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℃ with 5% carbon dioxide in humidified air. The supernatants were harvested and stored at -80℃ until assay. The incubation time of 48 h was chosen on the basis of previous kinetic studies indicating that this time provides a good estimate for the production of the cytokines assessed here (DeGroote D, Zangerle PF, Gevaert Y, et al., 1992). 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. friends and NK cell counts (r = .18).

Results

Correlation Analysis

The descriptive statistical characteristics of each variable are shown in Table 2. When examining these relationships, partial correlation coefficients, for which the effects of stressor variables (perceived demands, quantitative work load) were statistically controlled, were calculated to assess so-called variables that have an effect on immune system function according to previous research (age, alcohol consumption, smoking, amount of exercise) and their direct effects. As can be seen in Table 3, with respect to quantitative parameters of the immune system, a weak but positive correlation was obtained between support from family members and There were no statistically significant correlations observed between T cell counts, B cell counts and each aspect of social support. On the other hand, with respect to qualitative parameters of the immune system, weak but positive correlations were obtained between all aspects of social support and Th1/ Th2 balance(r=.16~.26 :table4). And there were weak but statistically significant correlations

between social support except from 'support from family members or friends' and IL-4(r=-.16~-.19). In the following analyses, analyses were conducted by controlling the effects of variables consisting of age, alcohol consumption, smoking and amount of exercise.

Interaction Between Social Support Affecting Immune System Function and Occupational Stressors

An analysis was conducted in which the interaction between social support having an effect on immune system function and occupational stressors was calculated using a two-way analysis of covariance in order to investigate buffering effects. This analysis was conducted by introducing variable consisting of smoking, age, alcohol consumption and amount of exercise n the form of covariables. As a result, interaction was observed between social support from family members and significant others and job demands with respect to T-cell counts ($F[2,74] = 3.216$, $p = .046$) (see Table 5 and Fig. 1). As a result of conducting a study using a simple test of main effect, although individuals having a high level of social support in the case of high job demands have higher T-cell counts than those individuals with a low level of social support (mean = 1625.7 vs. 1350.3, respectively; $p<.01$), in the case of low job demands, it was clearly determined that T-cell counts are not affected by the degree of social support (mean = 1485.6 vs. 1504.7; n.s.). There was no other statistically dominant interaction obtained for other immune system parameters or support from superiors or coworkers .

Discussion

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. A rapid progress has been made in understanding the immune mechanisms involved in autoimmune diseases, depression, asthma, HIV, and bacterial infections, and showed the contribution of Th1 and Th2 cells are significantly important to these mechanisms. The present study clearly showed that the subjects with high social support had relatively high Th1 function and added a new and very important knowledge to the studies of social support and health. This correlation was irrespective of support sources. We might safely say that social support has direct effect on Th1 and Th2 balance and shift the balance toward Th1. The Th1 dominant state observed here could be attributable to IL4 inhibition by support from supervisors and coworkers.

The previous findings (Cohen, S. et al, 1997) described that the low

prevalence of colds and other illnesses among individuals with high levels of support. Furthermore the life-prolonging effects was generated by supportive care for cancer patients (Fawzy, F. I., N. W. Fawzy, C. S. Hyun, R. Elashoff, D. Guthrie, J. L. Fahey & D. L. Morton., 1993). Therefore, health might be maintained as a result of social support which sustains Th1 dominance. In male employees as well, it is expected that social support results in a Th1 dominant state, which in turn typically creates good health.

Social support from superiors and coworkers suppressed IL-4 productions. This coincides nicely with the previous research that showed support from friends in school attenuated the learning stress and reduced the severity of bronchial asthma (Kang, D H. Coe, C L. Karaszewski, J. McCarthy, D O. , 1998). The support from coworkers in a work setting acts in the same way as support from friends in a school setting.

In addition to the above findings, the subjects with high support from family and significant others had high NK cell counts. This agreed with the findings of previous research (Miyazaki, T., et al.2001). In the present research, however, there is no correlation with NKCA, It is difficult to conclude that natural immunity is high when social support is high. High NK cell counts should be interpreted as having a high latent ability for emergency. Thus, the direct effects on immunity other than Th1 /Th2 balance differs by the variables of immune parameters and the sources of support.

In terms of buffering effects of social support, when there is a high mental demand, the subjects with high social support from family and significant others had higher T cell counts than those with low support from the same sources. The support from the family and significant others acts on T cell counts as a buffer of stressors. Occasionally social support is an important factor in order to predict the immune function when that individual perceives how difficult a particular stressor is. Only in a limited situation and limited dependent variables, the buffering hypothesis was applicable.

In what manner does social support acts on physical health? Although Cohen and Willis(1985) stated that the hypothesis could differ depending on the evaluation methods of social support, this prediction was not necessarily strictly applicable at all times. Our results showed that the mechanisms by which support affects immune function is not determined by evaluation methods. We cannot , however , resolve this problem directly, since only one aspect of support (perceived support) was measured. It was not simply a matter of an alternative judgment either by direct effects or buffering effects. It was suggested that which hypothesis is applicable depends on both the support source and dependent variables.

Since the results of this research were obtained from the male employees, it is difficult to generalize this result to other populations, such as females or the elderly.

However, this is rather an extensive research for male workers and provided a trustful knowledge. Levy (1990) mentioned that emotional support is an important aspect when predicting the function of the immune system. The majority of the support measured in this research was emotional and perceived social support. Future studies should be undertaken by using the combination of multiple social support evaluation methods and other populations.

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Table 1

Sample characteristics

Age at study entry, M(SD), years	37.9(9.1)
Education status, %	
Junior high school	15.2
Senior high school	35.1
College	4.2
University	16.8
Graduate school	2.6
Marital status, %	
Currently married	53.3
Never Married	17.8
Divorced	2.2
Separated	0.5

Table 2

Sample description of Major Variables

variable	n	M(range)	SD
Social support			
Total score of support	567	45.5(24-60)	6.8
Support from superior	568	15.0(4-20)	3.0
Support from coworkers	570	15.2(4-20)	2.7
Support from family members or friends	570	15.2(4-20)	3.0
Total score of Quantitative work load	563	37.6(23-55)	6.4
Total score of Job control	559	43.4(16-80)	11.6
Total score of Mental demand	570	14.5(5-20)	2.4
Indices of Immune system			
T cells(CD3+) (cells/μl)	210	1466.1(200.8-2859.6)	463.5
NK cells(CD3-/CD56 or 16+)(cells/μl)	210	1054.6(132.8-2847.7)	421
Natural Killer Cell(NK Cell) Activity (%)	324	51.0(7-73)	12.9
Interferon-Gamma(pg/μl)	251	95.2(1-522)	105.5
Interleukin-4(pg/μl)	254	12.1(0-83)	13.9

Table 3

Support from coworkers	-0.05	-0.02
Support from family members or friends	0.12	0.18 *

Note. Partial correlation coefficients adjusted for age,alchol intaking,smoking,excersize,stressor.

n=128

* p<.05. ** p<.01.

Table 4

Total score of Support	-0.03	-0.01	-0.19	**	0.26	**
Support from superior	-0.06	0.00	-0.16	*	0.16	*
Support from coworkers	0.04	0.00	-0.18	**	0.24	**
Support from family members or friends	-0.04	-0.02	-0.11		0.20	**
	_n_=308	_n_=236	_n_=239		_n_=236	

* _p_<.05. ** _p_<.01.

Table 5－1

		T-cell			B-cell			NK-cell	
	N	mean	SD	N	mean	SD	N	mean	SD
social support × job control									
high support/high job control	37	1562.	424.8	37	376.9	196.6	37	1175.3	451.6
high support/low job control	37	1515.	479.9	37	356.1	149.7	37	1074.3	445.1
low support/high job control	27	1557.	489.3	27	405.5	155.9	27	1002.3	371.5
low support/low job control	31	1335.	494.7	31	380.4	267.0	31	909.9	413.6
		N.S.			N.S.			N.S.	
social support × quantitative workload									
high support/high quantitative workload	37	1539.4	372.3	37	362.3	192.4	37	1162.1	399.9
high support/low quantitative workload	35	1552.6	534.2	35	373.6	159.6	35	1087.3	508.6
low support/high quantitative workload	26	1373.9	431.7	26	369.8	227.1	26	925.3	406.2
low support/low quantitative workload	31	1507.2	556.4	31	411.5	219.7	31	972.9	384.5
		N.S.			N.S.			N.S.	

* social support means 'support from family'
* controling for smoking,alchol intaking,exercise,age

Table 5

Changes in immune index for each four subgroup (support ×stressor)

	T-cell counts			NK-cell counts			NKCA		
	n	M	SD	n	M	SD	n	M	SD
support × job demand									
high support/high job demand	32	1625.7	422.9	32	1161.9	357.8	79	48.7	12.6
high support/low job demand	41	1485.6	462.9	41	1102.4	513.8	76	51.1	13.5
low support/high job demand	27	1350.3	401.0	27	959.1	383.1	58	51.8	14.2
low support/low job demand	31	1504.7	558.3	31	928.1	403.8	98	52.9	12.1
	social support × job demand; $F(2,160)=3.965, p=.021$				N.S.			N.S.	

	INF-γ			IL-4			Th1/Th2 balance		
	n	M	SD	n	M	SD	n	M	SD
support × job demand									
high support/high job demand	68	112.3	123.5	69	13.5	15.8	58	13.3	13.0
high support/low job demand	49	81.5	98.0	50	9.8	13.2	49	18.6	19.6
low support/high job demand	67	88.0	93.9	67	11.9	13.3	67	12.4	13.4
low support/low job demand	50	101.2	109.8	51	13.9	13.9	50	9.7	9.5
		N.S.			N.S.			N.S.	

Note. Analysis of covariance was administrated on each condition, controling for smoking, alchol intaking, exercise, age.
Interaction was only shown on Tcell counts.
Support='support from family members or friends' N.S.= No Significant

Daily Hassles, the amount of individual's stressors, increase human T cell apoptosis in vitro.

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Abstract

Background: Recent studies have shown that apoptosis is involved in stress responses. **Methods:** The current study examined the relationship between stress and in vitro apoptosis of peripheral blood lymphocytes in humans using a cross-sectional design. Daily subjective stress in 40 non-smoking men was analyzed quantitatively by a daily hassles questionnaire. Apoptosis of T lymphocytes was measured by flowcytometry using Annexin V/PI double staining method after 0, 12, and 24h of culture in the presence or absence of dexamethasone (DEX). **Results:** Results showed that apoptosis of T lymphocytes in vitro has a significant correlation with stress and age. Stress was positively correlated with percentage of apoptosis in T cells after 12h of culture irrespective of DEX treatment. Age was positively correlated with percentage of apoptosis in T cells after 0 and 12h of co-culture with DEX. **Conclusions:** These results indicate that age-related apoptosis and stress-related apoptosis of T cells are modulated through different mechanisms. This is the first study to show that in vitro lymphocyte apoptosis is influenced by daily stress in a dose-dependent manner.

Keywords : Human, Daily Hassles, T Lymphocytes, Apoptosis, Neuroimmunology.

Introduction

Psychoneuroimmunological studies have revealed that stressors can enhance or suppress immune responses, depending on the type and duration of various stressors [1-3]. Acute stress enhances immunity whereas chronic or exhausting stress suppresses it [2-5].

Tsuboi et al. have shown that destruction of lateral hypothalamus areas increases splenocyte apoptosis [6]. Yin et al. found that chronic restraint stress increases splenic lymphocyte apoptosis in a rodent model by modulating CD95 expression [7]. These studies suggest that apoptosis of lymphoid cells is involved when stress affects immune functions.

Eilat et al. reported that lymphocytes derived from patients with major depression undergo increased apoptosis compared with normal subjects [8]. However, many unresolved questions remain to be answered. Can categorical psychiatric disorders such as major depression induce apoptosis? Does stress regulates lymphocyte apoptosis in a dose-dependent manner? And which subpopulation of lymphocytes undergoes apoptosis as a result of mental disorders or stressors? In the present study we examined the dose-dependency of the effect of stress on in vitro apoptosis of human peripheral T lymphocytes.

Because human subjects were involved, we took special care to ensure the highest ethical standards in conducting this study. Therefore, subjective stress was assessed non-intrusively using a questionnaire, without exposing the subjects to physically and/or psychologically stressful environments. In this study, we used daily hassles as a measure of stress level. The Daily Hassles Scale is a widely used scale in psychological research, which assesses irritating demands and annoyances that people confront in the course of their daily life; such as housekeeping, repairing one's car, quarrels with one's close friends [9,10]. We detected apoptotic cell death on flowcytometry using FITC conjugated Annexin V combined with propidium iodide (PI). The data on the relationship between stressors and in vitro lymphocyte apoptosis was statistically analyzed.

Materials and Methods

Participants

The subjects were 40 non-smoking male workers recruited from a private company in Japan. They were administered the Daily Hassles Scale for Workers, a self-administered questionnaire for evaluating daily hassles [11]. We also obtained blood samples from the subjects after

obtaining their written informed consent.

Daily hassles questionnaire

The Daily Hassles Scale for Workers (DHS-W) [11] assesses twenty-two daily irritants and annoyances on the basis of Lazarus's stress model . A prior study [11] had evaluated the reliability and validity of this scale and shown that the DHS-W can adequately measure the level of psychological stressors [11]. Items chosen for inclusion on the DHS-W had been pretested and refined. A factor analysis of the pretested sample had suggested 5 scales that clustered around work-related problems, interpersonal conflict in the workplace, problems in family life, health-related problems, and having not enough time to spare. The final version of the scale had demonstrated adequate internal consistency (alpha-coefficients ranged from 0.69 to 0.83). The DHS-W is designed to measure both the frequency of hassles and the intensity of reaction to hassles experienced during the past 3 months and rated as 0-4 scales ranging from 0 (not occur or not at all a hassle) to 4 (frequently occurred or a great deal of a hassle). The 5 factors were used as scales and scored by summing the frequency and intensity ratings within the factor (the Frequency Score and the Intensity Score, respectively). Finally, the Total Frequency and Intensity Score by summing the scores across 5 factors were obtained. The Total Frequency Score was significantly correlated with the Center of Epidemiologic Studies Depression Scale (CES-D) and the General Health Questionnaire 12 (GHQ12), both instruments for evaluating mental status ($r = 0.39$, 0.47, respectively). The Total Frequency Score was employed as an amount of individual's stressors in data analysis.

Preparation of Peripheral Blood Lymphocytes

Heparinized blood samples were obtained between 8 and 11 a.m. and diluted with equal quantities of 0.9% NaCl within 6h of obtaining samples. The time intervals between sampling and analysis are due to the distance between the sampling site and the laboratory. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Lymphoprep (Nycomed Pharma, Norway), according to the manufacturer's instructions. The PBMC were washed twice and suspended in RPMI 1640 medium (Dainippon Pharmaceutical, Japan) supplemented with 10% FCS (Equitech-Biokerrville, US) and kanamycin (Meiji Seika, Japan). Viable cells were counted by tripan blue dye exclusion assay and prepared at a concentration of 1×10^6/ml.

Induction of lymphocyte apoptosis

For the induction of lymphocyte apoptosis, 10^{-7} M of dexamethasone (DEX) (Banyu Phama, Japan) was added to the cell suspension in 96-well flat-bottom plates (Beckton Dickson, US). The cells were cultured for 12 and 24h at 37 °C with 5% carbon dioxide in humidified air.

Cell Staining and flowcytometry

After 0, 12, and 24h of incubation, the cells were harvested and mixed with FITC-conjugated Annexin V, propidium iodide (PI), and ECD-conjugated CD3 (Beckman Coulter, France) at 4 °C for 30 min. The cells were gently washed and resuspended in phosphate buffered saline (PBS) and analyzed on four-color flowcytometry (EPICS-XL□, Beckman Coulter, France) using the standard method.

Statistical analysis

For statistical analysis, data on percentage of apoptosis of T cells were logarithmically transformed because of their skewed distribution. We excluded extreme biological data points on the percentage of in vitro apoptosis of T lymphocytes. Exclusion criteria were extreme values on the box-and-whisker plot. Thirty-seven data points were used in analyzing T cell apoptosis. We conducted repeated measures ANOVA with Bonforroni post hoc to examine time-dependent changes of lymphocyte apoptosis and paired t test to evaluate the effect of DEX. Pearson's correlation analysis was performed to confirm that apoptosis of T cells after 12 and 24h of culture reflect apoptosis before culture. Linear regression analysis was conducted to assess the association of stressors and age with percentage of apoptosis of T cells. All reported differences were significant at a $p < 0.05$, using two-tailed tests.

Results

Detection of in vitro apoptosis in T lymphocytes by flowcytometry using Annexin V and PI

Subpopulations of lymphocytes are usually assessed on viable cells in scattergram gated on Region A in figure 1. As shown in Region B, however, the cell population with a relatively low forward scatter and high side scatter emerged in the cultured, physically stressed, lymphocytes. This tendency was more prominent in DEX-treated cells than in untreated cells (data not shown). Cell shrinkage is one morphological feature of apoptosis. Dive, C. et al. showed that most of these cells (Region B, fig.1) are apoptotic, whereas nearly all of the cells that have not undergone any reduction in cell size (Region A, fig. 1) are not apoptotic [12].

Besides their findings, the previous studies showed that PS redistribution precedes cell shrinkage in cells undergoing apoptosis [14, 16]. When assessing apoptosis, it is necessary to measure the population including the cells that have not undergone any reduction in cell size (Region A, fig.1), as well as shrinking cells (Region B, fig.1).

Annexin V has been used as a probe for externalization of phosphatidylserine in various cell lines [13-15]. DNA binding dye, propidium iodide (PI), can assess permeability of plasma membrane that is a feature of late phase apoptosis or secondary necrosis. The present study measured apoptosis of lymphocytes using Annexin V/PI double staining. We measured early apoptosis (Annexin V+/PI-), late apoptosis or secondary necrosis (Annexin V+/PI+), and total apoptosis (Annexin V+/PI+ or PI-) on the population gated on Region C in figure1.

Dillon SR et al. demonstrated that PS exposed on B cells in vivo does not reflect early apoptosis. Percentage of apoptotic T cells was assessed in the present study.

Time points and Treatment with DEX

As shown in figure 2, a repeated measures ANOVA with Bonferroni post hoc showed that percentage of Annexin V+/CD3+ cells in Region C increased until 12h of culture and remained unchanged from 12 to 24h, though percentage of Annexin V+/PI+ T cells after 24h of culture in the absence of DEX decreased compared with that after 12h of culture, which may be due to long cultivation. The results suggest that percentage of in vitro apoptosis of T cells should not be assessed after 24h of culture but after 12h of culture. We also compared the proportion of apoptosis in DEX-treated cells with that in untreated cells to assess the effect of DEX on T cell apoptosis. Paired t test showed that lymphocytes treated with DEX underwent apoptotic cell death compared with those without DEX treatment (fig.2).

To confirm that percentage of apoptotic T cells after culture corresponds to that before culture, Pearson's correlation analysis was performed (table 1). The results showed that percentage of apoptotic T cells after 12 and 24h of culture (in vitro apoptosis) was positively correlated with that before culture (ex vivo apoptosis). Exceptionally, percentage of apoptotic T cells after 12h of culture in the presence of DEX was not correlated with ex vivo apoptosis. Incubation exerts a physical stress on lymphocytes but the results indicated that in vitro apoptosis of cultured T cells partially reflects percentage of ex vivo apoptosis

Figure 1

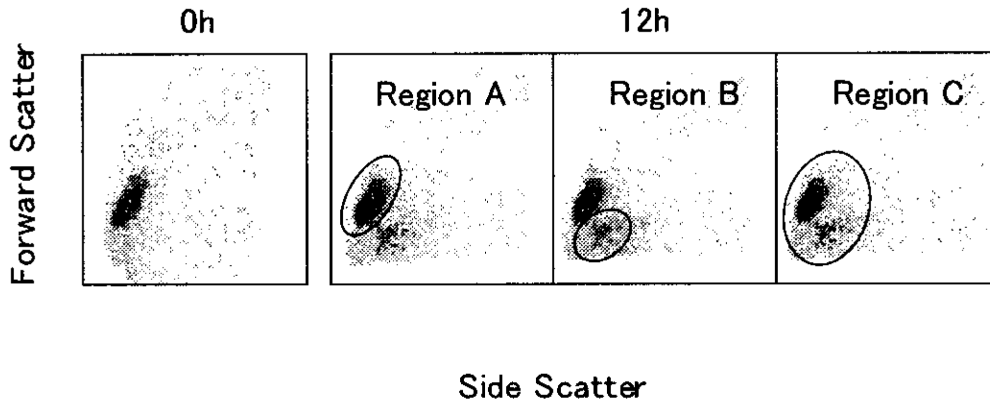


Figure 2

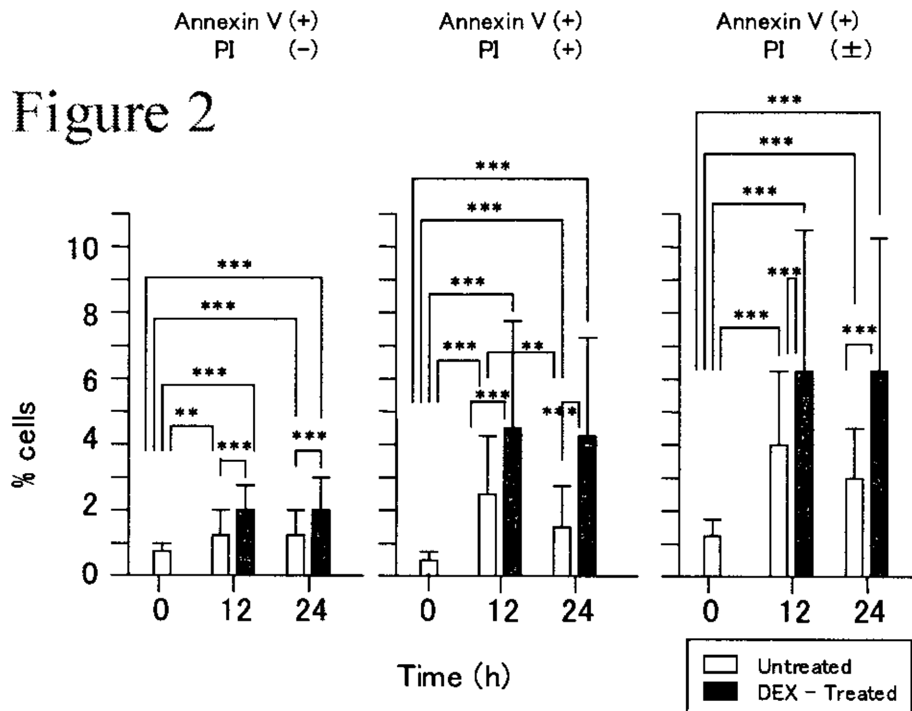


Table 1. Correlations between percentage of in vitro apoptosis of T cells before and after culture : Pearson's correlation coefficients

Table 1		12h		24h	
Annexin V	PI	Untreated	DEX-Treated	Untreated	DEX-Treated
+	-	0.601***	0.668***	0.510***	0.376*
+	+	0.395*	0.111	0.482**	0.397*
+	+ or -	0.379*	0.485**	0.456**	0.660***

$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Percentage of apoptotic T cells after 12 and 24h of culture was positively correlated with percentage of apoptosis before culture with the exception of 12h DEX-treated cells (Pearson' s correlation analysis).

Figure 3

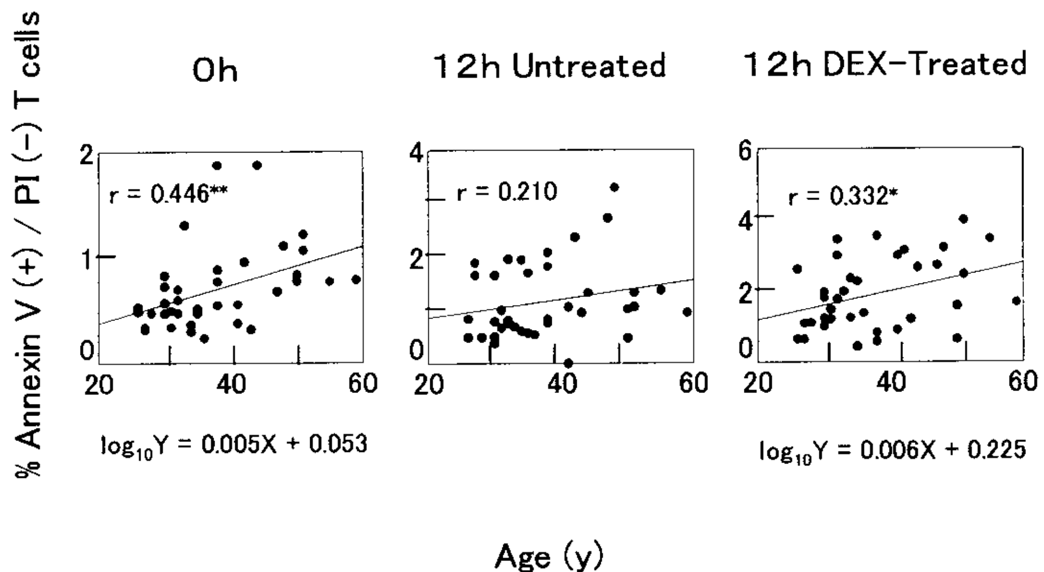


Table 2. Correlations between stressors and percentage of in vitro apoptosis of T cells: Pearson's correlation coefficients

Table 2			12h	
Annexin V	PI	0h	Untreated	DEX-Treated
+	-	-0.077	0.169	0.245
+	+	0.125	0.411*	0.361*
+	+ or -	-0.004	0.356*	0.342*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Stress level was measured using the Total Frequency Score of hassles obtained from the data on the Daily Hassles Scale for Workers (DHS-W). Stressors had a positive correlation with Annexin V+/PI+ and Annexin V+/PI+ or PI- T cells after 12h of culture regardless of DEX treatment.

Figure 4

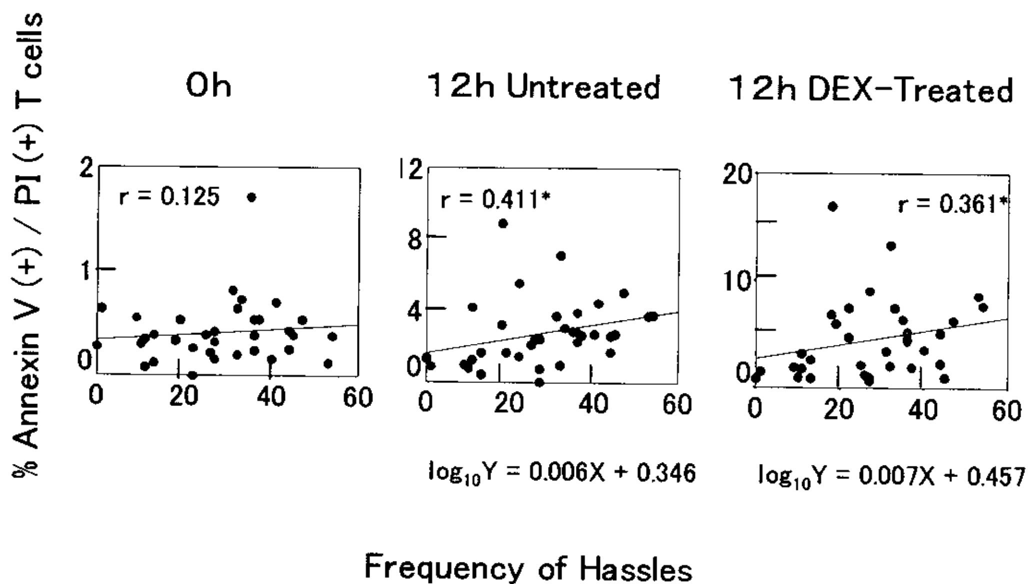
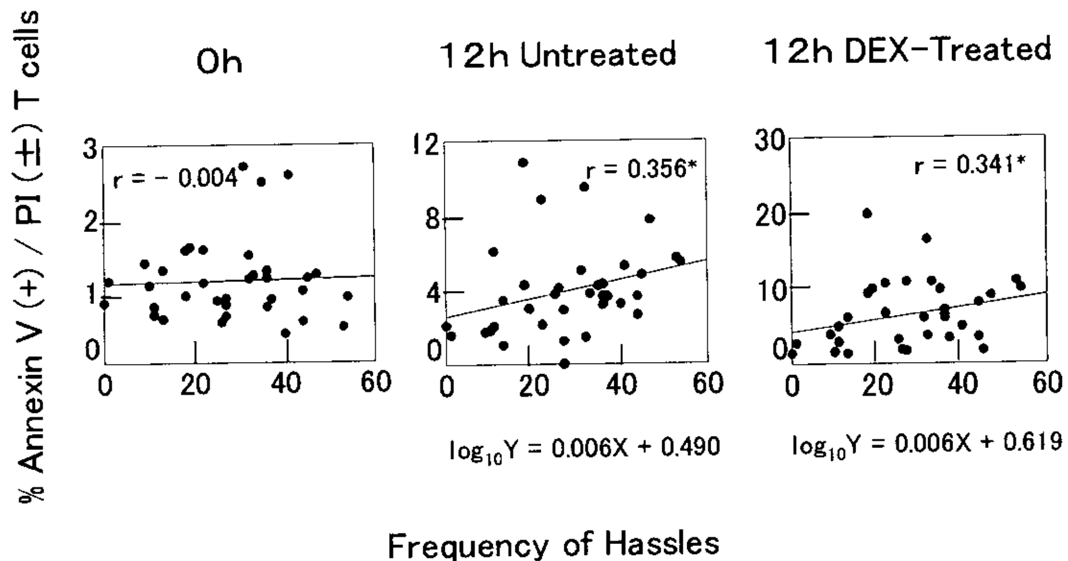


Figure 5



of T cells. Thus, among 4 combinations of culture time (12h vs 24h) and treatment (Untreated vs DEX-treated), 12h of untreated culture will be the best condition to assess the effects of stressors on in vitro apoptosis of T cells, which most reflects ex vivo apoptosis.

Aging increased apoptosis in T lymphocytes in vitro

Simple linear regression analysis showed that age was positively correlated with percentage of Annexin V+/PI- T cells (early phase apoptosis) after 0 and 12h of DEX-stimulation ($r = 0.446$, $p = 0.005$; $r = 0.332$, $p = 0.048$, respectively)(fig.3).

Stressors increased apoptosis in T lymphocytes in vitro

We assessed stress level using the Total Frequency Score of hassles from the data on the Daily Hassles Scale for Workers developed by Tomioka et al [11]. We performed simple linear regression analysis for evaluating if stressors can increase apoptosis of lymphocytes in humans. Stressors did not correlate with spontaneous apoptosis in T cells (table 2, fig. 4, 5). In cultured lymphocytes, stressors had a significant positive correlation with Annexin V+/PI+ (late apoptosis or secondary necrosis) (untreated: $r = 0.411$, $p = 0.014$; DEX-treated: $r = 0.361$, $p = 0.036$) and Annexin V+ (total apoptosis) (untreated: $r = 0.356$, $p =$

0.036; DEX-treated: $r = 0.342$, $p = 0.047$) T cells after 12h of culture regardless of DEX treatment (table 2, fig. 4, 5). Multiple linear regression analysis, controlling for age, showed that the significance was maintained (data not shown). The results suggested that stressors increase in vitro apoptosis in T lymphocytes.

Discussion

Major depression is a categorical entity, which has been implicated in increased apoptosis of peripheral blood lymphocytes. In contrast, the daily hassles score used in the present study is a continuous variable, which was positively correlated with T cell apoptosis in vitro. Previous studies have shown that the daily hassles score is a good predictor of the prognosis of several illnesses [17-21]. Thus, our findings extend the scope of the relationship between lymphocyte apoptosis and general mental health. Furthermore, this study specified the lymphocyte subsets that are involved in this relationship. The present data imply that accumulated daily hassles increase in vitro apoptosis of T cells in a dose-dependent manner.

Stressors did not correlate with spontaneous T cell apoptosis at 0h of culture, but did so with apoptosis of 12h-cultured T cells, irrespective of DEX treatment. This finding suggests that stress does not increase apoptosis in vivo, but altered some mRNAs expression and protein synthesis of T cells making them ready for apoptosis. Chronic restraint stress has been found to increase apoptosis and Fas expression of splenocytes in rats [7]. Involvement of Fas in stress-induced apoptosis is implicated in human lymphocytes as well, though the changes in the expression of Fas and Fas ligand in our study might be influenced by the long duration of culturing.

This study also demonstrated that peripheral blood T lymphocytes derived from elderly people undergo increased spontaneous apoptosis at 0h of culture and DEX-induced apoptosis after 12 h of culture compared with T cells from young people. The association between spontaneous apoptosis in T cells and aging found in the present study is supported by previous findings [22-24]. An increased expression of Fas and Fas ligand [25,26] and a decreased Bcl-2 [25] expression have been observed in T cell subsets from elderly humans compared with young controls. There is evidence that a decline in proliferation of T lymphocytes as well as altered gene expression of apoptosis promoting (fas) and inhibiting (bcl-2) factors are involved in increased apoptosis of T cells in aged humans. Expression of IL-2 receptors and secretion of IL-2