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アレキシサイミア傾向がT細胞のin vitro Apoptosisに与える影響

酒見正太郎 [1),2)]、中田光紀 [3)]、福西勇夫 [4)]、小牧元 [1)]、山村隆 [1)]、川村則行 [1)]

1） 国立精神神経センター
2） 東京大学医学部公衆衛生学教室
3） 産業医学総合研究所
4） 東京都精神医学総合研究所

要約
アレキシサイミアの人の生理学的ストレス反応の特異性を、末梢血T細胞のApoptosisをしらべることで評価した。TAS-20 の総得点と感情の同定困難の得点が、12 時間培養後の AnnexinV＋/PI＋/CD3＋（%）と正の相関があった。アレキシサイミア傾向（TAS-20、感情の同定困難）の高い人は、自覚ストレスのレベルに関係なく 12 時間培養後の AnnexinV＋/PI＋/CD3＋（%）が高値を示した。アレキシサイミア傾向（TAS-20、感情の同定困難）の低い人は、自覚ストレスのレベルの増加に伴い 12 時間培養後の AnnexinV＋/PI＋/CD3＋（%）が上昇した。これらの結果は、アレキシサイミア傾向の人の生理学的ストレス反応の特異性を示すものであると考えられる。

アレキシサイミアは感情の同定困難、言語化困難、想像的思考の欠如、外的思考を特徴とする性格特性である（Sifneos PE 1996）。アレキシサイミアは、一般人口ではあまり頻繁に見かけない性格特性であるが（Salminen JK et al. 1999）、心身症や精神疾患患者は非常に多くみられることが知られている（Parker JD et al. 1993, Taylor GJ et al. 1990）。そのため、多くの研究者がアレキシサイミアとストレスの関係をしらべてきた。

Martin JB et al.（1995）は、アレキシサイミアの人は特定の状況をストレスフルであると感知する能力にかけていること、それ故に、ストレスフルなイベントを普通の人より長く経験すると提言した。そして、かれらは、このことが身体的な機能不全をともない、ストレスが仲介する病気や症状の発症・進展につながると考えた。

このようなアレキシサイミアとストレスのつながりに関する理論は、様々な実証研究で支持されてきた。多くの研究が、アレキシサイミアの人は、交感神経活動が高いことを示した（Henry JP et al. 1992, Martin JB1985, Stone LA et al. 2001）。DST は、精神医学でうつ病などの病気の診断や治療のための生物学的マーカーとして研究されてきたが、Lindholm T et al.（1990）は、アレキシサイミアの人はDST陽性となることを示した。Dewaraja R et al.（1997）が、アレキシサイミアの人は、CD8＋細胞とNK細胞の数が少ないことを示した。これらの研究は、アレキシサイミアの人のストレス反応がアレキシサイミアでない人と異なっていることを表してい

る。そして、アレシシサイミアの人の特異なストレス反応が、精神科心療内科疾患の高頻度の発症につながることが示唆している。

Apoptosis は、組織から細胞を除去するプロセスであり、DNA 断片化、核細胞質の凝縮、細胞膜 blebbing を特徴としている。 Apoptosis は細胞のターンオーヴァー、embryogenesis、免疫機能など多岐にわたる生物学的プロセスにおいて重要な働きをしている。最近の研究が、うつ病で末梢リンパ球の Apoptosis が増えることが明らかにした(Eilat E et al 1999)。Yin D et al. (2000)が、ラットの実験で慢性抑圧性のストレスが脾臓細胞の Apoptosis を増やすことが見つけた。われわれは、先の研究で心理的ストレスが人の T 細胞の invitro Apoptosis の増加と関連があることを示した(Sakami S et al. 2002-2003)。しかしながら、われわれの知る限り、性格特性がリンパ球の Apoptosis に与える影響を調べた研究はない。

Stevenson JR et al. (2001)は、カテコラミンが α 受容体を介して Lymphoid organ の Apoptosis を誘導することを示した。アレキシサイミアにおける交感神経の過剰活動を考えると、アレキシサイミア傾向は、(たとえ仮にストレスが人のリンパ球の Apoptosis を増やすとしても)ストレスとは独立に、リンパ球の Apoptosis を多くすることが可能なのではないだろうか？ 情動反応の欠如を特徴とするアレキシサイミアは、ストレスによる免疫変化を修飾するのではないだろうか？

今回の研究の目的は、心理的ストレスとアレキシサイミア傾向が、人の末梢リンパ球の Apoptosis に与える影響を横断デザインでしらべることである。過去のアレキシサイミアのストレス反応に関する研究の大部分は、被験者にストレス刺激をあたえるような実験的デザインで行われた。今回の研究では、subjects をストレスフルな状況にさらないように、デイリーハッスルズ質問紙(Tomioka M et al. 2000)で主観的なストレスを評価した。アレキシサイミア傾向は、TAS-20(Fukunishi I et al. 1997)で評価した。末梢リンパ球の Apoptosis は AnnexinV/PI で評価した。AnnexinV は、B 細胞の Apoptosis の評価が不正確であるため(Dillon SR et al. 2000)、今回の研究では、T 細胞の Apoptosis のみ評価した。前述の仮説を確かめるために、われわれは、ストレスとアレキシシミアの二つの要因が人の末梢リンパ球の Apoptosis に与える影響を調べ、統計的に解析した。

方法

対象

対象は 40 人の非喫煙男性である。インフォームドコンセントを得て、質問紙の調査と採血を行った。

TAS-20

日本語版 TAS-20(Fukunishi I et al. 1997)を使ってアレキシサイミア傾向を調べた。(1)感情同定困難(2)感情言語化困難(3)外的思考の3つの因子が、アレキシサイミアの構造を反映している。TAS-20 の総得点とサブスケールの得点は連続変量としてデータ解析に用いた。

デイリーハッスルズ

The Daily Hassles Scale for Workers (DHS-W) [11] assesses 22 daily irritants and annoyances on the basis of Lazarus's stress model [9, 10]. 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 scored by adding up the frequency and intensity ratings mentioned for each factor (the Frequency Score and the Intensity Score, respectively). Finally, the Total Frequency and Intensity Score were calculated by adding up the scores of 5 factors. The Total Frequency Score was significantly correlated with the Center of Epidemiologic Studies Depression Scale [12, 13] (r = 0.39) and the General Health Questionnaire 12 [14, 15] (r=0.47). The Total Frequency Score was employed in data analysis.

免疫測定

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.

Cell Staining and flowcytometry

After 0 and 12h 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 II, Beckman Coulter, France) using the standard method.

統計解析

データ解析に先立ち、Apoptosis のデータは分布が歪だったが、箱ひげ図を基にして2つのデータポイントを除外したら正規分布が得られた。心理学的変数とApoptosis の間の関係を調べるために、ピアソンの相関分析をおこなった。ストレスとアレキシサイミアとこれらの交互作用をあらわす項が Apoptosis(%)に及ぼす影響を調べるために、階層的重回帰分

析をおこなった。独立変数は centered してから回帰式に投入した。最初のステップでハッスルズの頻度を投入した。第 2 ステップでアレキシサイミア傾向を投入した。次のステップでこれらの交互作用をあらわす項を投入して、アレキシサイミア傾向のグレードを基礎にして、ストレスが T 細胞の Apoptosis%と分別的に関係しているかどうかを調べた。年齢は回帰モデルに入れても T 細胞の Apoptosis%に有意な貢献をしなかったので、年齢はモデルから除去した。

すべての解析は、両側検定で $p<0.05$ を有意と考えた。

結果

表1にあるとおり、T 細胞を12時間培養すると、Early phase と Late phase の Apoptosis(%)は培養前より増加した。先のわれわれの研究の研究(Sakami S et al. 2002-2003)で示したとおり、12時間培養後の T 細胞の Apoptosis%は、0 時間培養後の Apoptosis%と中等度から高度の相関関係があった(表2)。このことは、培養は細胞に物理的なストレスを与えるが、in vitro Apoptosis は exvivo Apoptosis を部分的に反映していることを示している。

ハッスルズの頻度は、12 時間培養後の AnnexinV＋/PI＋/CD3＋(%)と正の相関があった(表 2)。アレキシサイミア傾向のスコアの中では、同定困難だけが、12 時間培養後の AnnexinV＋/PI＋/CD3＋%と正の相関があった(表 2)。これは、12 時間培養後の Late Apoptosis(%)が今回測定した心理的変数に鋭敏であることを示している。12 時間培養後の Late Apoptosis%に対するストレスとアレキシサイミア傾向の寄与を回帰モデルでしらべることにした。

回帰分析をするに当り、心理変数間の関係をしらべた(表2)。TAS-20 の総得点とサブスケールの間に中等度から強度の正の相関があった。同定困難と表出困難の間に中等度の正の相関があった。外的思考は他の2つのサブスケールとは関連がなかった。

表 3、4 は、12 時間培養後の AnnexinV＋/PI＋/CD3＋%に対する階層的重回帰の結果を示している。TAS-20 と3つのサブスケールは、2つの重回帰式に別に投入した。

ストレスと TAS-20 とそれらの交互作用項を独立変数として等式に入れた最終モデルでは、12 時間培養後の AnnexinV＋/PI＋/CD3＋(%)に対して、TAS-20 は主効果を持たなかったが、ストレスと TAS-20 の交互作用は marginally に有意であった(表 3)。ストレスは全てのステップを通じて 12 時間培養後の AnnexinV＋/PI＋/CD3%の増加と関連があった。

ストレスと TAS-20 のサブスケールのスコアの 12 時間培養後の AnnexinV＋/PI＋/CD3＋(%)に対する最終回帰モデルでは、同定困難が主効果とストレスと一緒に交互作用をもった(表 4)。その代わりに、ストレスによる Apotosis を増やす効果は marginally に有意だった。

ストレスと TAS-20、同定困難の交互作用のパターンを調べるために、高低のアレキシサイミア傾向をもつ人に対する回帰式の傾きの有意性をt検定で調べた。メディアンスプッリットをベースにして TAS-20 と同定困難を高低の 2 群に分けた。

図の1，2は交互作用をイラストレイトしている。TAS-20 が高い人では、ストレス頻度は 12 時間培養後の AnnexinV＋/PI＋/CD3＋(%)と関連がなかった(β

=-0.53、t=-0.21、p=0.84)。一方、TAS-20が低い人では、ストレス頻度が12時間培養後のAnnexinV＋/PI＋/CD3＋(%)の上昇と有意に関連していた(β=0.61、t=3.264、p=0.004)。同様に、同定困難のスコアが高い人では、ストレス頻度はApoptosisと関連がなく(β=-0.11、t=0.313,p=0.76)、同定困難が低い人では、ストレス頻度が12時間培養後のAnnexinV＋/PI＋/CD3＋%の上昇と有意に関連していた(β=0.68、t=2.69,p=0.02)。

Table 1. Descriptive statistics of psychological and immunological variables (n=38)

Variables	Mean	(SD	)Range
Age	37.82	(8.72	)26 -59
Total score of TAS-20	48.47	(7.65	)35 -66
Difficulty identifying feelings	12.05	(4.13	)7 -23
Difficulty describing feelings	14.21	(3.81	)8 -23
Externally-oriented thinking	22.21	(2.99	)16 -30
Frequency of daily hassles	28.06	(14.07	)0 -54
Apoptotic CD3+ cells (%)			
0h of culture			
Annexin V+/PI-	0.95	(1.41	)0.24 -9.05
Annexin V+/PI+	0.49	(0.46	)0 -2.05
12h of culture			
Annexin V+/PI-	1.36	(1.26	)0 -7.47
Annexin V+/PI+	2.58	(1.58	)0 -7.13

Table 2. Pearson's correlation coefficients among psychological and immunological variables

Variables	1	2	3	4	5
1. Age	—				
2. Total score of TAS-20	-0.24	—			
3. Difficulty identifying feelings	-0.21	0.75 ***	—		
4. Difficulty describing feelings	-0.21	0.81 ***	0.43 **	—	
5. Externally-oriented thinking	-0.06	0.49 **	-0.01	0.20	—
6. Frequency of daily hassles	0.13	0.33 *	0.31 [b]	0.41 *	-0.13
7. Annexin V+/PI- CD3+ (%) (0h)[a]	0.03	0.06	0.04	-0.02	0.10
8. Annexin V+/PI+ CD3+ (%) (0h)[a]	0.20	-0.04	-0.07	-0.06	0.08
9. Annexin V+/PI- CD3+ (%) (12h)[a]	0.08	0.09	0.14	-0.06	0.11
10. Annexin V+/PI+ CD3+ (%) (12h)[a]	0.15	0.22	0.34 *	0.08	-0.02

[a] incubation time (h). [b] p = 0.07

* p<0.05, ** p<0.01, *** p<0.001.

(Table 2. continued)

	6	7	8	9	10
7.	-0.15	—			
8.	0.05	0.54 ***	—		
9.	0.01	0.77 ***	0.62 ***	—	
10.	0.45 **	0.12	0.58 ***	0.55 ***	—

Table. 3 Results of hierarchical regression analysis for frequency of daily hassles and Total score of TAS-20 predicting Annexin V+/PI+ CD3+ cells (%) after 12h of culture (n=38)

Independent variables	β	t		F change	Cumulative adjusted R^2
Step1				8.43*	0.167
Frequency of daily hassles	0.44	2.90	**		
Step 2				0.32	0.151
Frequency of daily hassles	0.41	2.55	*		
Total score of TAS-20	0.09	0.56			
Step 3				3.69	0.212
Frequency of daily hassles	0.33	2.07	*		
Total score of TAS-20	0.11	0.72			
Hassles x TAS-20	-0.29	-1.92	[a]		

[a] p=0.06. * p<0.05, ** p<0.01.

Table. 4
Results of hierarchical regression analysis for frequency of daily hassles and factor scales of TAS-20 predicting Annexin V+/PI+ CD3+ cells (%) after 12h of culture (n=38)

Independent variables	β	t		F change	Cumulative adjusted R^2
Step 1				8.43*	0.167
Frequency of daily hassles	0.44	2.90	**		
Step 2				1.44	0.197
Frequency of daily hassles	0.45	2.71	*		
Difficulty identifying feelings	0.32	1.92	[a]		
Difficulty describing feelings	-0.25	-1.42			
Externally-oriented thinking	0.09	0.56			
Step 3				2.22	0.277
Frequency of daily hassles	0.29	1.71	[b]		
Difficulty identifying feelings	0.36	2.25	*		
Difficulty describing feelings	-0.24	-1.32			
Externally-oriented thinking	0.06	0.36			
Hassles x Difficulty identifying feelings	-0.42	-2.31	*		
Hassles x Difficulty describing feelings	0.13	0.72			
Hassels x Externally-oriented thinking	-0.16	-1.10			

[a] p=0.06. [b] p=0.09. * p<0.05

Figure 1

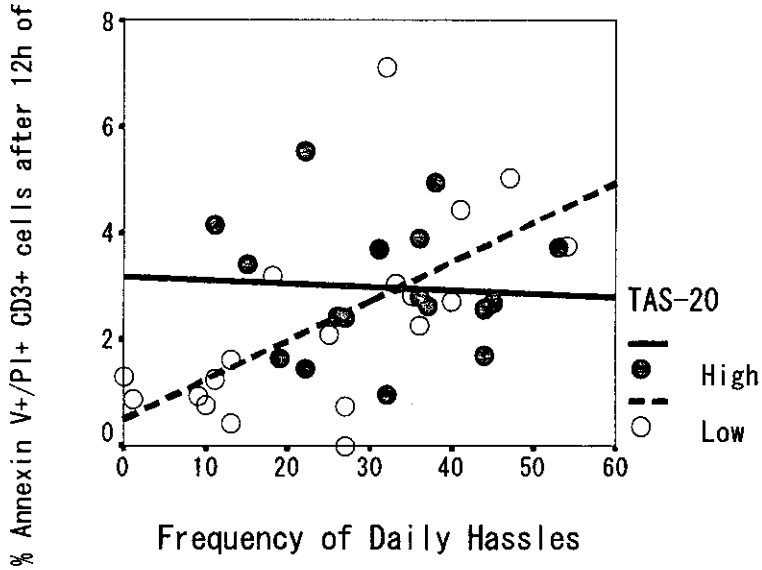
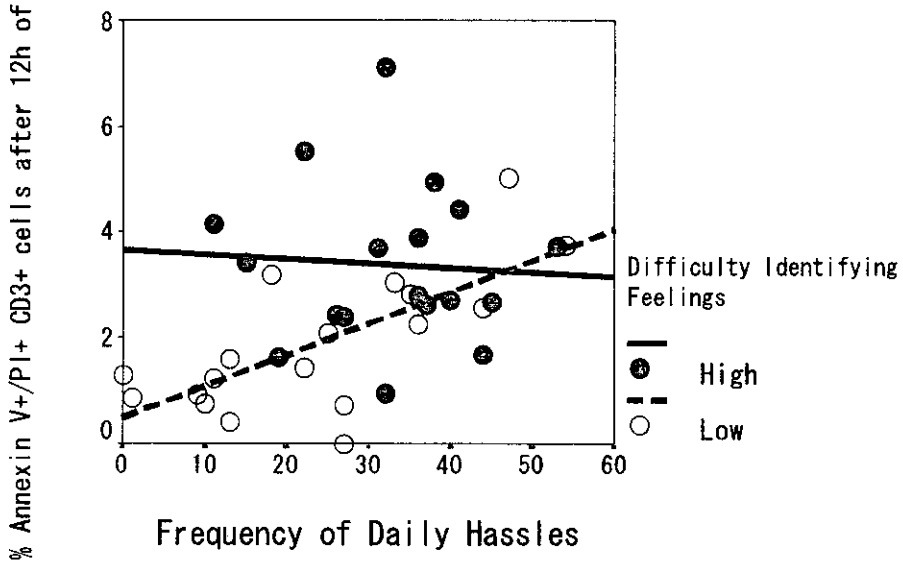


Figure 2



考察

この研究は、同定困難は、12 時間培養後の in vitro AnnexinV＋/PI＋/CD3＋(%)の上昇と関連していることを示した。同定困難のスコアの高い人のT細胞が、培養という物理的ストレスに対してより敏感なことを示唆した。

近年の動物研究が、カテコラミンがリンパ組織の Apoptosis を誘導することを示した(Stevenson JR et al.)。研究間で必ずしも結果に一致が見られないものの、アレキシサイミアはいくつかの研究で、ベースラインでの高い交感神経活動と関連していた(Henry JP et al. 1992, Martin JB1985, Stone LA et al. 2001)。これらの研究結果から考えると、アレキシサイミア傾向は、培養前の Apoptosis と関連がなかったという結果は予想に反する。Stevenson JR らの実験では、カテコラミン投与は、投与後 12 時間後の時点でコントロールに比べて 10 倍になる血中濃度を給した。われわれの研究では、高アレキシサイミア傾向における交感神経活動の上昇を調べていないが、たとえあったとしても、培養前の Apoptosis%の上昇と関連をもつのに十分なだけ高くはなかったのかもしれない。

12時間培養した後でも、同定困難は、Late phase Apoptosis (AnnexinV＋PI+)のようには Early phase Apoptosis (AnnexinV＋PI-)とは関連がなかった。理由は不明であり、今後の課題である。

TAS-20 のサブスケールの中で、同定困難だけが in vitro Apoptosis の上昇と関連があった。

アレキシサイミアを構成する要素(同定困難、表出困難、外的思考)と生理学的変化や病気との関係を明らかにした過去の研究は非常に少ない。Berthoz S et al.(2002)は、臨床的に健康な人を対象とした研究で、同定困難と表出困難の合計得点の高い人は、低い人と比べて情動反応を強く呼び起こすような刺激に対する fronto-cingulate cortices の活動性が違っていることを見つけた。

今回の研究では表出困難と in vitro Apoptosis(%)の間に関連は見られなかったが、同定困難と表出困難の間に中等度の相関があった。同定困難の高い人にみられた in vitro Apoptosis(%)の上昇は、情動をおこす刺激を処理する領域の機能異常と関連があるのかもしれない。

Wehmer F et al.(1995)は、同定困難や表出困難でなく、外的思考とベースラインの心拍数の上昇に関係があることを示した。今回の研究では、同定困難(α =0.84)や表出困難(α =0.69)に比べて外的思考の信頼性係数が極端に低かった(α =0.15)。Komaki G らは、日本語版 TAS-20 における、外的思考の信頼性係数の低さをしめした(unpublished data)。尺度の不正確さが、外的思考が invitro Apoptosis と関連がなかったことを部分的に説明しているのかもしれない。

先の研究で示したとおり、ストレスの頻度は 12 時間培養後の AnnexinV＋/PI＋/CD3＋(%)の上昇と関係があった。同定困難の低い人では、ストレスは12時間培養後の AnnexinV＋/PI＋/CD3＋(%)の上昇と関連があったが、同定困難の高い人では、ストレスは Apoptosis と関連がなかった。Berthoz S et al.(2002)は、同定困難(と表出困難)のレベルが高い人と低い人の間に活動性の違いが見られたのは、anterior cingular と mediofrontal cortices であり、limbic structure (amygdala 、hippocampal formation 、hypothalamus)にはこのような違いが認められなかったため、アレキシシミアは刺

激の感情的内容の評価に異常があると述べている。今回の研究で同定困難が高い人で、ストレスの頻度が in vitro Apoptosis と関係がなかったのは、ストレスの質的評価が同定困難の低い人と違っていたからかもしれない。ストレスに対する感情的な appraisal を測定することが、この仮説をサポートすると考えられる。

今回の研究は、アレキシサイミア傾向(とくに感情の同定困難)が末梢血 T 細胞の in vitro Apoptosis 増加と関連があることを示した。アレキシサイミア傾向の人では、自覚ストレスに関係なく in vitro Apoptosis が高いレベルにあった。アレキシサイミア傾向の人のストレスに対する情動過程の特異性を、生物的立場から示唆する結果であると考えられる。

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Suppression of cellular immunity and readjustment problems in subjects with a past history of posttraumatic stress disorder

Shotaro Sakami [1),2)], Toshio Ishikawa [1)], Nozomu Asukai [3)], Takashi Haratani [4)], Fumio Kobayashi [5)], Osamu Fujita [6)], Norito Kawakami [7)], Shunichi Araki [4)], Akira Fukui [8)], Hiroshi Iimori [9)], Noriyuki Kawamura [1)].

1) Department of Psychosomatic Medicine, National Institute of Mental Health, National Center of Neurology and Psychiatry, Japan.
2) Department of Public Health, Faculty of Medicine, University of Tokyo, Japan.
3) Department of Social Psychiatry, Tokyo Metropolitan Institute of Psychiatry, Japan.
4) National Institute of Industrial Health, Japan.
5) Department of Health and Psychosocial Medicine, Aichi Medical University School of Medicine, Japan.
6) Center for Health Care, Aichi University of Education, Japan.
7) Department of Hygiene and Preventive Medicine, Social and Environmental Life Sciences/Social Medicine and Longevity Sciences, Okayama University Graduate School of Medicine and Dentistry, Japan.
8) Department of Internal Medicine, Nagoya University School of Medicine, Japan.
9) Department of Internal Medicine, Nihon University School of Medicine, Japan.

Abstract

Objective: Increased rates of medical morbidity and compromised well-being and quality of life have been reported in posttraumatic stress disorder (PTSD) subjects. We examined lower immune function and readjustment problems in subjects in remission from past PTSD.

Method: Out of 1550 Japanese male workers, 12 with past PTSD were recruited through the Impact of Event Scale-Revised (IES-R) and Structured Clinical Interview for DSM-IV (SCID). We matched the controls by age and smoking habit and compared lymphocyte subset counts, NK cell activity, and interferon-γ (IFN-γ) and IL-4 production through phytohemagglutinin (PHA) stimulation. A self-administered questionnaire for evaluating sleep habits and a Generic Job Stress Questionnaire (GJSQ) were used to assess readjustment problems in subjects with past PTSD.

Results: The numbers of lymphocytes and T cells, NK cell activity, and total amounts of IFN-γ and IL-4, were significantly lower in the past PTSD group. Interpersonal conflict and depression were significantly higher in subjects with a past history of PTSD than those who had encountered traumatic events without developing PTSD. There were no statistically significant differences in other job related stresses. Subjects with a past history of PTSD had more nightmares than those with no history. However,

there was no difference in any tendency for perceived insomnia.
Conclusion: PTSD leaves a long lasting immunosuppression, and has long-term implications for health. The ongoing stress of PTSD appears to manifest itself in various forms of interpersonal conflict and nightmares many years after exposure to the trauma. These symptoms can be classified as intrusion and avoidance. Therefore in order to help patients suffering from PTSD, it may prove useful to manage their problems according to the basic concept of PTSD.
Keywords: past PTSD, immunity, readjustment.

Introduction

It has been suggested that PTSD not only has psychological, but also biological long lasting morbid effects. A 50-year prospective study demonstrated that combat exposure predicted early death and chronic illnesses by the age of 65 (1). A link between severe stress exposure and later physical diseases has also been mentioned (2). As yet, this assumption lacks biological evidence. A series of recent studies revealed various biological dysfunctions in the patients with current PTSD, such as low levels of 24-hour urinary cortisol excretion (3), high NK cell activity (4) and high CD4 and CD8 T cell numbers (5,6). No study, however, has focused on such chronic biological effects among those who had PTSD in the past, but who are now in remission.

Besides physical and psychological problems, several studies also examined to what extent the quality of life and well-being of PTSD patients were compromised in the context of social life. Among victims of Nazi persecution, survivors of death camps yield poorer academic and occupational achievement than those who had been in labor camps or in hiding (7). About half of the survivors of Nazi persecution suffer from insomnia and nightmares 50 years after the end of World War II (8). Patients who recovered from PTSD are defined as having a past history of PTSD. Except for Nazi Holocaust survivors (8, 9), there are a much smaller number of studies on past PTSD patients compared to studies on current PTSD patients. Further research on patients who recovered from PTSD is necessary for a wider understanding of the PTSD problem.

This study investigates the association between a past history of PTSD with any present immune dysfunction and readjustment problems at worksite (job stress and sleep problems), so as to detect any long lasting morbid effects of this disorder.

Methods

Participants

From July 1997 to April 1998, we provided a self-administered questionnaire for evaluating perceived job stress and sleep habits to 1,550 male workers randomly recruited from an electric equipment manufacturing company. From October 1998 to May

1999, a second survey was conducted on the same subjects whose aim was to establish the prevalence of subjects with PTSD among Japanese male workers and to assess their immune functions. We investigated readjustment problems among past PTSD patients selected from the latter research. Both of these studies were performed with written informed consent.

Diagnosing PTSD

We administered the Japanese versions of the Events Check List and the IES-R (10) to 1,550 male workers. Past exposure to traumatic events was examined through the Events Check List that comprised of 15 categories such as natural disasters, violence, bullying, etc. Those who had such exposure were screened for traumatic symptoms through the Japanese version of the IES-R (11), which has demonstrated 100% sensitivity for subjects with current and lifetime diagnosis of PTSD using the cut-off point of 24. Fifty-two male subjects with an IES-R score greater than 24 were interviewed by the Structured Clinical Interview for DSM-IV (SCID) (12) to diagnose current and past PTSD and other axis-I disorders. Exclusion criteria included a previous history of any axis I disorder other than PTSD, use of any psychotropic medication within 2 months prior to this study and any physical illness that needed treatment. Inter-rater kappa for the first twelve participants was 0.916.

We found 12 men who had a past history of PTSD (Table 1 and 2). The events that caused past PTSD were various: bullying, traffic accidents, witnessing cruel death, violence, fire, death of the family and flood. One subject had not developed PTSD before the first survey. The subject with no history of PTSD in the first study was eliminated from the data analysis on job stress and sleep problems.

For immunological assessment, we recruited as many control subjects as possible from those with exposure to trauma but whose IES-R scores were below 25, on a matched basis for age and smoking and found 48 controls for the 12 subjects with past PTSD (case: control =1:4). There was no significant difference in the number of years passed since the exposure to trauma between the men with (Mean: 9.48, SD: 1.55) and without past PTSD (Mean: 9.25, SD: 1.25) ($t = 0.03$, $p = 0.93$). Of 390 men with exposure to traumatic events and an IES-R score of < 25, 360 completed questionnaire on stress and sleep problems. To evaluate job stress and sleep problems in men with a past history of PTSD, these 360 subjects were selected as controls.

Table 1. Recruitment of the Subjects with a Past History of PTSD

	Male		Female		Total	
Initial subjects	1550		183		1733	
Subjects who completed the Events Check List and the IES-R	1009	(100%)	137	(100%)	1146	(100%)
Subjects who had experienced traumatic events	447		44		491	
Subjects with an IES-R score ≧25	57		11		68	
Subjects who agreed to go through SCID interview	52		9		61	
Current PTSD	3	(0.30%)	0	(0.00%)	3	(0.20%)
Past PTSD	12	(1.20%)	3	(2.20%)	15	(1.30%)

Table 2. Profiles of Men with a Past History of PTSD

Variable	Mean	(SD)
Age	37.7	(2.6)
Time since events occurred (years)	9.5	(1.6)
IES-R		
Total	37.0	(2.9)
Intrusion	13.9	(1.6)
Avoidance	13.4	(1.0)
Hyperarousal	9.8	(1.7)

Immunological assessment

The total numbers of WBC and leukocyte differential counts were determined by a Coulter counter (Beckman Coulter, Fullerton, CA). Lymphocyte subsets were determined by flowcytometry analysis (EPICS XL, Beckman Coulter, Fullerton, CA). Enumeration by flowcytometry included the following cells: T cells (CD3/FITC), B cells (CD19/PE), two types of T cell subsets (CD4/FITC, CD8/PE), and NK cells (CD3/ECD, CD16/FITC and CD56/PE) (Beckman Coulter, Fullerton, CA). PHA-stimulated IFN-γ and IL-4 productions were measured by ELISA kits (Biosource International, Camerilo, CA) as described previously (13, 14).

The NK cell activity assay was performed using K562 as target cells (E:T=20: 1), 51Cr release as the lysis indicator.

Generic job stress questionnaire

We measured perceived job stress using scales derived from the NIOSH generic job stress questionnaire (15, 16). Scales of job control, mental demand, quantitative workload, and social support were used in order to assess three dimensions of the demand-control-support model (17). In addition, interpersonal conflict, employment opportunities, and job future ambiguity, job satisfaction and depressive symptoms (the Center of Epidemiologic Studies Depression Scale, CES-D) were also assessed.

Sleep questionnaire

Five questionnaire items provided information about difficulty initiating sleep (DIS), difficulty maintaining sleep (DMS), early morning awaking (EMA), insufficient sleep, and nightmares during the past year.

Statistical Analysis

The results on immunological assessment were analyzed using the Student's t test with Levine's test. The results on job stress and sleep problems were analyzed using the Mann-Whitney U test. All differences were significant at a $p < 0.05$, using two-tailed tests.

Results

The numbers of lymphocytes, CD3+, CD4+, and CD8+ cells, NK cell activity, the total amounts of IFN-γ and IL-4, and those produced by T cell, were significantly lower in subjects with past PTSD than control subjects (Figure 1). The numbers of CD19+ ($t=-1.95$, $p=0.07$) and CD3-/CD56+ or CD56- ($t=-0.37$, $p=0.73$) were lower in subjects with past PTSD than control subjects though the differences did not reach the significance (Figure not shown).

As shown in Table 3, scale scores of interpersonal conflict and depression were significantly higher in subjects with a past history of PTSD than those who had never experienced it. There were no significant differences between PTSD patients and control subjects in any other variable including perceived job control, mental demand, quantitative workload, social support, and dissatisfaction with their job.

Table 4 shows that subjects with a past history of PTSD had more nightmares than those without PTSD. But there was no difference between the case and control group in perceived insomniac tendency.

Figure 1.
Means (SD) of Lymphocyte Subpopulations and Cellar Immunity in Men with a Past History of PTSD and Comparison Subjects. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

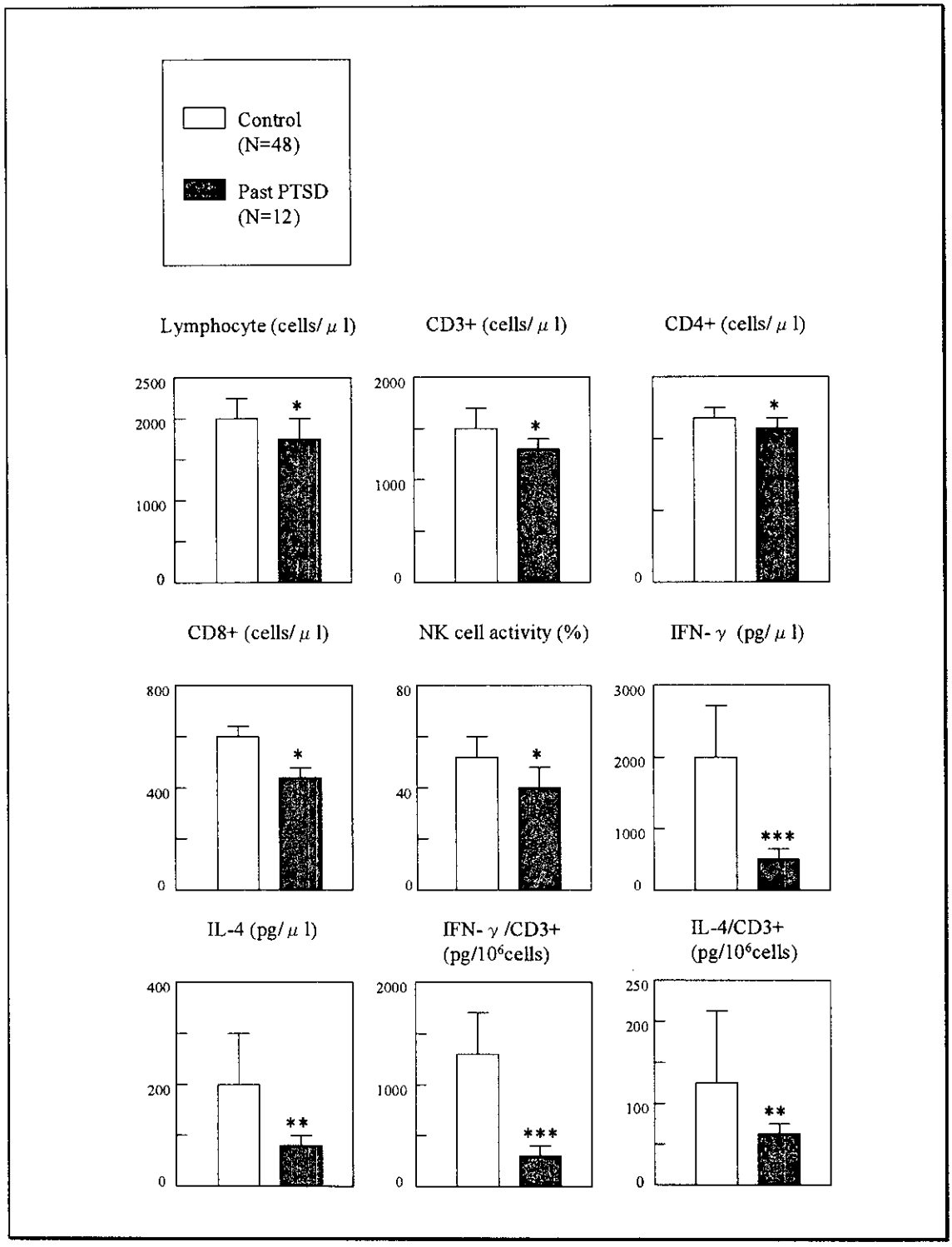

Table 3. Differences of Perceived Job Stress between Men with a Past History of PTSD and Comparison Subjects

Variable	Men with a past history of PTSD (N = 11) Mean	(SD)	Comparison subjects (N = 360) Mean	(SD)	*U*	*p*	
Age	35.4	(10.2)	38.2	(10.3)	1659	0.34	
Perceived control	43.5	(16.4)	43.3	(12.5)	1865	0.83	
Mental demand	14.3	(3.6)	14.3	(2.5)	1888	0.83	
Interpersonal conflict (cooperation)	23.9	(3.1)	20.1	(4.9)	985	<0.01	**
Interpersonal conflict (conflict)	25.2	(5.6)	20.3	(4.9)	934	0.01	**
Employment opportunity	12.4	(2.1)	11.7	(1.9)	1669	0.37	
Quantitative workload	38.8	(4.5)	37.3	(6.8)	1286	0.36	
Job future ambiguity	15.4	(4.0)	15.4	(3.7)	1957	0.95	
Job satisfaction	7.5	(1.4)	8.5	(1.8)	1169	0.06	
Social support from supervisor	13.2	(3.8)	14.6	(3.0)	1590	0.28	
Social support from coworkers	14.9	(2.8)	15.2	(2.7)	1797	0.61	
Social support from family/friends	16.2	(2.1)	15.0	(3.2)	1534	0.21	
CES-D	18.7	(8.4)	12.7	(6.5)	1162	0.01	**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4. Differences of Sleep Problems between Men with a Past History of PTSD and Comparison Subjects

Variable	Men with a past history of PTSD (N = 11) Mean rank	Comparison subjects (N = 360) Mean rank	*U*	*p*	
Difficulty initiating sleep (DIS)[a)]	172.6	184.9	1832.5	0.70	
Difficulty maintaing sleep (DMS)[b)]	206.2	176.7	1443.0	0.32	
Early morning awaking (EMA)[c)]	145.7	185.7	1536.5	0.19	
Insufficient sleep[d)]	215.8	185.1	1652.0	0.31	
Nightmares[e)]	116.4	187.1	1214.5	<0.01	*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

a) DIS: "How long does it take you to fall asleep?" (open-ended question)

b) DMS: "How many times do you tend to wake up a night?" (open-ended question)

c) EMA: "Do you tend to wake up early and lay in bed?" (1=very often/ 2=often/ 3=occasionally/ 4=never)

d) Insufficient sleep: "Do you sleep well?"(1=very well/ 2=well/ 3=normal/ 4=poor / 5=fairly poor)

e) Nightmares: "Do you have terrible dreams at night?" (1=very often/ 2=often/ 3=occasionally/ 4=never).

Discussion

Men with a past history of PTSD had a reduced number of T cell and T cell subset, cellular immunity (NK cell activity and IFN- γ), and humoral immunity (IL-4) than those without PTSD. Although the reported traumatic events were less threatening than holocaust or crime, the subjects strikingly suffered from global immunosuppression. Present PTSD has been claimed to activate cellular immunity, presumably due to the "fight or flight" up-regulatory immune response by "flashbacks" or episodes of reliving the stressor (4, 5). This supports our findings where, quite separate from the 12 past PTSD group, three current PTSD participants had a significantly higher NK cell activity (Mean: 52.7%, SD: 10.2%) compared to their controls (Mean: 48.2%, SD: 8.2%). Further investigations are required for elucidating the mechanisms of immunological up-regulation of current PTSD and down-regulation of past PTSD.

PTSD patients have a higher prevalence of psychiatric comorbidity such as depression, anxiety disorder, and alcohol/drug abuse. Patients with a past history of PTSD in this study did not have a personal history of major depression, yet nevertheless, they had a higher CES-D score than the control group. Their mean CES-D score exceeded the cutoff point (16.0) of the Japanese general population. This fact hints at the intriguing possibility that CES-D might be helpful for detecting almost half of the subjects with a history of PTSD. Hence a history of PTSD should be taken into account when screening for depression using the CES-D.

PTSD patients tend to suffer from nightmares and other insomnia related problems (8, 18). Concerning the insomniac tendencies of difficulty initiating and maintaining sleep as included in criteria D (hyperarousal), there were no significant differences between the PTSD and control groups. On the other hand, patients with a past history of PTSD reported more nightmares as included in criteria B (intrusion), than those without PTSD. The results indicate that intrusion still remains in patients who had a past history of PTSD even after hyperarousal symptoms had disappeared.

We investigated the readjustment problems of patients with a past history of PTSD at length. Our results pointed out that subjects with a past history of PTSD suffered interpersonal conflicts at the workplace compared to those without PTSD. Interpersonal conflict at the workplace is a scale designed to assess thoughts, emotions, and behaviors related to group memberships by using 16 items divided into two subscales, conflict (i.e. quarreling and disagreement with one another) and cooperation (i.e. helping and getting along with one another). Previous study indicated that both occupational factors (monotonous work and high educational level) and psychological (neuroticism, hostility, and low self-assurance) characteristics are predictive of interpersonal conflict at the