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White matter lesions as a feature of cognitive impairment, low vitality and other symptoms of geriatric syndrome in the elderly

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Aim: White matter lesions (WML) are common findings on magnetic resonance imaging (MRI) in elderly persons. In this study, we analyzed the relation of WML with global cognitive function, depression, vitality/volition, and 19 symptoms of geriatric syndrome in Japanese elderly patients who attended three university geriatric outpatient clinics.

Methods: Two hundred and eighty-six subjects (103 men and 183 women; mean ± standard deviation age, 74.5 ± 7.8 years) were included in this study. MRI scans were performed for the diagnosis of WML, and the severity of periventricular and deep white matter hyperintensities (PVH and DWMH) was rated semiquantitatively. Concurrently, all subjects underwent tests of cognitive function, depressive state and vitality, and were examined for 19 symptoms of geriatric syndrome.

Results: The study subjects showed cognitive decline, depression and low vitality, all to a mild extent. Univariate linear regression analysis showed a negative correlation between the severity of WML and cognitive function or vitality. Multiple logistic analysis revealed that the severity of WML was a significant determinant of cognitive impairment and low vitality, after adjustment for confounding factors such as age, sex and concomitant diseases. PVH and/or DWMH score was significantly greater in subjects who exhibited 13 out of 19 symptoms of geriatric syndrome. Logistic regression analysis indicated that WML were associated with psychological disorders, gait disturbance, urinary problems and parkinsonism.

Conclusion: WML were associated with various symptoms of functional decline in older persons. Evaluating WML in relation to functional decline would be important for preventing disability in elderly people.

Keywords: deep white matter hyperintensity, geriatric syndrome, periventricular hyperintensity, white matter lesion.

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Introduction

Brain magnetic resonance imaging (MRI) has markedly enhanced the chance of detecting characteristic hyperintense signals in the periventricular and subcortical areas on T2-weighted images, even in asymptomatic older persons.[1] These lesions are known as white matter lesions (WML), leukoaraiosis or white matter (periventricular and subcortical) hyperintensities.[2–4] WML, which accompany symptoms of gait abnormalities,[5–7] urinary symptoms[5,8,9] and cognitive impairment,[4,10,11] are reported to be associated with aging,[12–14] hypertension,[14] diabetes[15] and atherosclerosis.[5] There is poor understanding of the pathogenesis of the lesions, and it remains unknown whether WML are mere innocuous radiological changes that appear as a result of the aging process,[2,3,10] or whether they are one of the causal factors of the functional decline in elderly people.

Geriatric syndrome is a group of symptoms that are related to daily life, and the comorbidity triggers the loss of independence of elderly persons. Hence, evaluation of geriatric syndrome is important for the physical and mental care of the elderly. To address the pathological significance of WML in the global cognitive and psychological functions, and in geriatric syndrome in representative Japanese elderly subjects, we organized a group of geriatric outpatient clinics, and investigated the clinical manifestations of WML in those patients. Especially, we analyzed the relation of WML with global cognitive function, depressive state, vitality/volition and 19 symptoms of geriatric syndrome.

Methods

Subjects

This was a multicenter study performed at three different university geriatric outpatient clinics in Japan under the organization of a Longevity Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (H15-Choju-013). Two hundred and eighty-six consecutive subjects (103 men and 183 women; mean ± standard deviation [SD] age, 74.5 ± 7.8 years) were included in this study: 187 at Kyorin University Hospital, 74 at Chiba University Hospital, and 25 at Nagoya University Hospital, from January 2004 to January 2005.

The diagnosis of dementia was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The definition of hypertension was systolic blood pressure (BP) of more than 140 mmHg or diastolic BP of more than 90 mmHg, or receiving antihypertensive drugs. The definition of diabetes was glycosylated hemoglobin A1c of more than 6.5%, or receiving antidiabetic drugs. The definition of hyperlipidemia was total cholesterol of more than 5.72 mmol/L, triglyceride of more than 1.70 mmol/L, or receiving antihyperlipidemic drugs.

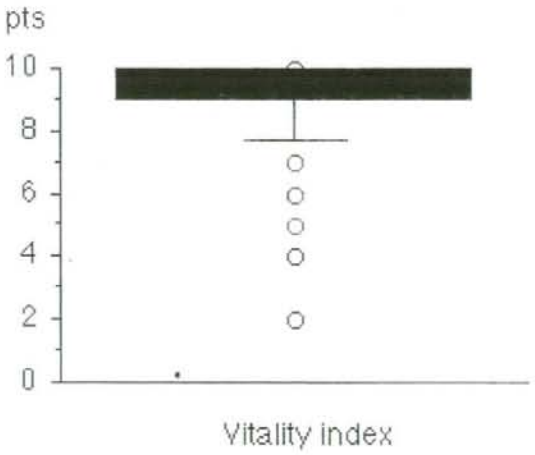

Figure 1 Distribution of vitality index. All subjects underwent assessment of vitality index as a measure of vitality related to activities of daily living (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0–10).

All subjects underwent the following assessment of global cognitive and psychological function. Cognitive function was evaluated by Mini-Mental State Examination (MMSE).[16] In this examination, we focused on calculation (serial subtraction of 7 from 100) to evaluate attention and working memory (part of the frontal lobe function). We also performed verbal fluency or word recollection test by asking the subjects to name as many vegetables as possible, which is also indicative of the frontal lobe function. Depression was evaluated by the 15-item Geriatric Depression Scale (GDS-15), which consists of 15 dichotomous questions for screening depressive symptoms in elderly subjects (range, 0–15).[17] Vitality index was used to measure vitality or volition in daily life (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0–10).[18] A full score can be maintained until one is severely disabled in cognition or function. The distribution of vitality index in the subjects of this study is shown in Figure 1.

We examined symptoms of geriatric syndrome: 19 dichotomous questions about hallucinations, delusions, insomnia, vertigo, paralysis, numbness, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, constipation, decreased appetite, weight loss, apathy, speech impairment, swallowing difficulty, tremor and muscle stiffness.

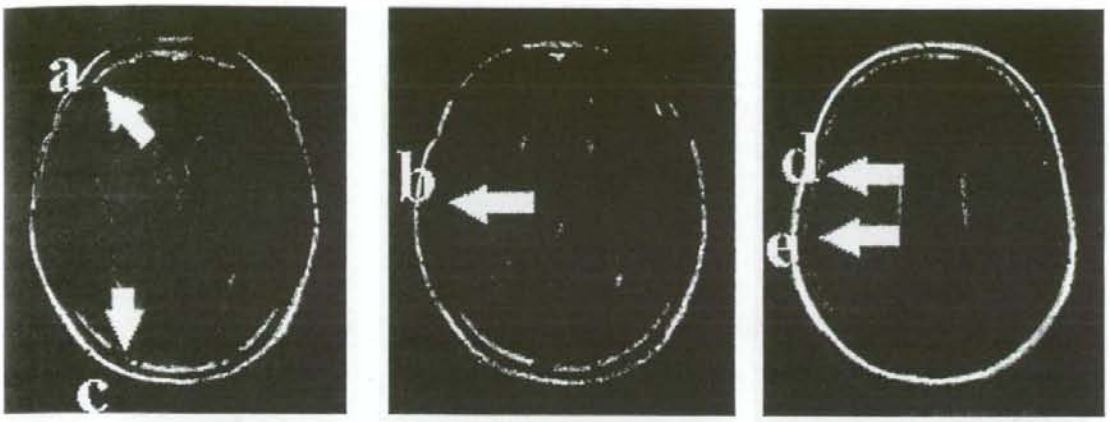

Figure 2 Evaluation of periventricular hyperintensity (PVH). PVH were evaluated in six regions in three slices: (a) adjacent to the frontal horns, (b) lateral ventricular body, (c) occipital horns, (d) frontal central semiovale in the parietal region and (e) occipital centrum semiovale in the parietal region in both hemispheres. Each area was rated as five grades according to the method of Junque *et al.*: (0) no hyperintensities; (1) <25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) >75%.[11] The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Magnetic resonance imaging

Magnetic resonance imaging scans were performed for the diagnosis of WML and cerebral infarction on 1.5-T scanners (Toshiba, Nasu, Japan). T1-weighted images (repetition time [TR], 496 ms; echo time [TE], 12 ms), T2-weighted images (TR, 4280 ms; TE, 105 ms), and fluid-attenuated inversion-recovery (FLAIR)-weighted images (TR, 8000 ms; TE, 105 ms; 5-mm slice thickness) were obtained in the axial plane. MRI images were examined to differentiate between WML, characterized by isointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images, and cerebral infarction, characterized by hypointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images.

White matter lesions were classified as periventricular hyperintensities (PVH), which adjoined the lateral ventricle, and deep white matter hyperintensities (DWMH), located in the deep white matter apart from the lateral ventricles.

Periventricular and deep white matter hyperintensity scores

Periventricular hyperintensities were evaluated in six regions in three slices: adjacent to the frontal horns, lateral ventricular body, occipital horns, frontal central semiovale in the parietal region, and occipital centrum semiovale in the parietal region in both hemispheres (Fig. 2). Each area was rated as five grades according to the systematic quantification method developed by Junque *et al.*: (0) no hyperintensities; (1) less than 25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) more than 75%.[11] The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Deep white matter hyperintensities were evaluated in the frontal, temporal, parietal and occipital lobes, and in the basal ganglia in both hemispheres (Fig. 3). Each lesion was rated as three grades according to the diameter by the study of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) more than 10 mm. The sum of all grades in five regions in both hemispheres was defined as the DWMH score.[4] Analysis was performed assuming that the white matter scores of PVH and DWMH were quantitative interval scales.

Statistical analysis

The relationship between two continuous variables such as MMSE, GDS-15 or vitality index, and WML (PVH or DWMH) score was analyzed by univariate linear regression analysis, and the correlation was analyzed by means of Pearson's simple correlation coefficients. Statistical significance was set at $P < 0.05$.

The relation of cognitive impairment or low vitality with PVH score or DWMH score was assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. Cognitive impairment and low vitality were defined as an MMSE score of 23 or less[19] and a vitality index of 9 or less, respectively. Odds ratios and 95% confidence interval were calculated from the, coefficients and their standard errors.

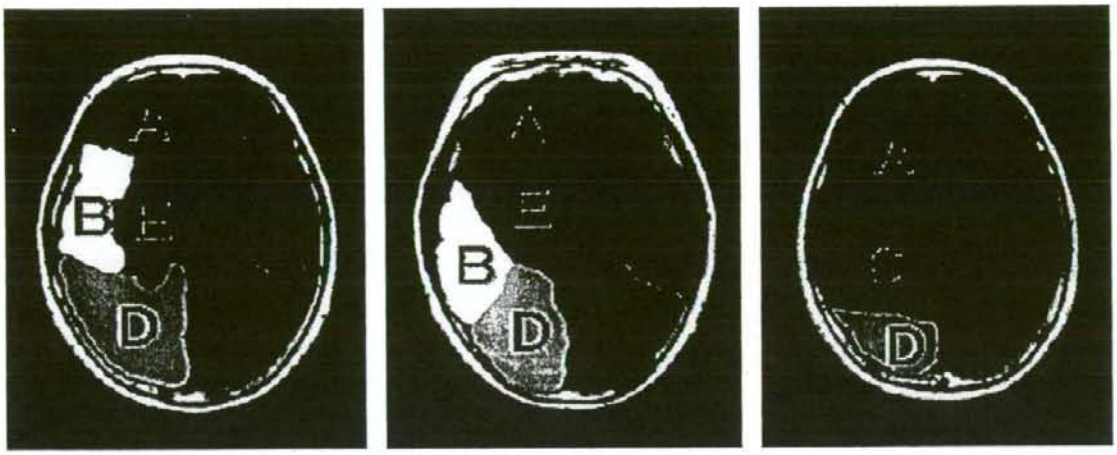

Figure 3 Evaluation of deep white matter hyperintensities (DWMH). DWMH were evaluated in the (A) frontal, (B) temporal, (C) parietal and (D) occipital lobes, and (E) in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to diameter by the method of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) >10 mm.[4] The sum of all grades in five regions in both hemispheres was defined as the DWMH score.

Periventricular hyperintensity score or DWMH score was compared between subjects who did or did not exhibit each symptom of geriatric syndrome and analyzed by Student's *t*-test. When the difference was considered to be significant ($P < 0.05$), the difference was further assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease.

Ethical considerations

This study was approved by the ethical committees of the institutes involved in this project. We explained this study clearly, and obtained written consent from all participants and their guardians (mainly family members). All the data were stored and analyzed carefully to preserve the subjects' anonymity and protect their privacy.

Results

Clinical data

The clinical characteristics of the study subjects are shown in Table 1. The mean age of subjects was 74.5 ± 7.8 years (mean ± SD), and subjects aged 65 or older comprised 88.1%. The mean body mass index was 21.8 ± 3.3 kg/m^2 and none of the subjects were obese. Of the subjects, 10.1% had experienced stroke or other cerebrovascular disease and 22.7% were smokers. Hypertension, diabetes and hyperlipidemia were present in 50.7%, 27.3% and 50.0% of the subjects, respectively.

White matter lesions

Periventricular hyperintensities and DWMH were observed in 77.7% and 96.7% of the total subjects, respectively. The mean score of PVH and DWMH was 5.5 ± 4.8 and 35.5 ± 39.8, respectively (Table 1). Pearson's correlation analysis showed a strong positive correlation between PVH score and DWHM score ($r = 0.56$, $P < 0.0001$). In relation to aging, a positive correlation was found between PVH score and age ($r = 0.34$, $P < 0.0001$), and between DWMH score and age ($r = 0.28$, $P < 0.0001$).

Cognitive and psychological assessment

The mean score of MMSE, GDS-15 and vitality index was 23.1 ± 5.3, 5.0 ± 3.5 and 9.4 ± 1.2 points, respectively, indicating that the subjects showed cognitive decline, depression and decreased vitality, all to a mild extent. Given that a score of 23 or below on MMSE is regarded as the presence of cognitive impairment,[19] 47.5% of the subjects fell into this category. The causes of cognitive impairment were Alzheimer disease (AD; 53.3%), vascular dementia (VaD; 16.4%), combined dementia of AD and VaD (9.0%) and other types of dementia (21.3%). Pearson's correlation analysis revealed a negative correlation between PVH score and MMSE, PVH score and vitality index, DWMH score and MMSE, and DWMH score and vitality index,

Table 1 Clinical characteristics of study subjects

	Prevalence ($n = 286$)	Mean ± standard deviation
Clinical characteristics		
Age (years)		74.5 ± 7.8
Women (%)	74.0	
Height (m)		1.55 ± 0.08
Bodyweight (kg)		52.4 ± 10.6
Body mass index (kg/m^2)		21.8 ± 3.3
Systolic blood pressure (mmHg)		135.3 ± 20.2
Diastolic blood pressure (mmHg)		76.3 ± 11.8
Prevalence of complications		
Hypertension (%)	50.7	
Diabetes (%)	27.3	
Hyperlipidemia (%)	50.0	
Past history of cerebrovascular disease (%)	10.1	
Smoking (%)	22.7	
Cognitive and psychological assessment		
Mini-Mental State Examination (0–30 points)		23.1 ± 5.3
Geriatric depression scale (0–15 points)		5.0 ± 3.5
Vitality index (0–10 points)		9.4 ± 1.2
White matter lesions		
Periventricular hyperintensities (points)	5.5 ± 4.8	
Deep white matter hyperintensities (points)	35.5 ± 39.8	

Table 2 Relationship between white matter lesions and global cognition (MMSE), depressive state (GDS-15) and vitality (vitality index)

	Linear regression	
	PVH score	DWMH score
MMSE	–0.380**	–0.272**
GDS-15	0.022	–0.066
Vitality index	–0.432**	–0.184*

Univariate linear regression analysis: *$P < 0.01$, **$P < 0.0001$. DVMH, deep white matter hyperintensity; GDS-15, 15-item Geriatric Depression Scale; MMSE, Mini-Mental State Examination; PVH, periventricular hyperintensity.

respectively (Table 2). It was also found that calculation (serial subtraction of 7 from 100) was negatively correlated with PVH score ($r = -0.156$, $P = 0.04$, data not shown), and verbal fluency (naming as many vegetables as possible) was negatively correlated with PVH score ($r = -0.216$, $P < 0.01$, data not shown). On the other hand, no significant correlation was found between PVH score and GDS-15, or between DWMH score and GDS-15. Multiple logistic analysis revealed that PVH score and DWMH score remained significant determinants of cognitive impairment (MMSE, ≤23) and low vitality (vitality index, ≤9) after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 3).

One hundred and ninety subjects reported symptoms of geriatric syndrome. The frequency is shown in Table 4. Frequent symptoms (>20%) were tripping (32.1%), constipation (26.3%), gait disturbance (23.2%) and pollakiuria (22.1%). Student's *t*-test showed that PVH score was significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, weight loss, apathy, swallowing difficulty, tremor and muscle stiffness. Multiple logistic analysis revealed that PVH score remained a significant determinant of hallucinations, tripping, pollakiuria, urinary incontinence, weight loss, apathy and swallowing difficulty after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 5). By the same method, DWMH score was

Table 3 Periventricular hyperintensity and deep white matter hyperintensity scores as determinants of cognitive impairment and low vitality

	PVH score			DWMH score		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Cognitive impairment	1.185	1.084–1.295	<0.001	1.010	1.001–1.021	<0.05
Low vitality	1.260	1.133–1.401	<0.0001	1.025	1.012–1.039	<0.001

Cognitive impairment and low vitality were defined as MMSE ≤23 and vitality index ≤9, respectively. Multiple logistic analysis was performed after adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; DVMH, deep white matter hyperintensity; OR, odds ratio; PVH, periventricular hyperintensity.

significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence and constipation. Multiple logistic analysis revealed that DWMH score remained a significant determinant of hallucinations, delusions, tripping, urinary incontinence and constipation after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 6).

Discussion

Elderly persons are affected by multiple chronic diseases. Once they are affected by serious illness, full recovery cannot be expected with medical treatment, because elderly patients are often trapped in a vicious circle of illness and poor quality of life (QOL). This is the reason why care and welfare contribute to the total well-being of the elderly. Physicians need to pay great attention to improving QOL as well as treating illness. Thus, it is important to comprehend the whole picture of their life by means of comprehensive geriatric assessment, which evaluates multiple aspects of an elderly person's life, such as activities of daily living, cognition, mood, vitality, communication and social environment.

The present study confirmed a negative correlation between the severity of WML and MMSE score. Multivariate analysis showed that the presence of WML was a significant risk factor for cognitive impairment, even after adjustment for confounding factors of age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease. The mechanism and the size and location of WML that impair cognitive function are not yet clear. However, from previous studies, it seems convincing that a reduction of blood flow in the frontal lobe plays an important role in cognitive impairment in elderly people who exhibit WML.[20,21] Clinical manifestations of WML include attention deficit and a decline in information-processing ability.[4,13,22] Junque *et al.* reported the reappearance of primitive reflexes, one of the symptoms of frontal lobe dysfunction, in patients with WML.[11] In this study, patients with PVH showed attention deficit (incapability of calculation) and verbal inarticulacy (naming less vegetables), implying the impairment of frontal lobe function. WML, as reported previously,[6,23] were negatively correlated with vitality. Multiple logistic regression analysis, using potential risk factors including advanced age as confounding variables, found that the presence of WML was an independent risk factor for low vitality. Additionally, a relation between PVH score and apathy, a significant symptom of geriatric syndrome, was also found. From previous studies showing the importance of frontal lobe function in vitality,[24–26] we assume that blood flow reduction in the frontal lobe may account for the apathy and low vitality in patients with WML. More precisely, WML disrupting the frontal-subcortical circuit may result in dysfunction in the anterior cingulate and dorsolateral prefrontal circuits, thereby leading to apathy and decreased vitality.[5,6,20] Increase in PVH score or DWMH score was not apparently correlated with depression, probably because depression is associated with many factors such as aging, female sex, hyperlipidemia and medication.[27–29] The subjects in this study were mostly elderly (88.1%) and female (74.0%). We assume that these confounding conditions made it difficult to prove a true relation between WML and depression. From analysis of the association of WML with geriatric syndrome, it appears that WML have a relation to psychiatric symptoms (hallucinations and delusions), gait abnormalities (gait disturbance, tripping and falls), urinary symptoms (pollakiuria and urinary incontinence) and possibly with parkinsonism (swallowing difficulty, tremor and muscle stiffness). It was reported that WML were related to gait abnormalities,[5–7] presumably caused by disruption of the frontal-subcortical circuit.[30] Some other studies suggested that parkinsonism is also a contributing factor to gait disturbance in patients with WML.[6,31] Interestingly, we found that both gait abnormalities and symptoms of parkinsonism were associated with WML.

The present study confirmed an association between WML and voiding dysfunction (pollakiuria and incontinence). It was reported that urinary dysfunction was derived from damage to the frontal-subcortical

Table 4 Comparison of periventricular hyperintensity and deep white matter hyperintensity scores between subjects who did or did not exhibit each symptom of geriatric syndrome

Geriatric syndrome	Prevalence (%)	PVH score Symptom Present	Absent	*P*-value	DWMH score Symptom Present	Absent	*P*-value
Hallucination	6.8	**8.5 ± 5.9**	**4.4 ± 4.7**	**<0.01**	**59.8 ± 43.9**	**28.6 ± 35.4**	**<0.01**
Delusion	9.5	**7.6 ± 5.2**	**4.4 ± 4.8**	**0.01**	**56.1 ± 37.6**	**28.2 ± 35.9**	**<0.01**
Insomnia	18.9	4.2 ± 3.6	4.7 ± 4.9	0.56	31.4 ± 36.0	31.3 ± 37.6	0.98
Vertigo	18.9	6.1 ± 6.5	4.4 ± 4.4	0.06	33.4 ± 38.1	30.7 ± 37.0	0.70
Paralysis	2.1	8.5 ± 4.8	4.6 ± 4.9	0.12	59.5 ± 47.2	30.1 ± 36.3	0.11
Numbness	16.6	5.1 ± 4.6	4.6 ± 4.8	0.62	34.6 ± 40.0	29.9 ± 36,0	0.52
Gait disturbance	23.2	**6.7 ± 5.1**	**4.2 ± 4.7**	**<0.01**	**43.3 ± 41.7**	**27.5 ± 34.9**	**0.01**
Tripping	32.1	**6.4 ± 4.5**	**3.9 ± 4.9**	**<0.01**	**42.1 ± 43.7**	**25.9 ± 32.4**	**<0.01**
Falls	17.9	**6.6 ± 4.9**	**4.3 ± 4.8**	**0.01**	**45.8 ± 43.1**	**28.0 ± 35.0**	**0.01**
Pollakiuria	22.1	**8.0 ± 5.8**	**3.8 ± 4.2**	**<0.01**	**41.5 ± 41.0**	**41.5 ± 41.0**	**0.04**
Urinary incontinence	13.8	**7.5 ± 5.1**	**4.3 ± 4.8**	**<0.01**	**52.4 ± 44.9**	**52.4 ± 44.9**	**<0.01**
Constipation	26.3	5.8 ± 4.3	4.4 ± 5.1	0.08	**44.5 ± 45.1**	**44.5 ± 45.1**	**<0.01**
Decreased appetite	14.7	6.1 ± 4.4	4.5 ± 5.0	0.12	42.1 ± 42.6	42.1 ± 42.6	0.11
Weight loss	14.2	**6.9 ± 4.1**	**4.4 ± 5.0**	**0.01**	40.7 ± 41.3	40.7 ± 41.3	0.15
Apathy	7.6	**7.4 ± 3.6**	**4.4 ± 5.0**	**0.03**	30.7 ± 28.1	30.7 ± 28.1	0.97
Speech impairment	2.7	5.6 ± 5.2	4.5 ± 4.7	0.62	35.3 ± 48.0	35.3 ± 48.0	0.80
Swallowing difficulty	14.7	**12.2 ± 4.4**	**4.5 ± 4.8**	**<0.01**	44.6 ± 34.6	44.6 ± 34.6	0.40
Tremor	5.3	**9.1 ± 6.5**	**4.4 ± 4.7**	**<0.01**	45.0 ± 38.1	45.0 ± 38.1	0.24
Muscle stiffness	3.2	9.2 ± 4.8	**4.5 ± 4.9**	**0.02**	48.7 ± 43.4	48.7 ± 43.4	0.23

PVH and DWMH score are shown as mean ± SD. Boldface values are statistically significant ($P < 0.05$ by Student's *t*-test). DVMH, deep white matter hyperintensity; PVH, periventricular hyperintensity.

Table 5 Periventricular hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.12	0.043	1.004–1.248
Tripping	1.11	0.005	1.032–1.194
Pollakiuria	1.17	0.001	1.067–1.278
Urinary incontinence	1.11	0.022	1.015–1.207
Weight loss	1.14	0.007	1.036–1.246
Apathy	1.14	0.027	1.015–1.276
Swallowing difficulty	1.35	0.019	1.050–1.741

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

Table 6 Deep white matter hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.017	0.020	1.003–1.032
Delusion	1.016	0.024	1.002–1.030
Tripping	1.011	0.020	1.002–1.020
Urinary incontinence	1.016	0.008	1.004–1.028
Constipation	1.011	0.025	1.001–1.021

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

circuit.[5,20] In relation to the symptoms of parkinsonism (swallowing difficulty, tremor and muscle stiffness), this association was previously explained by dysfunction of the frontal-subcortical circuit.[6,31] The importance of this lesion was also suggested by a study showing that swallowing difficulty occurs with dysfunction of internuncial neurons that link the brainstem to the cerebral cortex.[32]

Considering the cause of manifestation of geriatric syndrome in patients with WML, it appears that damage to associative pathways in the frontal and subcortical regions due to ischemic hypoperfusion is an important mechanism.[5,20,21] It is necessary to localize the responsible connecting pathway for each symptom by a sophisticated approach in the future.

In conclusion, we showed that WML were associated with cognitive impairment, low vitality and geriatric syndrome of psychological disorders, gait disturbance,

urinary problems and parkinsonism. Evaluating WML in relation to geriatric syndrome and building a preventive measure against WML is an important future task for maintaining the independence of elderly people.

Acknowledgments

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介護予防に対する医療関係者の役割

Participation with medical staffs in a preventive care

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介護予防は，日常生活自立機能，基本的日常生活動作，認知機能など多くの要素別の機能低下の多段階を理解し，何を予防するかを知ることから始まる．

介護予防は，慢性疾患のケアから，障害の段階の理解を経て，社会サービス受給への概念に変化した．

虚弱の進展因子として，疾患，液性因子，生活習慣と統一した概念としてホメオスタシス破綻などの考え方がある．これらの考え方を踏まえて，簡便な虚弱者のスクリーニング方法が開発されている．

介護予防の失敗は，地域の高齢者の自主的な参加要件である，高齢者自身の役割付与，予防の意義の説明，「選択と楽しみ」のいずれにも配慮がなかったことによる．

介護予防事業を根本的に改善するためには，医療関係者が協力して科学的アプローチを行うことが必要である．

key words

介護予防
評価
介入方法
医療関係者の役割
転倒予防

はじめに

介護保険におけるコンセプトは「地域における自立支援」と「地域で要介護者を支える」の2点に集約されてきた．介護保険制定後5年間に介護認定者が200万人から倍増し，特に要支援，要介護Ⅰといった，「自立支援」を図るべき対象が激増し，「介護保険料の値上げ」が避けられなくなってきていることが，「介護予防」の概念の導入に関係している．

改正の要点は，従来の要支援と要介護Ⅰに対し，認知症や脳血管障害，症状の不安定な対象を除き，筋力トレーニングや活力賦活(アクティビティーデイ)などを行う「要支援Ⅰ，要支援Ⅱ(新設)」を選別し，「介護予防事業」で経費を賄うというものである．

新しい介護予防事業のサービスの選定根拠が十分科学的に担保されておらず，一部の少数例のデータによって，虫食い的なサービスモデルが提唱されていることが最も危惧される点である．栄養，口腔ケア，筋力トレーニングなど重要な視点であることは間違いないが，高齢者の多様な病態と機能低下の学問的関連を，十分反映した施策が求められる．この点の不足は，介護予防プログラム参加者が悲惨なほど少ない現実によって証明された．本総説では，虚弱，要支援，要介護などの用語についても改めて歴史的な概念の変遷を整理し，医療チームが「どのような状態をどうやって予防するのか」という基本的な疑問を考える．

介護予防：何を予防するのか

1．介護の多様性

介護保険の介護は，生活支援と身体介護に分けられる．

生活支援は，家事援助とも言い，独居あるいは，家族の家事代行が不十分な認定者に対して，買い物，掃除，洗濯，炊事，通院などを手助けするものであり，「手段的日常生活動作能力(IADL：Instrumental Activities of Daily Living)」(表1)の代行をしている．

身体介護には，寝返り，移動の介助や排泄支援，清拭などといった，「基本的日常生活動作能力(Basic Activities of Daily Living：BADL)」(表1)の介助と，床擦れ処置，オムツ交換，摂食介助などといった，褥瘡，尿失禁，

表1　基本的日常生活動作能力

生活支援	身体介護	
独居高齢者の生活自立要因 ＝手段的日常生活動作能力	最低限の自立(sADL)	移動の介護(mADL)
交通機関の利用	食事	寝返り
買い物	排尿・排便	起立
金銭管理	入浴	歩行屋内
料理	整容	歩行屋外
家事	更衣	階段昇降
洗濯	口腔衛生	
熱源の取り扱い		
服薬管理		
電話		

[江藤文夫：ADL20．日老医誌 29(11)：841-848，1992]

嚥下障害などの「老年症候群のケア」が含まれる．

したがって，介護予防という概念は，IADL 依存の予防，BADL 低下予防，および老年症候群の発症・悪化予防という極めて幅の広い概念にならざるを得ない．

このことが，一般に介護予防の意味をわかりにくくし，一部は健康増進などの生活自立のみと捉えたり，一部は寝たきり予防という BADL 低下予防を念頭に置く傾向がみられる．

また，欠けている能力を賦活する介護サービスとして共同生活，リハビリテーションがあり，前者は手助けを受けながら共同で作業を行うことによって IADL を維持し，後者は BADL の改善・維持を主な目的としているが，認知症(認知症)やうつなどにも効果が期待され，「認知機能・情緒」といった精神機能に対する介護の形態を含んでいる．

このように，介護予防は，日常生活自立機能，基本的日常生活動作，認知機能など多くの要素別の機能低下の多段階を理解することによって，はじめて，対象が「何を予防すべき」段階であるかを理解することになる．

2. 介護予防対象者に対する考え方の変遷

虚弱や要介護者という概念は1980年以降に出現した比較的新しい概念である．それ以前の捉え方を振り返ると，「介護予防対象者」の全体像が見事に浮かび上がる．

高齢者の包括的な評価の創始者である Majory Wallen は，要介護者に対し，1940年に「慢性疾患に対するケア」という概念を発表した[1]．その後，疾患ー障害ー能力低下ー不利というリハビリテーションの基本的概念の中で，能力低下した対象が虚弱や要介護者という捉え方が広まり[2]，介護保険の創設当時の最近まで通常の捉え方であったと思われる．1980年代には福祉サービスの発展や，医療ソーシャルワーカーの増加と社会的活躍により，虚弱者は，福祉的サービスの受給者であるという考え方も出てきた[3]．

このように，疾患論的捉え方，障害論的捉え方，社会サービス的捉え方が，歴史的に「虚弱者」に対する概念の変遷と発展的積み重ねであり，これらを重層化した構造として，高齢者の QOL 構造が理解されるようになった．

さらに，前虚弱者の早期発見というテーマが世界的に重要になってきた[4]．すなわち，介護予防対象者は，臓器障害として医学的に評価され，運動器の機能低下が理学的に評価され，生活自立が評価されたうえで，支援内容や量が評価されなければならないことは自明である．

このなかで，サルコペニアは特に中核的所見として重要と考えられている．

介護予防－危険因子（リスクファクター）の解析

1．ホルモン，液性因子

高齢患者の虚弱や障害，有害事象を反映する血清マーカーとして，テストステロン値の低下[5]，DHEA値の低下[6]，早朝におけるコルチゾール・DHEA硫酸塩比の上昇[7]，高感度CRP，IL-6上昇[8]，総コレステロールの減少[9]，血清アルブミン値の低下[10]など，多くの因子が指摘されている．

我々も，テストステロン値やDHEA値の低下がADLの低下と相関し，また認知機能や意欲とも正の相関をもち[11]，運動によって認知機能が改善する成績を得ている[12]．

これらの指標は虚弱の指標として単独で提唱されている．しかし，神経，内分泌，栄養，動脈硬化，炎症など多角的視点のなかで総合的に捉える必要があるだろう．

2．疾患要因

寝たきりの原因疾患の年代別解析では，65～74歳の前期高齢者では脳血管障害が最も重要な危険因子である．75～84歳の後期高齢者では，脳血管障害に認知症や転倒・骨折が加わり3大危険因子となる．85歳以上の超高齢者では，衰弱といった「疾患によらない要因」の重要性が急速に増す．

転倒・骨折を例にとると，我々が全国7都道府県の60歳以上2,162名の転倒率を調査した成績では，65歳以上で加齢とともに転倒率が上昇する[13]（図1）．仮に30％以上の高頻度の転倒率をもつ集団に対して，転倒予防事業を行うならば，75歳以上の後期高齢者を対象とすればよいことがわかる．

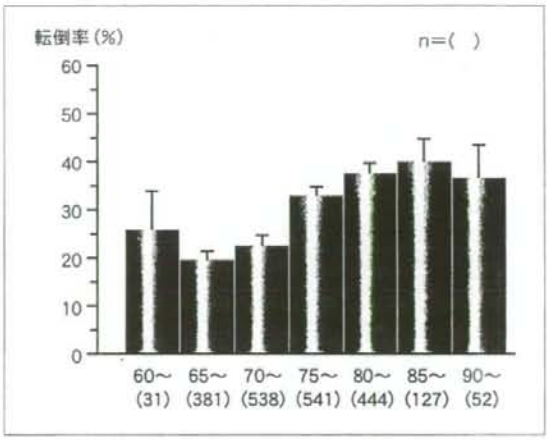

図1　高齢者（60歳以上）の過去1年における転倒率

3．生活自立要因

BADLは年齢とともに低下するが年々改善し，縦軸にADLをとり，横軸に年齢をとって折れ線グラフでつないだカーブは年々矩形化し，生命予後と同様，健康寿命は改善している[13]．どのような活動度がより早期に低下するかについては，移動系では階段昇降，セルフケア系では入浴や排尿があげられ，食事は最後まで保たれる機能である[12]．

前期高齢者では，階段昇降などの筋力アップが課題であり，後期高齢者では，入浴，排泄自立，超高齢者では，嚥下障害などに対する摂食嚥下が，自立要因の年齢別の低下に着目した視点といえる．

4．ハイリスクアプローチ

寝たきりになりやすい群を早期にスクリーニングすることが可能ならば，「ハイリスクグループ＝高危険集団」として特定し，早期に介入しようとする考え方である．この考え方の原点は，生活習慣病におけるハイリスク集団の特定にある．前期高齢者の寝たきり原因の第1位である脳血管障害においては，高血圧，糖尿病などの疾患や加齢，男性など避け得ぬ要因と，日本酒に換算して2合以上の飲酒，喫煙などといったライフスタイルの要因が縦断研究によって明らかにされている．一方認知症では，代表的なアルツハイマー型認知症において，ApoE ε4の遺伝的危険因子と高血圧が危険因子であることが明らかにされ，栄養学的にも，野菜不足，肉食過多などのライフスタイルの影響が注目を浴びている．

虚弱に対する最近の包括的アプローチ研究では，複数の生体システムに同

表2　高知県香北町における総合機能評価と介入事業

1) 健康関連アンケート調査(65歳以上全高齢者)：ADL，視力，聴力，老研式活動能力，うつ，福祉サービス利用，QOL
2) 包括的機能健診(75歳以上全高齢者)：認知機能(MMSE)，歩行能力，身体柔軟性，指先巧緻性
3) 運動教室
4) 家庭血圧測定
5) 定期健診，訪問看護
6) 保健・福祉・医療調整会議(現行のケアカンファランス)
7) 健康関連講演会(年2回)

時発生して虚弱を引き起こす障害に注目している．多数の生理的組織が症候的，臨床的機能不全の限界に近づき，複数の系統において予備能力の限界を超えた結果生じる症状または症候群[14]という考え方で，極めて老年症候群に近い考え方である．実際の測定方法としては，運動系機能として，握力，up & go テスト，トレッドミル，6分間歩行などを行い，認知機能として認知機能(MMSE)，バランス機能として片足立ち試験，栄養状態として BMI，周囲径などがあげられている．これらは，「高齢者総合的機能評価ガイドライン」[15]に推奨した方法と図らずも一致している．同様の考え方に，虚弱は自立と終末期の中間点とみなす考え方で，ハイリスクの因子として，75歳以上の高齢，ADL および IADL 障害・依存状態，転倒・骨折，多剤投与，慢性病，認知機能低下，抑うつ，栄養障害を指摘している[16]．これも，老年症候群に対する総合的機能評価がハイリスクグループの検出に有効な指摘である．

介護予防－介護予防はなぜ失敗したのか

介護保険制度創設前の成功事例(香北町研究)によれば，健康予防活動(表2)による介入によって，基本的日常生活活動の増大と老人医療費の抑制という，理想的な結果が得られているが，介護予防開始後の自治体の特定高齢者事業の参加者率の低さは目を覆わんばかりである．

医療関係者が予防の意義を熱心に説明することにより，介護予防事業参加者が5倍になった成績も得ている．地域の高齢者が自主的に参加するためには，役割を付与し，予防の意義を十分に説明することが必要であり，「選択と楽しみ」がない予防事業は失敗している．

新しい介護予防のグランドデザイン

寝たきり予防介入研究による検証－問題点は何か？

我々の研究班では，寝たきり予防介入研究によって以下の介入効果と課題を得ている[15]．

1．転倒予防の効果的な方策は何か

我々は，認知症患者240名を対象に転倒の特異的に多い時間帯にスタッフ配置の工夫と個別ケアプラン充実による転倒予防の試みを行い，転倒および骨折の半減効果を認めた．また，我々は，運動を定期的に行っている地域在住中高年者4,500名において転倒予防に役立つ運動の性質，頻度，時間を解明した．

2．在宅維持条件の解明

高橋は，地域在住高齢者全3,097名について，5年間(1999～2004年)の追跡調査を行い，男性の COPD，変形性関節症，女性の糖尿病，膝関節疾患など，従来寝たきりの直接疾患と考えられていなかった慢性疾患が，軽微な ADL 低下を起こすことを指摘し，医療関係者に注意を喚起している．

3．介護予防対象者をどう選ぶべきかを解明

地域(高橋：大三島町1,838名)で軽度介護者，施設(鳥羽：特養など1,200名)でも J2～ A2 レベルの高齢者において自立度が縦断的に低下する率が高いことを確認した．要支援レベル以下でも介護予防の重要性が示唆される成績である．

4．注目されている小規模介護施設の課題を解明

山田らは，グループホームの高齢者を対象に ADL 低下を6ヵ月まで縦断的に確認し，新しい小規模多機能施設における課題(リハビリテーションや

運動療法の導入の必要性)を解明した．運動介入がグループホームの介護予防に効果的であることがはじめて明らかになった．

5. 従来の介護保険制度における介護事業の強化が介護予防に有効な対象者の特徴を明らかにした

松田らは，ケアプランや自治体レベルの施策で，移動介助の例では，介護力強化が寝たきり予防に効果があることを横断調査により解明した．

6. より早期の介護予防 ーパワーリハビリテーションの問題点

松田らは，マシンを用いたいわゆるパワーリハビリは，短期的には改善効果が期待できるが，リハビリ専門職による対象者のコンプライアンスも含めた筋力トレーニングの可否，介入中に生じ得るリスク評価が必要であり，また，1回2～3時間で8～10名程度の高齢者に筋力トレーニングを提供するのが限界であり，またリスク管理の点から1台に1人のスタッフが付くという体制を取ることが望ましい．そのためコスト的には非常に高いものになることを指摘している．

7. 運動継続の効果を検証

我々は，均整柔軟体操の効果を大規模縦断的に検証した．そして自立高齢者を増やし，要支援への移行を予防阻止する観点(介護予防)から，開発した虚弱者の活力を測定する機能評価表を用いて調査したところ，体操教室の全国的組織(体操三井島システム：2,600名：18～84歳)に対する大規模縦断研究1年目の成績で，運動による活力度(IADL，交流，運動機能，健康意識，うつ)の向上を示した．このなかで，後期高齢者では週2～3時間程度の運動最適時間も示している．また，1年以上継続した例では，転倒率が30％から15％に減少し，この効果は7年間の運動継続者でも転倒率の程度が変わらなかった[12]．

以上の総括から，運動介入に関しては，「個人が楽しめる運動」を「年齢に適した運動強度と時間」を設定することにより，持続可能な介入となり，理想的な早期介護予防が実現される．

欧米に比べ，安価で楽しめるスポーツの場所，種類の豊富さが少ないわが国の現状に対し，「介護予防は高齢者の余暇文化水準を図る物差し」との考え方が重要で，安易にどの自治体でも新しい施設にトレーニングマシンが並び，数年後に誰も使用しなくなる悪夢だけは避けなくてはならない．

転倒・骨折は高齢者における寝たきり要因の第3位であり，大腿骨頚部骨折はその90％以上が転倒によって生ずる[1]．転倒は，骨折しなくても意欲やADLを低下させる[2]．地域住民におけるADL依存の危険因子として，転倒は約2倍のリスク[3]であり，転倒予防は寝たきり予防に重要である．

転倒予防

地域・施設共通の寝たきりの危険因子である転倒予防について述べる．

従来，転倒危険因子の抽出は，特定のフィールドでの横断的，あるいは縦断的解析によってなされているが，抽出された危険因子は，身体的脆弱性，歩行機能の低下など共通の危険因子がある一方，めまいや痴呆などについては成績が一致していない．従来の転倒危険因子は，病歴，現症，血液検査，生活能力などの簡便な検査，専門調査員による測定検査，特殊な機器を用いた検査などが統一性なく調査され，一般健康診断に適応できるかどうかの観点に著しく欠けていた．その欠点を補うため厚生労働省研究班「転倒ハイリスク者の早期発見の評価方法作成ワーキンググループ」によって簡易な「転倒スコア」が完成し，実用に付されている(表3)．

おわりに

介護予防のコンセプトは，生活習慣病によるイベント予防が不十分で，脳梗塞や骨折が増加し，これらによる機能低下のエビデンスの構築はようやくここ数年で重要な学問として市民権を得てきた状況である．

従来の医学では，イベントがエンドポイントでそれ以降の医療やケアに無関心なほとんどの臓器別の専門医から，イベント後のADL低下者や認知機能の低下者は，少なくとも一部では「病院での厄介者」扱いされてきた．これらに対して老年医学やリハビリテーション医学が真剣に対応してきたといっても過言ではない．介護予防はど

表3　転倒スコア

1) 過去1年に転んだことがありますか
「はい」の場合，転倒回数（　回）
2) つまずくことがありますか
3) 手すりを使わないと階段の上り降りができませんか
4) 歩く速度が遅くなってきましたか
5) 横断歩道を青のうちに渡りきれませんか
6) 1キロメートルくらい続けて歩けませんか
7) 片足で5秒くらい立つことができませんか
8) 杖を使っていますか
9) タオルは固く絞れませんか
10) めまい・ふらつきはありますか
11) 背中が丸くなってきましたか
12) 膝が痛みますか
13) 目が見えにくいですか
14) 耳が聞こえにくいですか
15) 物忘れが気になりますか
16) 転ばないかと不安になりますか
17) 毎日，お薬を5種類以上飲んでいますか
18) 家の中で歩くとき，暗く感じますか
19) 廊下・居間・玄関によけて通る物がありますか
20) 家の中に段差がありますか
21) 階段を使わなくてはなりませんか
22) 生活上，家の近くの急な坂道を歩きますか

使用方法：質問項目で「はい」の項目数を合計する．「はい」の項目数が増加すると転倒率が高くなる．10項目以上で，感度，特異度とも70%で転倒を予測可能．
各項目は，転倒危険者に対するケアプランにも使用する(図2)．

	看護	理学療法士・リハ医師	一般医師
評価	つまづきの有無 立ち上がる様子 移動の時間 タオルを絞れる 猫背 コミュニケーション めまい 服薬遵守	歩行観察 つま先の上がり UP&Go 試験 握力	異常歩行 R/O 麻痺 筋強剛 下腿周囲径 CT/DEXA 認知能 血圧変動 睡眠薬 抗不整脈
予防	転倒場面による ケアプラン 歩行・移動支援 見守り	転倒予防体操 靴の処方	趣味の運動 履物チェック 栄養指導 骨粗鬆症薬 ビタミン D

図2　チーム医療・看護・介護の共通言語＝老年症候群
CGA/老年症候群を通じたチーム医療の一例(転倒)

のような機能の段階においても機能低下防止が図られなくてはならない．

医療関係者は，情報を多職種で共有し，臓器の壁を超えて介護予防に務めなければならない．

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β_2-Adrenergic receptor regulates Toll-like receptor-4-induced nuclear factor-κB activation through β-arrestin 2

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Summary

Toll-like receptors (TLRs) play an important role in innate immunity while, β_2-adrenergic receptors (β_2AR) provide the key linkages for the sympathetic nervous system to regulate the immune system. However, their role in macrophages remains uncertain. Here, we demonstrate the cross-talk between β_2AR and TLR signalling pathways. Expression of β_2AR was down-regulated by TLR4 ligand lipopolysaccharide (LPS) stimulation. To investigate the physiological consequence of this down-regulation RAW264 cells, a macrophage cell line, were transfected with a β_2AR expression vector (RAWar). Both LPS-stimulated inducible nitric oxide synthase (NOS II) expression and NO production were markedly suppressed in the RAWar cells. The activation of nuclear factor-κB (NF-κB) and degradation of the inhibitor of NF-κB (IκBα) in response to LPS were markedly decreased in these cells. The level of β-arrestin 2, which regulates β_2AR signalling, was also reduced in RAW264 cells after stimulation with LPS, but not in RAWar cells. Overexpression of β-arrestin 2 (RAWarr2) also inhibited NO production and NOS II expression. Furthermore, we demonstrated that β-arrestin 2 interacted with cytosolic IκBα and that the level of IκBα coimmunoprecipitated by anti-β-arrestin 2 antibodies was decreased in the RAW264 cells but not in RAWar or RAWarr2 cells. These findings suggest that LPS-stimulated signals suppress β_2AR expression, leading to down-regulation of β-arrestin 2 expression, which stabilizes cytosolic IκBα and inhibits the NF-κB activation essential for NOS II expression, probably to ensure rapid and sufficient production of NO in response to microbial attack.

Keywords: β_2-adrenergic receptor; monocytes/macrophages; nitric oxide; nuclear factor-κB; toll-like receptor

Introduction

The ability of the innate immune system to recognize and respond to microbial components has been chiefly attributed to a family of type I transmembrane receptors termed Toll-like receptors (TLRs) that are expressed abundantly on antigen-presenting cells such as macrophages and dendritic cells and can discriminate among the distinct molecular patterns associated with microbial components.[1,2] The TLR-initiated activation of nuclear factor-κB (NF-κB) is essential for the regulation of inducible nitric oxide synthase (NOS II) and several proinflammatory cytokines, which are produced in response to invading pathogens. The NO produced by NOS II has a number of important biological functions, including roles in host defence against intracellular pathogens and tumour-cell killing. Although this basic definition is still accepted, over the past decade NO has been shown to play a much more diverse role not only in the immune system but also in other organ systems, including both beneficial and detrimental effects.[3,4] For example, the systemic inflammatory response syndrome, which includes

severe septic shock and multiple organ system failure, remains a leading cause of death in critically ill patients. Therefore, it is necessary to clarify the molecular mechanisms of TLR-initiated signalling that lead to NO production in response to microbial components.

Nuclear factor-κB is found predominantly in the cytoplasm complexed with members of the inhibitor of NF-κB (IκB) family. The release of NF-κB from IκB proteins is an essential step in the generation of transcriptionally competent NF-κB. The consensus is that IκB proteins mask the nuclear localization signals of NF-κB proteins, thereby regulating NF-κB activity, primarily by limiting their nuclear translocation. Recent studies, however, have indicated that IκBα is detected in both the nucleus and cytoplasm and that although the NF-κB complexes shuttle between the nucleus and cytoplasm under all conditions, they are unable to bind DNA because of their association with proteins of the IκB family.[5–7] Nuclear IκBα is not sensitive to signal-induced degradation. Therefore, following stimulation, NF-κB activities are dependent on the level of cytoplasmic NF-κB/IκBα complexes.

Recently, we demonstrated that the level of β_2-adrenergic receptor (β_2AR) expression influences TLR4 signalling.[8] β_2AR is a member of a family of G protein-coupled receptors (GPCRs) and is the key link involved in immune system regulation via the sympathetic nervous system.[9,10] Primary and secondary lymphoid organs, such as the thymus, spleen and lymph nodes, receive extensive sympathetic/noradrenergic innervation, and lymphocytes, macrophages and many other immune cells bear functional β_2AR. Therefore, β_2AR stimulation regulates pro-inflammatory cytokine production, lymphocyte traffic and proliferation, and antibody secretion through cyclic adenosine monophosphate (cAMP) generation and protein kinase A (PKA) activation.[10,11] However, the role of β_2AR in the TLR signalling pathway in macrophages remains vague. On the other hand, arrestins are cytosolic proteins that play a critical role in the regulation of GPCR signalling.[12,13] Recent studies have shown that they also interact with their partner molecules in a variety of signalling pathways, including NF-κB signalling.[14–16] In the present study, we investigated the physiological consequence of the down-regulation of β_2AR expression in macrophages and analysed the cross-talk between the signalling of β_2AR and TLRs.

Materials and methods

Cell culture

The murine macrophage cell line RAW264 (RCB0535) was purchased from RIKEN Cell Bank (Ibaraki, Japan) and cultured as described in our previous study.[17] The cells were stimulated with 1 μg/ml lipopolysaccharide (LPS) from *Escherichia coli* 055 (Sigma-Aldrich, St Louis, MO). Cell viability was assessed using the trypan blue dye exclusion test and cell size was measured by flow cytometric analysis of forward light scatter characteristics using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA).

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as described elsewhere.[18] The NF-κB oligonucleotide probe (5′-AGT TGA GGG GAC TTT CCC AGG-3′) was purchased from Promega (Madison, WI) and labelled with biotin at its 3′ end. The nuclear protein (2 μg) and excess amounts of labelled oligonucleotide probes were incubated in 20 μl EMSA buffer [20 mM HEPES, pH 7·6, 10 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid (EDTA), 0·2% Tween, 30 mM KCl, 1 μg poly (dI-dC), 1 μg poly L-lysine] at room temperature for 15 min, electrophoresed in 7% polyacrylamide gels, transferred onto the Biodyne Plus Membane (Pall BioSupport Division, Port Washington, NY), and cross-linked in ultraviolet light. To detect signals, the blots were incubated with streptavidin–horseradish peroxidase conjugate in a blocking reagent for 15 min and with a chemiluminescent reagent for 5 min. The blots were then exposed to Kodak X Omat AR film (GE Healthcare Bio-Science, Piscataway, NJ).

Western blotting analysis

Cell membrane proteins were prepared using the Plasma Membrane Protein Extraction Kit (Bio Vision, Mountain View, CA). Cytoplasmic protein extracts were prepared as described previously (30). The protein concentration was determined using the Bradford reagent (BioRad, Hercules, CA), and equal amounts of membrane proteins or cytoplasmic proteins were loaded. The samples were separated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred on to polyvinylidene difluoride membranes (Applied Biosystems, Foster City, CA). The membranes were blocked with 10% non-fat dried milk in Tris-buffered saline and incubated with goat polyclonal antibodies against β_2AR, goat polyclonal antibodies against β-arrestin 2, or rabbit polyclonal antibodies against IκBα and NOS II (Santa Cruz Biotechnology, Santa Cruz, CA); this was followed by incubation with appropriate secondary antibodies (horseradish peroxidase-conjugated rabbit anti-goat or goat anti-rabbit immunoglobulin G; Dako, Kyoto, Japan). To ensure equal protein loading, the membranes were incubated with rabbit anti-actin or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology) for the detection of cytoplasmic or cell surface GAPDH[19] after stripping. Immunoreactivity was visualized using an enhanced chemiluminescence reagent (ECL; GE Healthcare Bio-Science).

Immunoprecipitation

The cells were lysed with lysis buffer (20 mM Tris–HCl, pH 7·6, 150 mM NaCl, 2 mM EDTA, 0·5% Nonidet P-40 and protease inhibitors). The samples were clarified by centrifugation at 21 000 *g* at 4° for 30 min. The protein concentration was determined using the Bradford reagent (Bio-Rad). β-Arrestin 2 was immunoprecipitated with anti-β-arrestin 2 monoclonal antibodies (Santa Cruz Biotechnology) from equal samples, followed by treatment with 10 μl protein G–Sepharose beads (GE Healthcare Bio-Science). After extensive washing, the complexes were analysed by SDS–PAGE and Western blotting by using rabbit polyclonal antibodies against IκBα.

Determination of nitrite concentration

Nitrite in the cell culture supernatants was measured using the assay system of Ding *et al.*[20] The nitrite concentration was calculated by comparison with sodium nitrite, which was used as a standard. In some experiments, 200 μM pyrrolidine dithiocarbamate (PDTC, Sigma) was added to the cultures.

Determination of intracellular cAMP concentration

Cells were cultured with or without LPS for 6 hr and were stimulated with Salbutamol (1×10^{-6} M) for the final 30 min. Cell supernatants were then removed and cells were lysed. Intracellular cAMP was determined with a commercially available enzyme immunoassay (GE Healthcare Bio-Science).

Real-time polymerase chain reaction (PCR)

Total cellular RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and aliquots of 2 μg were reverse-transcribed with ReverScript I (Wako Pure Chemical Industries, Osaka, Japan) and an oligo-dT(15-mer) (Roche Diagnostics, Indianapolis, IN) at 42° for 50 min. The complementary DNAs (cDNAs) were amplified by PCR under the following conditions using the oligonucleotide primers and cycles listed in Table 1: 94° for 30 seconds, 55° for 30 seconds, and 72° for 30 seconds for NOS II and 18S ribosomal RNA (rRNA), and 94° for 30 seconds, 60° for 30 seconds, and 72° for 30 seconds for total and transfected β_2AR and β-arrestin 2. The quantity of the cDNA template included in these reactions and the number of amplification cycles were optimized to ensure that the reactions were stopped during the linear phase of product amplification, thus permitting semiquantitative comparisons of messenger RNA (mRNA) abundance between different RNA preparations.

β_2AR and β-arrestin 2 plasmid constructs and stable transfection

Full-length murine β_2AR (β_2ar) and β-arrestin 2 (*βarrestin2*) cDNAs were obtained by PCR using the primers 5′-GCTGAATGAAGCTTCCAGGA-3′ (sense) and 5′-GCCTGTATTACAGTGGCGAG-3′ (antisense) for β_2AR and 5′-GGCGGGCGGAGGGCGGCGAG-3′ (sense) and 5′-CGTCCTAGCAGAACTGGTCA-3′ (antisense) for β-arrestin 2. The amplified β_2AR and β-arrestin 2 fragments were subcloned into the pGEM-T Easy vector (Promega) and then into *Not*I-digested pcDNA4 (Invitrogen, Carlsbad, CA). The amplified PCR products were sequenced using an automatic DNA sequencer (Applied Biosystems). The plasmid DNA used for transfection was prepared using the EndoFree Plasmid Kit (Qiagen). RAW264 cells were transfected with the pcDNA4 vector, pcDNA4-$\beta_2$ar, or pcDNA4-βarrestin2 using LipofectAMINE Reagent (Invitrogen). Selection was initiated in a medium containing 500 μg/ml Zeocine (Invitrogen).

Luciferase assays

The full-length murine NOS II promoter fragment was cloned into the pGL3-enhancer luciferase reporter gene vector (Promega) (pGL3-NOS II) as described previously.[21] RAW264 cells were transfected using the LipofectAMINE Reagent with constructs containing the luciferase reporter gene, and luciferase activity was determined using the Dual Luciferase Assay System Kit (Promega) as described elsewhere.[21] Activity was normalized relative to an internal cotransfected constitutive control (*Renilla* luciferase expression vector, pRL-TK; Promega). In some

Table 1. Oligonucleotide sequences used for polymerase chain reaction

	Forward	Reverse	Cycle
β_2AR	GGAGCAGGATGGGCGGACGG	GCCTTCCATGCCTGGGGGAT	34
Transfected β_2AR	GGAGCAGGATGGGCGGACGG	TGGTGATGGTGATGATGACC	34
β-arrestin 2	GCAGCCAGGACCAGAGGACA	CCACGCTTCTCTCGGTTGTC	35
NOS II	CTTCCGAAGTTTCTGGCAGCAGCG	GAGCCTCGTGGCTTTGGGCTCCTC	26
18S	GAGAAACGGCTACCACATCC	CCCAAGATCCAACTACGAGC	26

β_2AR, β_2-adrenergic receptor; NOS II, nitric oxide synthase II.

experiments, RAW264 cells were transiently cotransfected with the NF-κB-responsive promoter reporter–luciferase construct pNF-κB-Luc (Clontech, Palo Alto, CA) or pGL3-NOS II and pcDNA4-$\beta_2$ar or IκBα dominant-negative vector pCMV-IκBαM (Clontech).

Statistical analysis

Student's *t*-test for unpaired samples was used to compare two means. For more than two groups, statistical significance of the data was assessed by analysis of variance. Where significant differences were found, individual comparisons were made between groups using the *t*-statistic and adjusting the critical value according to the Bonferroni method. Differences were considered significant at $P < 0{\cdot}05$. Data in the text and figures are expressed as means ± SEM.

Results

Preventing the down-regulation of β_2AR inhibits LPS-stimulated NOS II expression

Levels of both β_2AR protein and β_2AR mRNA were markedly decreased in RAW264 cells following LPS stimulation (Fig. 1a). To investigate the role of β_2AR down-regulation in response to LPS, a stable β_2AR transfectant (RAWar) and a vector control (RAWvec) were established. Although the levels of both β_2AR protein and mRNA expression were notably decreased in RAWvec cells following LPS stimulation, the down-regulation of β_2AR expression was prevented in the RAWar cells (Fig. 1b). The transfected β_2AR protein did not have a tag sequence capable of modifying β_2AR function so the protein levels of only transfected β_2AR could not be analysed. The mRNA levels of transfected β_2AR were low in unstimulated RAWar cells but markedly increased in the cells following LPS stimulation (Fig. 1c). In our previous study, we showed that the levels of both protein and mRNA of transfected cDNA cloned into the pcDNA4 vector were low in unstimulated RAW264 cells but were markedly increased in the cells following LPS stimulation.[17] Therefore, it appears that total β_2AR expression in unstimulated RAWar cells was not much higher than in RAWvec cells and that the decrease in intrinsic β_2AR expression in the LPS-stimulated RAWar cells was masked by the increased expression of transfected β_2AR as the result of the LPS stimulation. Although, the intracellular cAMP concentration in RAWar cells stimulated with salbutamol was similar to that in RAWvec cells, LPS

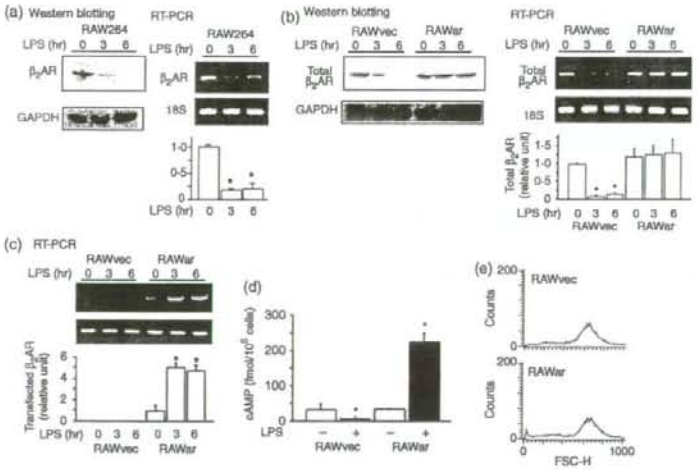

Figure 1. Lipopolysaccharide (LPS) stimulation down-regulates β_2-adrenergic receptor (β_2AR) expression. (a) RAW264 cells were stimulated with LPS. The protein levels of β_2AR and GAPDH (loading control) in the plasma membrane were analysed by Western blotting (left panel). The β_2AR messenger RNA (mRNA) and 18S ribosomal RNA (rRNA; loading control) were analysed by reverse transcription–polymerase chain reaction (RT-PCR; right upper panel). Bar graphs show the relative intensity of the PCR bands from three separate experiments (mean ± SEM) (right lower panel). *$P < 0{\cdot}01$ versus 0 hr. (b) RAW264 cells were transfected with the β_2*ar* construct or vector alone. The protein levels of β_2AR and GAPDH (left panel) and mRNA expressions of β_2AR and 18S rRNA (right upper panel) were analysed as in (a). Bar graphs show the relative intensities of the PCR bands from three separate experiments (mean ± SEM) (right lower panel). *$P < 0{\cdot}01$ versus 0 hr. (c) mRNA expressions of β_2AR and 18S rRNA (upper panel) were analysed as in (a). Bar graphs show the relative intensities of the PCR bands from three separate experiments (mean ± SEM) (lower panel). *$P < 0{\cdot}01$ versus 0 hr. (d) Cells were cultured with or without LPS for 6 hr and were stimulated with salbutamol (1×10^{-6} M) for the final 30 min. Then, intracellular cyclic AMP concentrations were analysed. *$P < 0{\cdot}05$ versus without LPS. (e) Cell size was measured by flow cytometric analysis of forward light scatter characteristics (FSC).

stimulation decreased the accumulation of intracellular cAMP in RAWvec cells but increased it in RAWar cells (Fig. 1d), suggesting that the transfected β_2AR was functionally active. Similar histograms of the distribution of forward light scatter characteristics were observed in RAWvec and RAWar cells, suggesting that the β_2AR transfection did not alter the cell size (Fig. 1e). In addition, cell viabilities were more than 98% in both cells.

The effects of forced β_2AR expression on NO production were examined. The nitrite concentration in the culture supernatants of the LPS-stimulated RAWar cells was considerably lower than in the culture supernatants of the RAWvec cells (Fig. 2a). After stimulation with LPS for 6 hr, a distinct 130 000 molecular weight NOS II protein band was observed in the RAWvec cells but not in the RAWar cells (Fig. 2b). Although a protein band corresponding to NOS II was observed in the RAWar cells after stimulation with LPS for 24 hr, the expression level was apparently lower than in the RAWvec cells. Similar results were obtained on reverse transcription PCR analysis of NOS II mRNA expression (Fig. 2b).

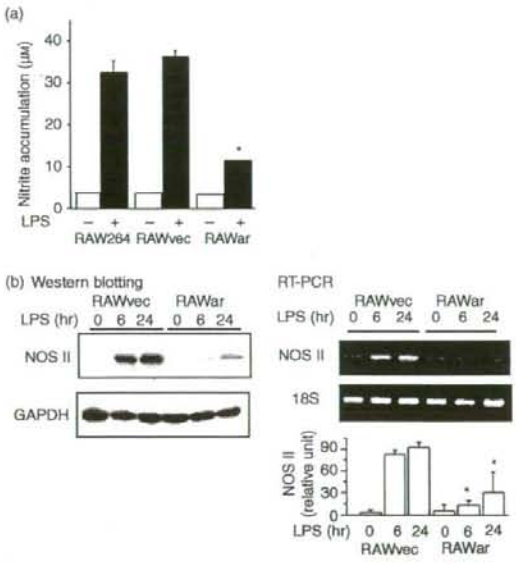

Figure 2. Forced β_2-adenergic receptor (β_2AR) expression suppresses nitric oxide (NO) production and nitric oxide synthase II (NOS II) expression. (a) Cells were stimulated with lipopolysaccharide (LPS) for 24 hr, and nitrite accumulation in the supernatants was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. *$P < 0{\cdot}001$ versus LPS-stimulated RAW264 or RAWvec cells. (b) The protein levels of NOS II and GAPDH (left panel) and messenger RNA expressions of NOS II and 18S ribosomal RNA were analysed as in A (right upper panel). Bar graphs show the relative intensity of the polymerase chain reaction bands from four separate experiments (mean ± SEM) (right lower panel). *$P < 0{\cdot}01$ versus corresponding RAWvec cells. Data shown are representative of three or four separate experiments.

Preventing the down-regulation of β_2AR inhibits LPS-stimulated NF-κB activation.

Next, the effects of forced β_2AR expression on NF-κB activation in response to LPS were analysed. As illustrated in Fig. 3(a), marked NF-κB activation was observed in the RAWvec cells stimulated with LPS for 3 and 6 hr but not in the RAWar cells. The level of cytoplasmic IκBα was decreased in the RAWvec cells after LPS stimulation for 6 hr but this level was not decreased in the RAWar cells (Fig. 3b). To further confirm the role of β_2AR in LPS-stimulated NF-κB activation, the effects of forced β_2AR expression on NF-κB-dependent gene transcription were analysed. NF-κB-mediated-luciferase reporter activity (Fig. 3c) and NOS II promoter activity (Fig. 3d) after stimulation with LPS were inhibited in cells that were cotransfected with the pcDNA4-$\beta_2$ar construct (AR) as well as in cells cotransfected with pCMV-IκBαM (DN-κB). These findings suggested that β_2AR functions as a negative regulator of NF-κB activation by inhibiting IκBα degradation in LPS-stimulated macrophages. Previously, it has been shown that PDTC blocks NF-κB activation by inhibiting IκBα degradation and subsequently the translocation of NF-κB subunits to the nucleus.[22] To elucidate the effects of NF-κB activation on the expression of the responsive gene, *Nos2*, PDTC was added to the RAW264 cell cultures at several time-points after the addition of LPS, and accumulation of NO in the supernatants was analysed after LPS stimulation for 24 hr. As illustrated in Fig. 3(e), when PDTC was added to cultures at 0–9 hr after the addition of LPS, the NO concentrations in these cultures were markedly lower than those in cultures stimulated with LPS for 24 hr without PDTC (right column), indicating that continuous NF-κB activation is essential for adequate NOS II induction.

β_2AR regulates NF-κB activation through β-arrestins

As β-arrestin 2 has been reported to interact with IκBα,[15,16] we examined whether β-arrestin 2 participates in the β_2AR-mediated regulation of IκBα degradation and NF-κB activation in response to LPS. The expression of β-arrestin 2 was also down-regulated in the LPS-stimulated RAW264 cells (Fig. 4, left panels). Forced β_2AR expression abolished the down-regulation of β-arrestin 2 expression (middle panels), suggesting that β-arrestin 2 expression was regulated by β_2AR. Deletion of β_2AR by small interfering RNA (siRNA) decreased β-arrestin 2 expression (data not shown), supporting the theory that β-arrestin 2 expression is regulated by β_2AR. To investigate the role of β-arrestin 2 down-regulation in response to LPS, a stable β-arrestin 2 transfectant (RAWarr2) was