indirect aggression scale were 0.729 and 0.737 respectively (n=3420: Study 1). The test retest reliability of overt and obvious direct aggression scale and covert and latent indirect aggression scale were 0.884 and 0.841 respectively (n=111: Study2). These results indicate the adequate consistency and reliability of the O-CAI (Guilford, 1954).

Factor Validity of O-CAI (Study 1).

To analyze the factor validity of the overt and obvious direct aggression scale and the covert and latent indirect aggression scale, a principal component factor analysis was conducted using varimax rotation. We used three criteria to determine the number of meaningful components (1) an eigen value greater than 1.00, and inspection of the scree plot, (2) the proportion of the variance accounted (total factorial variance greater than 40% of the total variance), and (3) the interpretability criteria. Results indicated that the third factor had an eigenvalue less than 1.00. Therefore, we decided on a two-factor solution (see table2). Factor 1 closely corresponded to the scale concept of obvious direct aggression and Factor 2 to the scale concept of latent indirect aggression. We also conducted a confirmatory factor analysis by using AMOS, version

Table 1

Mean scores ,SD and item-total correlation coefficients of the O-CAI items

Item Number	M	SD	Item-total correration
1	2.85	1.24	0.45
2	2.75	1.15	0.49
3	2.87	1.12	0.51
4	3.28	1.16	0.54
5	3.33	1.21	0.42
6	2.35	1.09	0.53
7	2.60	1.24	0.51
8	2.33	1.00	0.47
9	2.98	1.29	0.56
10	3.30	1.21	0.47

Table 2

Results of factor analysis of O-CAI

Item	Obvious direct aggression	Latent indirect aggression	Communality
1 When I lose my temper, I take it out on something.	**0.46**	0.30	0.30
2 I ask questions from a know-all to embarrass him/her.	0.24	**0.53**	0.34
3 I have a shorter temper than others.	**0.62**	0.14	0.41
4 I make ironical remarks to people I dislike.	0.41	**0.52**	0.44
5 I speak up when I am angry	**0.49**	0.18	0.28
6 When I am ordered, I take halfway measures on purpose to vent my anger.	0.23	**0.60**	0.41
7 When I am yelled at, I shout back.	**0.53**	0.31	0.37
8 I ignore people I dislike much with others.	0.15	**0.57**	0.35
9 When I cannot bear my anger, I use abusive language.	**0.58**	0.35	0.46
10 I imagine revenge, but in fact I do nothing.	0.29	**0.48**	0.32
Eigenvalue	3.84	1.08	
Proportion (%)	38.43	10.81	