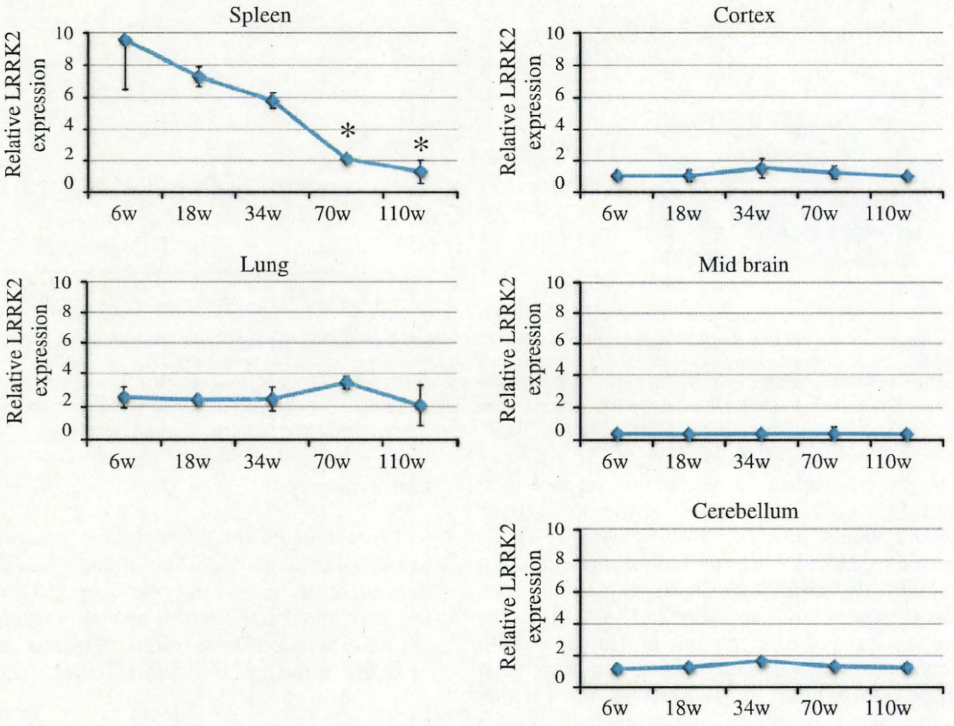

Fig. 2. Relationship between expression of LRRK2 mRNA and aging. RNA was isolated from spleen, lung, cortex, midbrain, and cerebellum of mice aged 6, 18, 34, 70, and 110 weeks, and subjected to quantitative PCR. Relative *LRRK2* expression normalized by *GAPDH* is shown. Stars represent statistical comparisons by one-way ANOVA ($n = 3$); * $p < 0.05$.

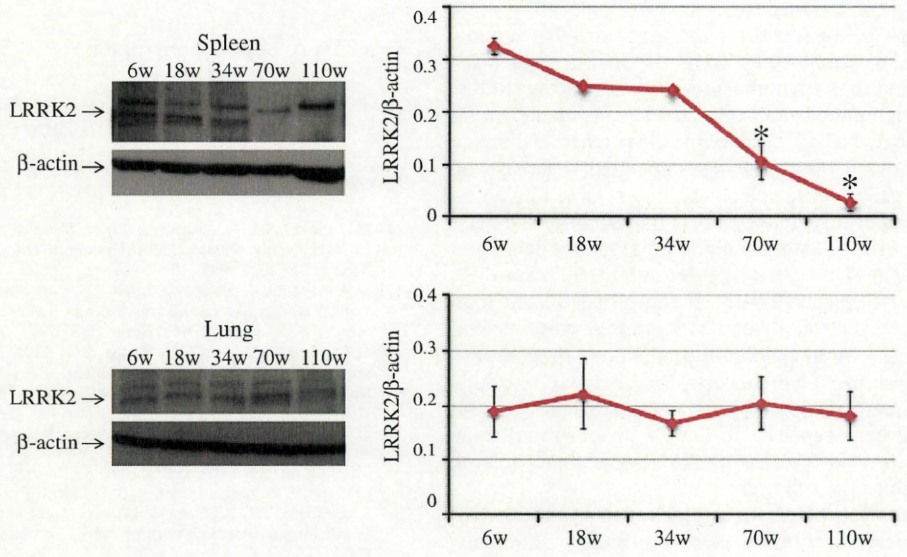

Fig. 3. Age-related change of the LRRK2 protein level in spleen. Tissue lysates were prepared from spleen and lung of mice aged 6, 18, 34, 70, and 110 weeks and subjected to Western analysis using AT106. LRRK2 protein levels normalized by β-actin are shown. Stars represent statistical comparisons by one-way ANOVA ($n = 3$); * $p < 0.05$.

gated antibodies directed against cell surface markers, i.e., CD3 (T lymphocytes), CD19 (B lymphocytes), and CD11b (macrophages), and investigated the expression of LRRK2 in each population. Western analysis with AT106 demonstrated that B lymphocytes were the major LRRK2-expressing cell population in the spleen (Fig. 4A). Macrophages expressed the LRRK2 protein weakly, but T lymphocytes showed no expression. Finally, we found that expression of the mRNA for the B lymphocyte marker CD19 exhibited an age-dependent decrease (Fig. 4B). These results suggested that the age-dependent decrease of LRRK2 expression in the spleen can be explained by a decrease in the number of splenic B lymphocytes.

Discussion

LRRK2 was originally identified as the causal molecule of autosomal-dominant familial PD, PARK8 [1–4]. Although the kinase activity has been demonstrated using several candidate

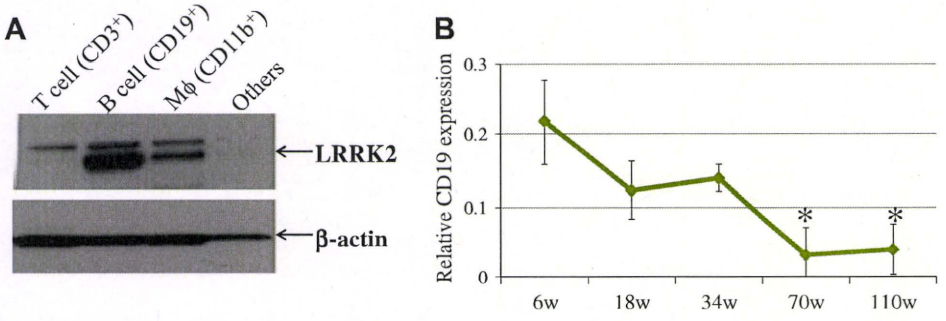

Fig. 4. LRRK2 expression in spleen cell populations. (A) Western analysis of LRRK2 expression in spleen cell populations. T lymphocytes, B lymphocytes, and macrophages were purified with magnetic bead-conjugated antibodies directed against each cell surface marker. Lysates of these cells and other residual spleen cells were subjected to Western analysis using the anti-LRRK2 antibody AT106. (B) Age-related change in the expression of *CD19* mRNA in spleen. RNA was isolated from the spleen of mice at various ages and subjected to quantitative PCR. Relative *CD19* mRNA expression normalized by *GAPDH* is shown. Stars represent statistical comparisons by one-way ANOVA ($n = 3$); * $p < 0.05$.

substrates [19–23], the true substrate or the true physiological function of LRRK2 is still unknown. Analysis of the organ/tissue distribution of LRRK2 would provide valuable clues as to its function. In early studies, LRRK2 was reportedly expressed ubiquitously, but subsequently, differences in its expression level among organs and tissues became apparent [6,13,14,18]. In the present study, we analyzed the expression of LRRK2 at both the mRNA and protein levels in various organs, and found that LRRK2 expression in kidney, lung, and spleen was much higher than that in brain, being consistent with previous reports [13,14,18]. It is suggested, therefore, that LRRK2 may play some important roles in these organs. Histochemical analysis with AT106 and two additional anti-LRRK2 antibodies revealed immunoreactivity in bronchioles of the lung and proximal renal tubules of the kidney, further suggesting some specific function in these areas (data not shown).

In the present study, we found for the first time that among cells in the spleen, B lymphocytes were the major population expressing LRRK2, and that T lymphocytes did not express LRRK2. Although human B-lymphoblastoid cell lines have been shown to express LRRK2 strongly [16], it has been unclear whether human T lymphocytes express LRRK2. The age-dependent decrease of LRRK2 expression in the mouse spleen can be explained by a decrease in the number of B lymphocytes, as indicated by the age-dependent decline of the cell surface marker CD19, although there is some controversy regarding the age-dependent decrease of B lymphocytes in mouse spleen [24–27]. It can be postulated that LRRK2 may play a specific role related to B lymphocytes, but not to T lymphocytes, e.g., immunoglobulin production, antigen presentation, or other forms of B lymphocyte-specific signal transduction. Human B-lymphoblastoid cell lines expressing the R1441C mutant LRRK2 have been reported to have a lower growth rate than those expressing wild-type LRRK2 [28], suggesting a role of LRRK2 in cell growth or the cell cycle.

In PD patients, analysis of whom originally led to the discovery of LRRK2, increased serum levels of anti-melanin antibody and IgG deposition in the substantia nigra have been found [29,30]. In addition, accumulating evidence indicates that microglia, the brain equivalent of macrophages known to express LRRK2, play crucial roles in the pathogenesis of PD [31–33]. Indeed, LRRK2 expressed in neurons may serve as a key molecule in neurodegeneration, but LRRK2 may also contribute to the progression of PD as a form of immune-response-related molecule, for example directly or indirectly regulating the humoral immune responses of B lymphocytes and the production of inflammatory mediators by macrophages and microglia. Investigations to clarify the functional role of LRRK2 in the immune system are currently in progress.

Conclusions

Expression of LRRK2 decreased markedly in mouse spleen in an age-dependent manner. This finding was explained by an age-dependent decrease of B-lymphocytes, the major LRRK2-expressing cell population in the spleen. T lymphocytes showed no LRRK2-expression.These results suggest a possibly novel function of LRRK2 in the immune system, especially in B lymphocytes.

Acknowledgments

This study was supported by Kitasato University (All Kitasato Project Study, No.18-1) and the Graduate School of Medical Sciences, Kitasato University (Integrative Research Program, 2008–2009).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.01.041.

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Journal of Neuroimmunology 229 (2010) 123–128

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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LRRK2 is expressed in B-2 but not in B-1 B cells, and downregulated by cellular activation

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ARTICLE INFO

Article history:
Received 19 May 2010
Received in revised form 22 July 2010
Accepted 23 July 2010

Keywords:
Leucine-rich repeat kinase 2
B-1 cells
B-2 cells
Parkinson's disease
PARK8
Downregulation

ABSTRACT

LRRK2, the causal molecule of familial Parkinson's disease, is expressed strongly by one of the B cell subsets, B-2 cells, but not by the other subset, B-1 cells, in the mouse peritoneal cavity, spleen, and peripheral blood. Bone marrow pre-B cells or T cells exhibited little LRRK2 expression. LRRK2 expression was dramatically downregulated upon activation of B-2 cells with various types of stimulation. These results suggest that LRRK2, whose true function has not yet been clarified, may play some important role(s) in the development and function of B cells, particularly the maintenance of B-2 cells in a resting status.

1. Introduction

Leucine-rich repeat kinase 2 (LRRK2) is the causal molecule of autosomal dominant familial Parkinson's disease, PARK8, which was originally defined in a study of a large Japanese family, the Sagamihara family (Funayama et al., 2002, 2005; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). LRRK2 is a large complex protein with an approximate molecular mass of 260 kDa and contains multiple domains including the LRR (leucine-rich repeat), ROC (Ras of complex), COR (C-terminal ROC), kinase, and WD40 domains (Meylan and Tschopp, 2005; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). The binding of GTP to the ROC domain, as well as dimer formation, is known to increase the kinase activity of LRRK2 (Deng et al., 2008; Ito et al., 2007; Smith et al., 2006). Although it has been reported that LRRK2 phosphorylates itself (autophosphorylation), and its potential interactors include moesin, eukaryotic initiation factor 4E-binding protein (4E-BP), β-tubulin, and mitogen-activated kinase kinase (MKK) 3, 6, and 7 (Gandhi et al., 2008; Hsu et al., 2010; Imai et al., 2008; Jaleel et al., 2007; West et al., 2007), the true substrate and true function of LRRK2 remain unknown.

Analyses of LRRK2 expression in human and rodent brain have demonstrated that LRRK2 is expressed in various regions including the substantia nigra, putamen, cortex, and cerebellum (Higashi et al., 2007a,b; Melrose et al., 2006; Paisan-Ruiz et al., 2004; Simon-Sanchez et al., 2006; Westerlund et al., 2008; Zimprich et al., 2004). It is also known that LRRK2 expression is much higher in the spleen, lung, kidney, and testis than in other organs, including the brain (Biskup et al., 2007; Larsen and Madsen, 2009; Maekawa et al., 2010; Westerlund et al., 2008). In particular, we have recently reported that B cells are the major LRRK2-expressing cell population in mouse spleen (Maekawa et al., 2010). Macrophages express LRRK2 weakly, but T cells show no expression. These results suggest a possibly novel function of LRRK2 in the immune system, especially in B cells.

B cells are the effectors of humoral immunity, and are classified into two subsets, B-1 B cells and B-2 B cells (or simply B-1 cells and B-2 cells, respectively) according to differences in developmental lineage and function (Berland and Wortis, 2002). B-1 cells develop primarily during the fetal stage as well as in the perinatal phase, whereas B-2 cells are produced from bone marrow during postnatal life (Hardy and Hayakawa, 2001; Herzenberg, 2000). B-1 cells are defined by their expression of the pan-T cell surface glycoprotein CD5, and have a $CD43^{+}$, $CD23^{-}$, $CD45R^{lo}$, immunoglobulin (Ig) M^{hi}, and IgD^{lo} immunophenotype (Berland and Wortis, 2002; Hardy and Hayakawa, 2001). They constitute a substantial fraction of B cells in the peritoneal and pleural cavities, and 5–10% of those in the spleen, but are absent from lymph nodes

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doi:10.1016/j.jneuroim.2010.07.021

(Hayakawa et al., 1983). In contrast, B-2 cells are conventional B cells characterized as $CD5^{-}$, $CD43^{-}$, $CD23^{+}$, $CD45R^{hi}$, IgM^{lo}, and IgD^{hi} (Berland and Wortis, 2002; Hardy and Hayakawa, 2001). They constitute a major fraction of B cells in spleen and lymph nodes (Berland and Wortis, 2002). B-1 cells spontaneously, in the absence of antigen stimulation, secrete IgM that is reactive with bacterial carbohydrate antigens and autoantigens (Berland and Wortis, 2002; Hayakawa et al., 1984). In contrast, B-2 cells produce IgM and IgG that are reactive with huge variety of foreign antigens only after antigen stimulation (Baumgarth et al., 1999). In keeping with this, typical B-1 Ig genes have fewer N insertions than those of most B-2 cells, and do not contain somatic mutations (Berland and Wortis, 2002; Hardy, 2006; Herzenberg, 2000).

In the present study, as one approach for elucidating the possible role of LRRK2 in B cells, we investigated whether LRRK2 expression differs between B-1 and B-2 cell subsets in various immune tissues. Because our previous Western blotting analysis using polyclonal anti-LRRK2 antibody revealed the need for careful discrimination of protein bands other than the full-length LRRK2, we quantified LRRK2 mRNA expressed by each of the B cell subsets. We found that LRRK2 mRNA was expressed at a much higher level in B-2 cells than in B-1 cells in the peritoneal cavity, spleen and peripheral blood. Furthermore, the expression of LRRK2 in B-2 cells was dramatically down-regulated by various types of stimulation. These results suggest that LRRK2 may have a functional role that is strongly restricted to resting B-2 cells, and not to B-1 or activated B-2 cells.

2. Materials and methods

2.1. Animals

C57BL/6 and alymphoplasia (aly/aly) mice were purchased from CLEA Japan, Inc. (Tokyo Japan). Female mice at 7–16 weeks of age were used for this study. Mice were cared for and handled in accordance with the guidelines of the Animal Experimentation and Ethics Committee of Kitasato University.

2.2. Lymphocyte purification and immunofluorescence staining

Whole lymphocytes were obtained by spleen disruption, blood collection, peritoneal cavity washout, and bone marrow washout. The cell suspensions were depleted of erythrocytes by hypotonic lysis at 4 °C, except for peripheral blood, from which erythrocytes were depleted by density gradient centrifugation using Lymphosepar II™ (IBL, Gunma, Japan). Lymphocyte subsets were purified on a magnetic activated cell-sorting (MACS™) separation column using magnetic microbeads coated with anti-CD5, -CD19, -CD43, and -CD90.2 monoclonal antibodies (MAbs) (Miltenyi Biotec, Gladbach, Germany). After MACS separation, cell subpopulations were stained for flow-cytometric analysis using combinations of the following fluorochrome-conjugated MAbs: CD3 (fluorescein isothiocyanate: FITC), CD5 (FITC), CD19 (spectral red: SPRD), CD23 (phycoerythrin: PE), CD43 (PE), CD45R/B220 (PE), and CD90.2 (PE). All of these antibodies were purchased from Beckman Coulter (Miami, FL). The stained cells were analyzed with an EPICS XL flow cytometer and EXPO32™ software (Beckman Coulter).

2.3. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from each of the separated cell subsets using TRIzol™ Reagent (Invitrogen, Carlsbad, CA), and cDNA was synthesized using a ThermoScript RT-PCR System (Invitrogen). Murine LRRK2 mRNA expression was assessed using the following combination of PCR primers: 5′-TCTGGCTGGAACCCTGCTAT-3′ and 5′-AACTGGCCATCTTCATCTCC-3′ (product size: 155 bp). As the internal control, murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was amplified with the following combination of primers: 5′-GAGGCCGGTGCTGAGTATGTCGTG-3′ and 5′-TCGGCAGAAGGGGCGGAGAT-3′ (product size: 116 bp). The qRT-PCR was performed in 25-μl volumes of triplicated reaction mixture containing 1 μl of cDNA, 12.5 μl of SYBR Green I Dye (Applied Biosystems, Foster City, CA), and primer pairs using a 7500 Real-time PCR System (Applied Biosystems).

2.4. B cell stimulation

MACS-sorted B cells (2×10^5) were cultured in 0.2 ml of RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum, 10 mM HEPES (pH 7.25), 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin in 96-well U-bottom microtiter plates. They were stimulated with either a mixture of phorbol 12-myristate 13-acetate (PMA) (Sigma) and ionomycin (Sigma), lipopolysaccharide (LPS) (Sigma, St. Louis, MO), or $F(ab')_2$ fragments of goat anti-mouse IgM (anti-IgM) antibody (Beckman Caulter) for 24 h.

3. Results

3.1. Isolation of B cell subsets from immune tissues

B cells are classified into B-1 and B-2 cell subsets according to their process of development, phenotype, and function. To compare the expression of LRRK2 mRNA in B-1 and B-2 cells, we separated the two subsets from the peritoneal cavity of a C57BL/6 mouse, the original site from which B-1 and B-2 cells had originally been defined. B-1 cells were isolated with anti-CD5 antibody-conjugated magnetic microbeads after depletion of T cells using anti-CD90.2 antibody-conjugated magnetic microbeads (Fig. 1). Because the B-1 cell-depleted fraction still contained a substantial number of $CD5^{+}$ cells, instead of using it as the source of B-2 cells, we isolated B-2 cells as the CD43-negative fraction using anti-CD43 antibody-conjugated magnetic microbeads according to Hein et al. (Hein et al., 1998), as CD43 is expressed on a wide variety of leukocytes including T cells, granulocytes, macrophages, plasmacytes, and B-1 cells, but not on resting B-2 cells.

Flow-cytometric analysis indicated that the cells in the B-1 and B-2 fractions prepared from the peritoneal cavity had each of the characteristic phenotypes, i.e., almost all cells in the B-1 fractions were positive for CD19 (98.4%), CD5 (96.0%), and CD43 (94.1%), but few of them were positive for CD23 (9.3%) and $CD45R^{hi}$ (24.0%); almost all cells in the B-2 fractions were positive for CD19 (98.2%), $CD45R^{hi}$ (96.0%), and CD23 (84.5%), but few were positive for CD5 (1.4%) and

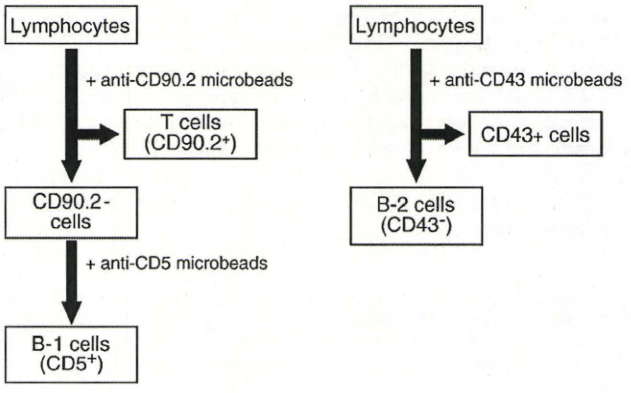

Fig. 1. Schematic representation of the isolation procedures for B-1 and B-2 cells.

Table 1
Flow-cytometric analysis of cell subsets separated from C57BL/6 and aly/aly mice.

Strain	Tissue	Subsets	B-1/B-2 ratio[a]	CD19$^+$	CD5$^+$	CD43$^+$	CD23$^+$	CD45R^{hi}	CD90.2$^+$	CD3$^+$
C57BL/6	Peritoneal cavity	B-1 cell	1:16	98.4%	96.0%	94.1%	9.3%	24.0%	2.9%	ND
		B-2 cell		98.2%	1.4%	14.8%	84.5%	96.0%	ND	0.3%
		T cell		21.5%	80.7%	ND	ND	ND	63.7%	85.2%
	Spleen	B-1 cell	1:120	81.0%	17.5%	20.5%	48.8%	76.2%	12.5%	ND
		B-2 cell		94.7%	1.3%	2.2%	87.4%	99.2%	ND	0.6%
		T cell		8.4%	91.9%	ND	ND	ND	95.4%	90.2%
	Peripheral blood	B-1 cell	1:20	ND	64.9%	ND	ND	60.9%	ND	ND
		B-2 cell		87.1%	6.6%	1.9%	90.9%	92.5%	ND	1.4%
		T cell		9.5%	90.8%	ND	ND	ND	91.0%	90.2%
	Bone marrow	Pre-B cell		73.4%	2.9%	5.7%	63.6%	54.9%	ND	0.7%
		T cell		35.1%	39.8%	ND	ND	ND	53.8%	41.3%
aly/aly	Peritoneal cavity	B-1 cell	1:7	96.8%	86.7%	84.4%	ND	ND	ND	ND
		B-2 cell		98.4%	5.2%	1.7%	12.3%	83.0%	ND	ND
		T cell		34.6%	96.0%	ND	ND	ND	33.3%	94.0%
	Spleen	B-1 cell	1:16	87.1%	13.2%	24.1%	43.8%	87.8%	ND	ND
		B-2 cell		90.9%	2.2%	2.4%	71.3%	91.2%	ND	0.6%
		T cell		5.2%	93.5%	ND	ND	ND	96.2%	94.4%

ND: Not determined.
[a] Estimated from the B-1 and B-2 cell numbers obtained.

CD43 (14.8%) (Table 1, Fig. 2). The B-2 cells prepared from spleen and peripheral blood exhibited the phenotypes identical to those of B-2 cells prepared from the peritoneal cavity, whereas the B-1 fraction prepared from these tissues was less pure than that prepared from the peritoneal cavity, possibly due to a markedly low B-1/B-2 ratio (Table 1, Fig. 2). We also prepared CD43-negative B cells from bone marrow (pre-B cells) that exhibited a phenotype, except for CD23, similar to that of B-2 cells. The bone marrow CD5-positive B cells were too few to analyze.

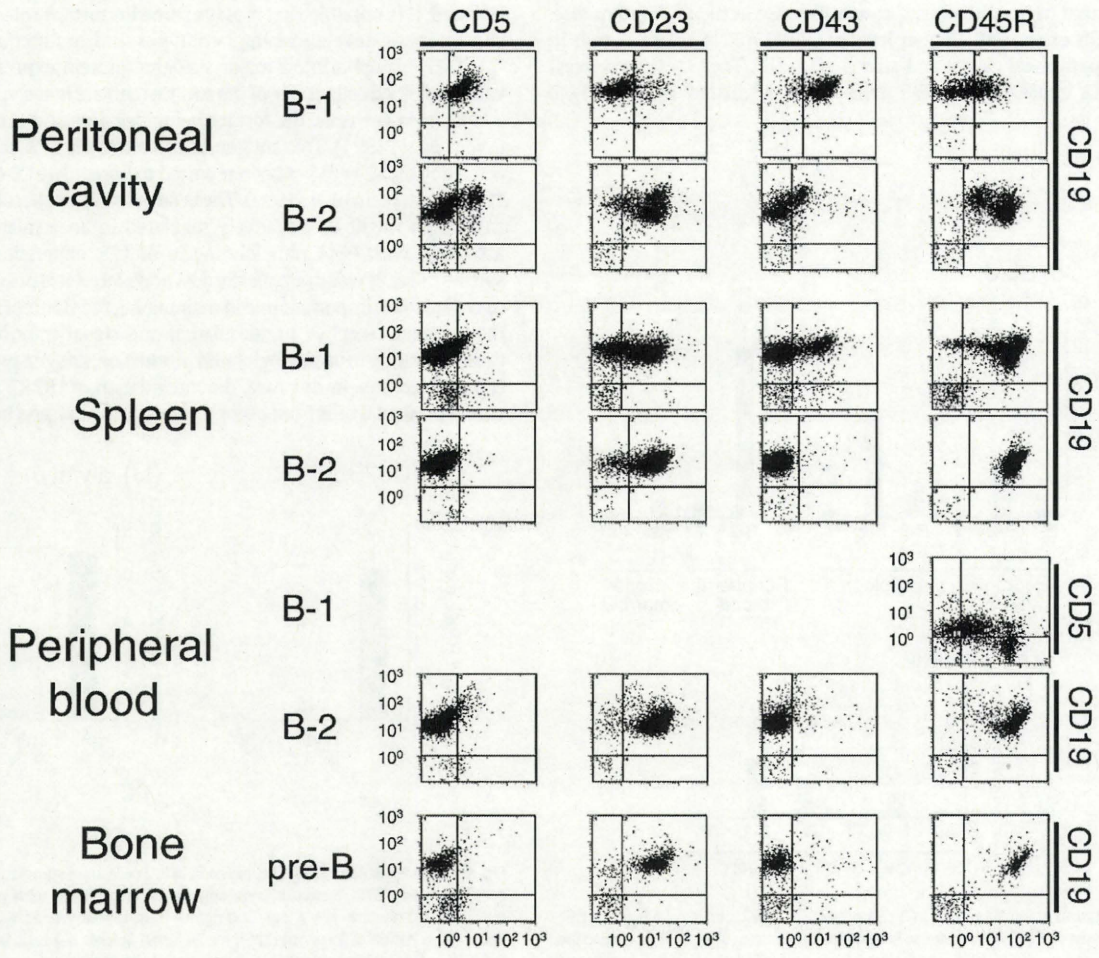

Fig. 2. Flow-cytometric characterization of isolated B cell subsets from spleen, peripheral blood, peritoneal cavity, and bone marrow. Each B cell subset was obtained by magnetic activated cell-sorting (MACS) as described in Materials and methods. B cell subsets from each tissue were characterized by flow-cytometric analysis using immunofluorescent antibody staining for CD5, CD19, CD23, CD43, and CD45R. Data for one of three independent experiments are shown.

3.2. LRRK2 mRNA expression in B-1 and B-2 cell subsets

LRRK2 mRNA expression in B-1 and B-2 cells as well as T cells isolated from the peritoneal cavity, spleen, and peripheral blood of the C57BL/6 mouse was investigated by qRT-PCR. This revealed that the B-2 cells expressed a much higher level of LRRK2 mRNA than the B-1 cells in all three tissues (6.7-, 3.8-, and 12.8-fold for the peritoneal cavity, spleen, and peripheral blood, respectively) (Fig. 3A). These results suggested that LRRK2 may have a more active function in B-2 cells than in B-1 cells. In contrast to the B-2 cells, the bone marrow pre-B cells expressed a much lower level of LRRK2 mRNA, suggesting that LRRK2 had a differentiation-related expression profile. In accordance with our previous study of spleen T cells (Maekawa et al., 2010), T cells isolated from either of these tissues expressed little or no LRRK2 mRNA.

The alymphoidplasia (aly) mouse strain has a naturally occurring point mutation in the NF-kB inducing kinase (NIK) gene that results in defective development of lymph nodes and Payer's patches (Miyawaki et al., 1994). Because the aly mouse has been reported to have an increased B-1/B-2 ratio, we investigated whether this strain exhibits a LRRK2 expression profile among B cell subsets different from that of the C57BL/6 mouse. As reported previously (Fagarasan et al., 2000; Shinkura et al., 1999), the B-1/B-2 ratio in the aly mouse was markedly high in both the peritoneal cavity and spleen (2.3- and 7.5-fold, respectively, in comparison with the C57BL/6 mouse, Table 1). Nevertheless, B-1 and B-2 cells prepared from peritoneal cavity and spleen of the aly mouse exhibited CD profiles similar to those of equivalent cells prepared from the corresponding tissues of the C57BL/6 mouse (Table 1). The qRT-PCR indicated that in the aly mouse, as was the case in the C57BL/6 mouse, the B-2 cells expressed a higher level of LRRK2 mRNA than B-1 cells in both the peritoneal cavity and spleen (Fig. 3B). These results suggest that LRRK2 expression by B-2 cells is not regulated by the NF-κB pathway.

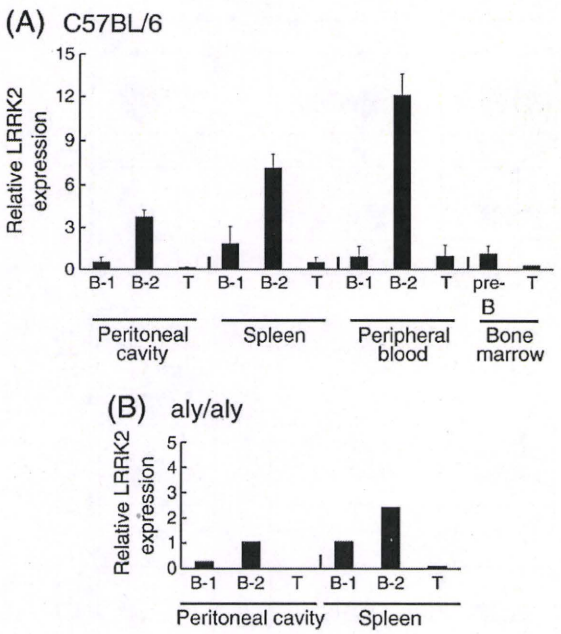

Fig. 3. Expression of LRRK2 mRNA in B cell subsets. B-1, B-2, and T cells were purified from each tissue of the C57BL/6 mouse (A) and the aly mouse (B) by MACS, as described in Materials and methods. RNA was isolated from each of the pooled cell populations from 10 mice. The amount of LRRK2 mRNA was measured by qRT-PCR and normalized relative to the expression of GAPDH. The average of three independent experiments is shown.

3.3. Downregulation of LRRK2 expression by activation of B-2 cells

As described above, we isolated B-2 cells as the CD43-negative B cell fraction. CD43 (sialophorin) is expressed at high levels on the surface of wide variety of leukocytes, including B-1 cells and activated B-2 cells, but not on resting B-2 cells. Based on speculation that the difference in LRRK2 expression between B-1 and B-2 cells is related to the activation status of B cells, we next investigated whether LRRK2 expression by B-2 cells is influenced by activation. Splenic B-2 cells from the C57BL/6 mouse were activated with either PMA plus ionomycin or LPS for 24 h. It was found that LRRK2 expression by B-2 cells was almost completely downregulated by activation (Fig. 4A). The LRRK2 downregulation was independent of the NF-κB pathway mediated by NIK, as LRRK2 mRNA expression by B-2 cells from the aly mouse was also downregulated by activation with anti-IgM or LPS (Fig. 4B). Our results suggest that LRRK2 in B-2 cells may have an important functional role in maintaining B-2 cells at the resting stage.

4. Discussion

In the present study, we found for the first time that LRRK2, a complex kinase that had been originally identified as the causative molecule of familial Parkinson's disease, is expressed differentially between the two B cell subsets, B-1 and B-2. B-2 cells (conventional B cells) isolated from the peritoneal cavity, spleen and peripheral blood expressed LRRK2 mRNA at a much higher level than B-1 cells. Although the true physiological function of LRRK2 has yet to be clarified, it is possible that it plays some important roles related to the characteristic development, phenotypes, and/or functions of B-2 cells.

CD43 (sialophorin), a major sialoglycoprotein expressed by a wide variety of blood cells, is one of the surface markers that can discriminate B-2 cells from B-1 cells, the former being negative and the latter positive (Wells et al., 1994). This antigen is also a marker of B-cell activation, as activation of B-2 cells has been reported to upregulate CD43 (Gulley et al., 1988; Rosenstein et al., 1999). These facts led us to speculate that LRRK2 expression might be negatively regulated in an activated state. Upon activation with PMA plus ionomycin or LPS, expression of LRRK2 by splenic B-2 cells was dramatically downregulated. It is possible that LRRK2 may play some important role in maintaining the status of B-2 cells so that they remain receptive to stimulation and signal transduction, such as those operating in immunoglobulin production, antigen presentation, and cell proliferation. In this case, downregulation of LRRK2 after activation would prevent B cells from becoming overactivated, and being induced to

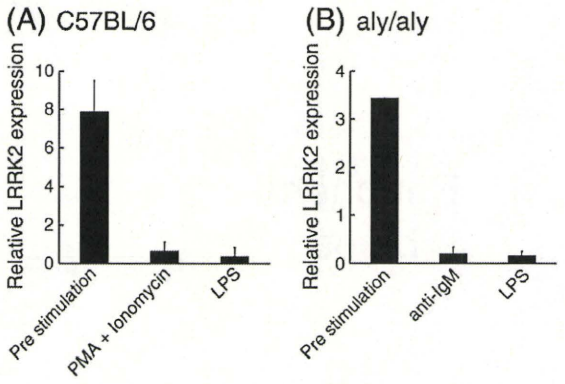

Fig. 4. Downregulation of LRRK2 expression in B-2 cells upon stimulation. (A) Splenic B-2 cells from the C57BL/6 mouse were stimulated in triplicate with either phorbol 12-myristate 13-acetate (PMA) at 300 ng/ml plus ionomycin at 600 ng/ml, or with lipopolysaccharide at 25 μg/ml (LPS), for 24 h. (B) Splenic B-2 cells from the aly mouse were stimulated with either $F(ab')_2$ fragments of either goat anti-mouse IgM at 15 μg/ml (anti-IgM), or with LPS, for 24 h. RNAs were isolated from pre-stimulated and stimulated cells, and the amount of LRRK2 mRNA was measured by qRT-PCR. Expression of LRRK2 normalized relative to GAPDH is shown.

undergo apoptosis. On the other hand, LRRK2 expression in B-1 cells may be sustained at a low level by spontaneous activation, as B-1 cells are known to have constitutively activated extracellular signal-regulated kinase and secrete immunoglobulin spontaneously in the absence of exogenous antigenic stimulation (Berland and Wortis, 2002; Dasu et al., 2009; Durand et al., 2009; Holodick et al., 2009). The bone marrow pre-B cells, although CD43-negative, expressed only a very low level of LRRK2. This is in accordance with their positivity for the activation markers, interleukin-7 receptor and CD25, thereby indicating a degree of activated status in the process of B cell development (Hardy and Hayakawa, 2001).

The NIK-mutant aly mouse, having an abnormally high B-1/B-2 ratio (Fagarasan et al., 2000; Shinkura et al., 1999), exhibited features of LRRK2 expression that were similar to those of the C57BL/6 mouse, i.e., higher expression in B-2 than in B-1 cells and downregulation after activation. These facts suggest that expression of the LRRK2 gene may not be regulated by the NIK–NF-κB pathway. Because three different forms of stimulation – PMA plus ionomycin, LPS, and anti-IgM antibody – similarly downregulated the expression of LRRK2, transcription factors functioning downstream of, and in common with these types of stimulation, such as p38 and c-Jun N-terminal kinase, could be possible regulators of LRRK2 gene expression. MKK6, an upstream kinase of p38, reportedly increases the intracellular level of LRRK2 (Hsu et al., 2010).

The molecular mechanism whereby mutant LRRK2 causes neurodegeneration in familial Parkinson's disease has not been conclusively clarified. The difficulty comes from the fact that the true function of LRRK2 is not known, although its kinase activity has been demonstrated using potential interactors such as moesin, 4E-BP, β-tubulin, and MKK3, 6, and 7 (Gandhi et al., 2008; Hsu et al., 2010; Imai et al., 2008; Jaleel et al., 2007). Investigation of the functional role of LRRK2 in B-2 cells from an immunological viewpoint could provide valuable clues, different from those obtained by neurological analysis, to clarifying its true biological function and its pathogenetic role in Parkinson's disease.

In patients with Parkinson's disease, increased serum levels of anti-melanin antibody and IgG deposition have been found in the substantia nigra (Double et al., 2009; Orr et al., 2005). It is possible that LRRK2 may contribute directly or indirectly to progression of the disease by regulating the humoral immune responses of B-2 cells. An interesting issue to be clarified is whether or not B-2 cells expressing mutant LRRK2 exhibit a normal immune response. It has been reported that B-lymphoblastoid cell lines carrying LRRK2 mutations show impaired growth (Tan et al., 2008). On the other hand, invasion of $CD8^+$ and $CD4^+$ T cells in the brain and alterations of the peripheral T-cell population (increased $CD8^+$ and decreased $CD4^+$ $CD25^+$) reportedly observed in Parkinson's disease patients indicate the contribution of a cellular immune response to progression of the disease (Baba et al., 2005; Brochard et al., 2009). Thus, it is apparent that Parkinson's disease is not only a lesion of neurons *per se* but also has an immune inflammatory character. LRRK2 is a candidate molecule that could play a role in the process of both neural dysfunction and immune inflammation in Parkinson's disease.

Acknowledgments

This study was supported by Kitasato University (All Kitasato Project Study, No. 18-1) and the Graduate School of Medical Sciences, Kitasato University (Integrative Research Program, 2008–2009).

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薬物治療のまとめ

坂本 崇　村田美穂

ジストニア治療における薬物治療の位置づけ

もともとわが国においては，薬物治療はジストニア治療のほぼ唯一の選択肢であった．そのためか，今から5年ほど前まではジストニア治療の現場で最も多く行われている治療法ということになっていた[1]．しかしながら，欧米においてはジストニア治療となると第一選択はボツリヌス治療，そして外科治療すなわち深部脳刺激であり，薬物治療の効果はそれに及ぶものではないとされてきている[2]．この解離は，ボツリヌス神経毒素製剤の厳格な使用制限とそれに伴うボツリヌス治療普及の遅延というわが国の特殊事情によるものと推察されるが，昨今はボツリヌス治療・深部脳刺激に関する研究が進み，また臨床経験の蓄積によって治療成績が向上し，幅広く臨床科医の間にもその知識が普及しつつあることは事実であろう．しかしながら，なおボツリヌス治療・深部脳刺激については一部の施設に集中するきらいもあり，現実的には最もアクセスのしやすい薬物治療に何らかの指針が得られることが望ましい．平成21年度厚生労働省の研究班ではまさにその部分を課題として取り組んでいるが，この先も班研究が継続されることになれば，さらに踏み込んだ指針作成を目指したいと考える．

薬物治療の実際

それでは，実際の薬物治療はどのように行われるべきであろうか．

発症5年以内の20歳未満のジストニア患者においては薬物治療の有効率が70%に達するという報告があるが[3]，20歳以上の患者でも発症から時間が短い場合，軽症の場合には薬物治療が十分に奏効することがある．具体的には，眼瞼痙攣患者の場合，若倉の分類[4]でいうⅠ(訴えに対応した他覚的所見が得られない)，Ⅱ(軽瞬・速瞬が不規則または強瞬しかできない)に相当する軽症例においては薬物治療のみで十分な症状改善が得られることが少なくない．

これに比べると痙性斜頸等のジストニアでの成績は劣っており，早期からのボツリヌス治療が推奨されている[5]．この場合も，ボツリヌス神経毒素製剤の使用開始までには登録手続きで1週間前後を要することがあるので，特に薬物治療を試したことがない患者の場合には後述の薬剤を(何種類か)試してみるのは決して悪いことではないと考える．筆者の経験でも，数自体は決して多くはないが，ボツリヌス治療開始までの薬物治療が思いのほか良好な結果をもたらし，最終的にボツリヌス治療は行わずに軽快した例も認められた．

さらに，今後の展開として重要なのは，ボツリヌス治療や外科治療との併用という観点であろう．薬物治療での反応がいま一つであったために，ボツリヌス治療ないし外科治療に「進む」という考え方は治療戦略として当然の選択であるが，だからといって薬物治療が完全に放擲されるわけではないことは強調しておきたい．すなわち，ボツリヌス治療の効果が続いている場合には内服量を減らし，効果が減弱してきたときに増やすといった調整を行うことによって，より有効なコントロールが可能となる．また，外科治療に際しても，特に脳深部刺激の刺激強度調節に応じて適宜薬物の調整を行うことも有用であろう．さらに，副作用等の理由でボツリヌス治療や外科治療の効果が十分に得られない場合もあり，そうしたときに薬物治療の併用を考慮するのは何ら不自然なことではなく，むしろ大いに検討すべき問題である．

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0289-0585/10/¥500/論文/JCOPY

薬物治療各論

わが国のジストニア薬物治療に関しては1980年代[6]，2004年[1]に大規模な調査が行われているが，薬剤のレパートリー自体は大きく変化していない．以下に代表的な薬剤について述べる．なお，現時点でわが国で「ジストニア」に対する保険適用のある薬物は全くない，すなわち全てoff-label useとなる．

1．抗コリン薬：trihexyphenidyl(アーテン®)

ボツリヌス神経毒素製剤と比較する報告では，平均16.25 mg(4～24 mg)の服用で37.5%の有効率があったとされている．これは薬物治療の成績として最も良好な値であり，文献上唯一ジストニアに対する有効性が示された薬剤となっている[7]．また，大量投与が分節性・全身性ジストニアに有効であるとも報告されている[8]．しかしながら少量でも口渇は必発症状であり，緑内障・尿閉のリスクのある患者には原則禁忌である．さらに，認知機能の低下(ぼーっとする，集中力がなくなったといういい方をすることが多い)を訴える患者も多く，本来は最高60～90 mg程度まで使用したいところ，内服増量・継続を断念する理由となってしまう点が残念である．分2～3の定期的な服用が効果的である．

biperiden(アキネトン®)は抗コリン作用がmildで精神科領域で好んで用いられることが多い印象があるが，trihexyphenidylの切れ味には乏しい[9]．

2．Benzodiazepine系：clonazepam(リボトリール®，ランドセン®)，diazepam(セルシン®)，etizolam(デパス®)

多くの副作用は眠気・ふらつきで，それも慣れることによって耐えることができるようになるため，最も使いやすい薬といってよいかもしれない．0.5～1.5 mgあたりから開始，3～4.5 mgあたりが平均的な維持量である．筆者は分2～3の定期的な服用に加えて，そのときの症状に応じて生活のリズムに合わせて適宜頓用することを勧めており，好評である．また，眠気の問題に対しては就眠前に睡眠薬として用いることも効果的である．

最近のトピックスとしてはzolpidem(マイスリー®)が著効する症例の報告がみられている[10]．報告例の使用量は50 mgと多量だが，通常の5～10 mgの内服が有効な場合もあり，今後注目に値する．

3．GABA作動薬：baclofen(リオレサール®，ギャバロン®)

いわゆる抗痙縮薬として従来から用いられているが，その効果を疑問視する声も少なくない[11]．さらに，副作用としての脱力感や眠気が出やすいこともあり，第一選択薬としては見劣りするといわざるを得ない．むしろ二次的な凝りやこわばりとそれに伴う痛みに対して用いるのが適切であろう．

バクロフェンポンプによる痙性対麻痺の管理は注目を集めているところだが，ジストニアに対してもその有効性は報告されている[12]．全体に小児例での有効性の報告が多い．深部脳刺激の進歩に伴ってその有用性は一歩後退している感が否めない．

GABA作動薬として最近開発されたのがgabapentin(ガバペン®)である．動物モデルのレベルでは有効性を報告するものもあるが[13]，臨床応用ではむしろジストニアを誘発する例も散見され，注意が必要である．

4．そのほかの薬剤

mexiletineの有効性はわが国から報告されており，選択肢の一つとして考慮すべき薬剤といえる[14]．

L-DOPAはドーパ反応性ジストニアには絶対的な第一選択薬であるが，一般のジストニアの場合無効，むしろ悪化させることがあるので注意を要する[15]．ドーパミン枯渇薬であるtetrabenazineで有効例の報告があり[16]，ドーパミン拮抗薬の中のperphenazine，tiapride，risperidoneにも有効性を認めるとの報告があるが一般的ではない．

薬物治療の実際(自験例から)

1．セルシン®大量が有効であった体軸性ジストニア

35歳男性．もともと統合失調症で10歳代後半より多量の抗精神病薬を内服している．20歳代半ばから身体の歪みが目立つようになり，当科初診時には体軸の側屈がほぼ

90度に達していた．ボツリヌス治療や外科治療は本人が拒否，セルシン®内服を試すことになった．1日当たり6mgから開始，副作用もなかったため増量していった．24mgの段階で身体が伸ばしやすいと感じるようになり，捻転も軽減がみられた．本人希望でボツリヌス治療を開始，100単位傍脊柱筋へ施注し，側屈が30度前後まで改善している．

2．アーテン®は有効だが副作用のため中断せざるを得なかった上肢ジストニア

42歳男性，海外オーケストラでコンサートマスターをしている．楽器を演奏しづらいと感じ，ドイツの専門医を受診，手指の過屈曲のおこる音楽家のジストニアと診断された．ボツリヌス治療で過度の脱力が生じたために内服治療を開始，リボトリール®は無効だったが，アーテン®4mgで劇的な症状改善がみられ，演奏活動を再開することができた．しかしながら，舞台上でぼーっとしてしまう，楽譜に集中しづらい等の問題があり，アーテン®2mgに減量，以降は1%リドカインの局注を繰り返している．

3．リボトリール®で完全に症状の改善した眼瞼痙攣

72歳女性．目がまぶしいと感じて眼科受診するも年齢相応の加齢性変化のみ，眼鏡調整を繰り返したが大きな改善なし．家人のみた新聞記事で眼瞼痙攣のことを知って神経内科受診．強収縮後の開瞼障害は軽度であるが感覚トリック存在，軽度の眼瞼痙攣と診断し，本人希望で2週間後にボツリヌス治療を予約，それまでの間リボトリール®を内服するよう指示した．1週間0.5mgを内服しているときは特に変化なかったが，1mgに増量してからはまぶしさがなくなっている．ボツリヌス治療は中止，3ヵ月ほど定期的な内服を行った後は自己調節とし，現在は外出時のみ0.5mg使用している．

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A Phase I Study of Aromatic L-Amino Acid Decarboxylase Gene Therapy for Parkinson's Disease

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Gene transfer of dopamine-synthesizing enzymes into the striatal neurons has led to behavioral recovery in animal models of Parkinson's disease (PD). We evaluated the safety, tolerability, and potential efficacy of adeno-associated virus (AAV) vector–mediated gene delivery of aromatic L-amino acid decarboxylase (AADC) into the putamen of PD patients. Six PD patients were evaluated at baseline and at 6 months, using multiple measures, including the Unified Parkinson's Disease Rating Scale (UPDRS), motor state diaries, and positron emission tomography (PET) with 6-[^{18}F]fluoro-L-*m*-tyrosine (FMT), a tracer for AADC. The short-duration response to levodopa was measured in three patients. The procedure was well tolerated. Six months after surgery, motor functions in the OFF-medication state improved an average of 46% based on the UPDRS scores, without apparent changes in the short-duration response to levodopa. PET revealed a 56% increase in FMT activity, which persisted up to 96 weeks. Our findings provide class IV evidence regarding the safety and efficacy of AADC gene therapy and warrant further evaluation in a randomized, controlled, phase 2 setting.

Received 25 January 2010; accepted 5 June 2010; published online 6 July 2010. doi:10.1038/mt.2010.135

INTRODUCTION

Dopamine replacement has been the standard pharmacotherapy for motor impairment in Parkinson's disease (PD). Although virtually all patients benefit from levodopa at an early stage of the disease, severe loss of nigrostriatal nerve terminals in advanced PD leads to profoundly decreased activities of dopamine-synthesizing enzymes, including aromatic L-amino acid decarboxylase (AADC), an essential enzyme that converts levodopa to dopamine. Failure to respond to levodopa therapy may result from a reduction in AADC activity, decreased dopamine storage capacity in synaptic vesicles, postsynaptic changes in striatal output neurons, and abnormalities of nondopaminergic neurotransmitter systems.[1,2] Systemic administration of high-dose levodopa enhances oscillations in motor performance and complications, including hallucinations, due to dopaminergic stimulation of the mesolimbic system.

One potential treatment for advanced PD is gene therapy to restore striatum-selective dopamine production. In addition to AADC, tyrosine hydroxylase, which converts L-tyrosine to levodopa, and guanosine triphosphate cyclohydrolase I, which catalyzes biosynthesis of the essential tyrosine hydroxylase cofactor, tetrahydrobiopterine, are necessary for efficient synthesis of dopamine.[2] Viral vector–mediated gene transfer of these dopamine-synthesizing enzymes has been shown to achieve behavioral recovery in animal PD models, with efficient transduction of striatal neurons that escape degeneration.[3–6] When tyrosine hydroxylase and guanosine triphosphate cyclohydrolase I are expressed in the striatum, levodopa can be synthesized continuously. This strategy would be useful for reducing motor fluctuations associated with intermittent levodopa intake. Gene transfer of AADC alone in combination with oral levodopa administration would be a safer strategy for initial clinical trials. In the latter approach, the patients still need to take levodopa to control motor symptoms, but excess production of dopamine could be avoided by reducing the dose of levodopa. We assessed the safety, tolerability, and the potential efficacy of intraputaminal infusion of recombinant adeno-associated virus (AAV) serotype 2 vector encoding human AADC (AAV-hAADC-2) in patients with mid- to late-stage PD. We also examined whether the short-duration response to levodopa, the antiparkinsonian response that parallels the plasma levodopa levels, would change after gene therapy.[7]

RESULTS

Patient disposition and baseline characteristics

Six patients (4 men, 2 women), mean age 60 (range, 51–68) years, were enrolled (Table 1). The mean disease duration was 10 (range, 5–18) years, and time on levodopa was 9.3 (range, 5–15) years. The average baseline daily levodopa and levodopa equivalent doses were 642 and 808 mg, respectively.

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Table 1 Patients' baseline characteristics

Subject	Age (years)	Sex	Disease duration (years)	Time on levodopa (years)	Levodopa dose (mg)	Levodopa equivalents (mg)
A-1	51	M	11	9	600	900
A-2	63	M	9	9	450	650
A-3	66	F	7	7	500	700
A-4	58	M	11	11	700	700
A-5	68	F	18	15	1,000	1,100
A-6	56	M	5	5	600	800
Mean (SD)	60 (6.5)	67% M	10 (4.5)	9.3 (3.4)	642 (196)	808 (169)

Abbreviations: F, female; M, male.
Patients are listed in the order in which they received treatment. Levodopa equivalents were estimated as follows: 100 mg of levodopa with a dopa-decarboxylase inhibitor is equivalent to 0.8 mg talipexole, 1 mg pergolide, 1 mg pramipexole, and 1.5 mg cabergoline.

Primary end point

The procedure was well tolerated. All patients completed all protocol-defined visits. One patient (patient A-2) had a venous hemorrhage in the right frontal lobe just below a burr hole that was found on CT scan 3 days after infusion. The patient used his left arm less frequently than his right arm for 3 weeks; this was assumed to reflect mild frontal lobe dysfunction and resolved completely. Mild, transient headache around the burr holes was present for 2 days after surgery in all patients. There were no significant laboratory test abnormalities. All patients had mildly increased titers of anti-AAV2-neutralizing antibodies 6 months after treatment, which tended toward baseline concentrations thereafter (Table 2).

Table 2 Changes in neutralizing AAV2 antibody titers in sera following gene therapy

Subject	Pre	2 weeks	6 months	1 year
A-1	1:2	1:4	1:4	1:4
A-2	<1	1:32	1:4	1:2
A-3	1:32	1:64	1:64	1:32
A-4	1:32	1:32	1:256	1:64
A-5	1:4	1:32	1:32	1:32
A-6	<1	1:16	1:32	1:32

Abbreviations: AAV, adeno-associated virus.
Titers are determined by *in vitro* assay and represented as "1:" dilutions.

Clinical evaluations

The clinical results are summarized in Table 3. Intraputaminal AAV-hAADC-2 infusion significantly improved both total and motor scores of the unified Parkinson's disease rating scale (UPDRS) in the OFF state. Five of six patients showed substantial improvement in UPDRS motor ratings in the OFF state (Figure 1). Changes in the UPDRS ON state and the percent of ON state hours in a day were not significant. One patient with relatively mild motor symptoms at baseline did not improve on UPDRS (A-3 in Figure 1). However, this patient showed a remarkable increase in mobile time as measured by the diaries (28% at baseline to 58% at 6 months after gene transfer; Figure 2). The daily dose of levodopa was unchanged in two patients (A-2 and A-5) and reduced in three patients (A-1, A-3, and A-5) at 6 months. Patient A-6, who had daytime sleepiness, preferred to reduce pramipexole instead of levodopa after gene therapy.

The last three patients underwent the levodopa test after our institutional review board confirmed the safety of AADC gene transfer in the first three patients. The short-duration response to levodopa did not change significantly after gene therapy in these three patients, though UPDRS motor scores at 6 months showed slight improvement at 30 minutes in patient 5 and at 120 minutes in patient 4 after levodopa intake (Figure 3). Significantly higher peak plasma levodopa concentrations were observed in these two patients after gene therapy.

The mini-mental state examination (MMSE) and geriatric depression scale (GDS) scores did not change significantly.

Table 3 Clinical outcomes of six patients

	Baseline	6 months	*P* value
UPDRS Total OFF	53 (12.4)	38 (10.1)	0.049*
UPDRS Total ON	15 (7.2)	10.7 (2.9)	0.262
UPDRS Part III (Motor) OFF	25.3 (9.4)	13.7 (6.0)	0.024*
UPDRS Part III (Motor) ON	5.2 (4.6)	1.8 (1.5)	0.120
Percent day spent in mobile state	48.8 (12.9)	55.4 (14.8)	0.348
Daily levodopa equivalents dose, mg	808 (169)	707 (233)	0.097

Abbreviations: OFF, off-medication state; ON, on-medication state; UPDRS, Unified Parkinson's Disease Rating Scale.
Data are presented as means (SD). The UPDRS scores in each patient did not change during the 2 months of the screening period.
*$P < 0.05$.

PET analysis

PET imaging revealed increased 6-[^{18}F]fluoro-L-*m*-tyrosine (FMT), a tracer for AADC, activity 4 weeks postoperatively, which persisted at 6-month evaluation (Figure 4). The mean increase in FMT uptake from baseline in the combined (right and left) putamen at 24 weeks was 56%. Two patients (A-1 and A-2) who had PET scans 96 weeks after surgery showed persistently increased FMT uptake. In these two patients, motor performance in the OFF state also maintained its improvement at 96 weeks.

DISCUSSION

Extensive preclinical studies on both rodent and nonhuman primate models of PD have shown that AAV vectors can express exogenous genes for a long time in the brain target areas without

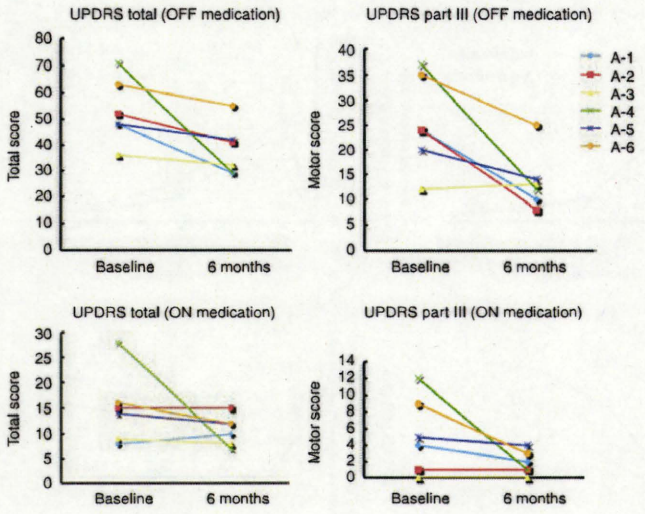

Figure 1 Changes in UPDRS scores. Absolute changes in scores from baseline to 6 months for individual patients. OFF, off-medication state; ON, on-medication state; UPDRS, Unified Parkinson's Disease Rating Scale.

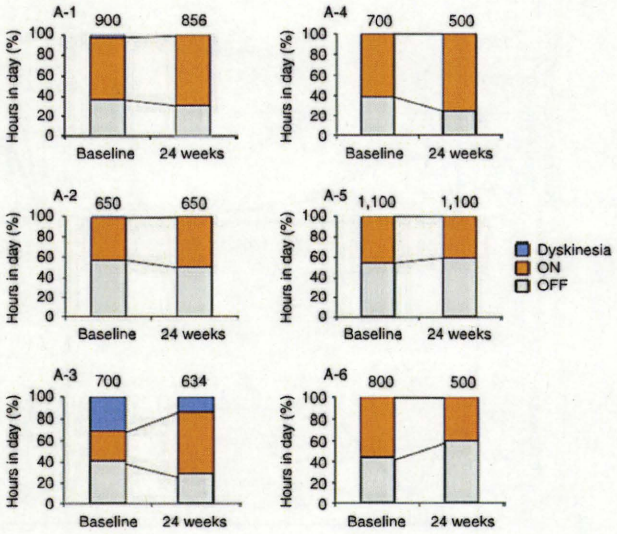

Figure 2 Evaluation of patients' diaries and daily doses of levodopa equivalents. For each 30-minute interval throughout the day, the patients recorded whether they were mobile (ON), immobile (OFF), or asleep. They also recorded the time with troublesome dyskinesias (Dyskinesia). The graph shows the percentage of hours in a day spent in each condition at baseline and at 6 months. The numbers on the bars indicate the mean daily doses of levodopa equivalents (mg). OFF, off-medication state; ON, on-medication state.

significant toxicity.[3,4,6,8,9] Recently, three phase I clinical trials of gene therapy for advanced PD demonstrated that AAV vector–mediated gene delivery into the subthalamic nucleus or putamen was safe and tolerable.[10–13] In this study, the safety of the AAV vectors for clinical use in the human brain was confirmed. Although one patient developed a venous hemorrhage in the subcortical white matter along the trajectory, it is well known that cerebral bleeding occasionally occurs in association with surgical procedures for deep brain stimulation in which electrodes are inserted into the basal ganglia through the frontal lobe white matter.[14,15] PET imaging in this patient showed that putaminal AADC expression was not affected by the subcortical venous hemorrhage and persisted up to 96 weeks. Thus, the venous hemorrhage was probably due to the surgical procedure and not gene transduction.

Although the present trial was a small, open-label study, and the nonblinded, uncontrolled analysis limits the interpretation, the initial efficacy outcomes are encouraging. Our patients showed improved motor performance in the OFF state. Levodopa has a relatively short plasma half-life (60–90 minutes), and antiparkinsonian effects observed after levodopa administration have generally been recognized as short- and long-duration responses. The short-duration response roughly parallels the plasma levodopa concentrations and is thought to be closely linked to dyskinesia, whereas the long-duration response builds up over weeks and improves trough (worst) motor performance in the OFF state.[7] Because the pattern of the short-duration response to levodopa did not change after gene therapy in our patients, the beneficial effect on the OFF state appears to be attributed to augmentation of the long-term response to levodopa.[16] In the preclinical studies with animal models of PD, AAV vectors mainly transduced medium spiny neurons that have dopamine receptors, and extracellular dopamine was increased in the striatum after administration of levodopa.[5,17] The mechanism underlying the long-duration response is not sufficiently understood, and future study is necessary to determine how nonphysiologic production of dopamine in the striatal neurons could enhance the response. It has been reported that the sustained long-duration response to levodopa is greater in patients treated with higher single doses of levodopa.[18] Thus, it is likely that increased dopamine in the putamen after gene transfer may enhance the stable long-duration response. Motor fluctuations in PD are associated with increased response to levodopa with a deeper trough in motor performance, rather than shortening of the response. Improving trough or OFF state motor function by augmenting the long-term response would likely reduce motor fluctuation.[16] Two of three patients in whom the short-duration response to levodopa was studied showed increased peak plasma levodopa concentrations after gene therapy. This finding may simply reflect variable absorbance of levodopa, and it remains to be elucidated whether changes in gastrointestinal absorption could be related to better motor performance in the OFF state.[19]

Activities and levels of AADC mRNA and protein are profoundly reduced in advanced PD,[2] but there are still several types of AADC-containing cells in the striatum, such as serotonin neurons, intrinsic dopamine neurons, AADC-containing "D" neurons, and glial cells.[20] These cells may act as a local source of dopamine. However, dopamine produced in nondopamine cells may not be taken up into dopamine cells and stored in synaptic vesicles, as dopamine transporter and vesicular monoamine transporter 2 are also reduced in advanced PD. The functional efficacy of dopamine produced from exogenous levodopa in these cells may be limited, at least in primates.[2,3] Striatal output neurons, main targets in AADC gene therapy, play a principal role in dopamine modulation of motor function in the basal ganglia. Dopamine synthesized in the striatal neurons themselves may more easily stimulate both synaptic and extrasynaptic receptors.

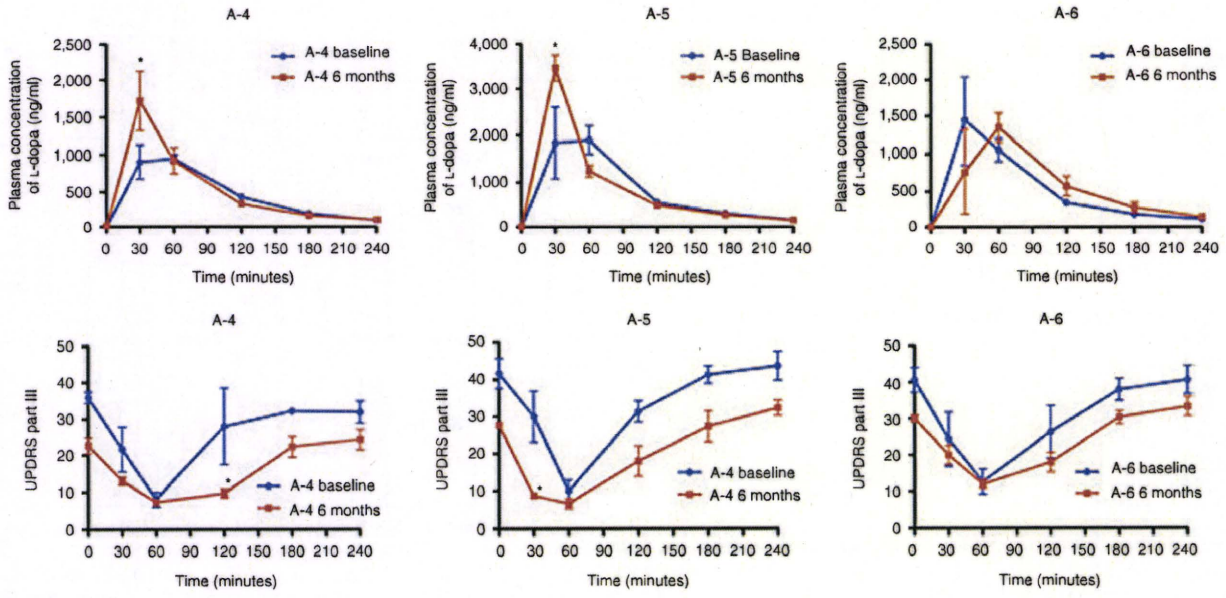

Figure 3 Short-duration response to levodopa. Comparison of short-duration response to levodopa before (blue) and after gene therapy (brown) in three patients (A-4, A-5, and A-6). Patients took 100 mg of levodopa with 25 mg benserazide orally after 20 hours without dopaminergic medication. Values represent means and SE of three trials. Upper panels: plasma levodopa levels; lower panels: Unified Parkinson's Disease Rating Scale motor scores. $*P < 0.05$.

Results of a similar phase I protocol were reported recently for the 10 patients treated with AAV-hAADC-2 (ref. 10). That study used the same vector preparations as this study. The subjects were divided into two groups that received the same or one-third dose of the vector used in this study, respectively. Although the present patients had slightly milder initial symptoms, the patients treated with the same dose of vector in the two studies showed similar improvement in the OFF state and putaminal FMT uptake on PET. These findings provide independent confirmation of the safety, tolerability, and potential efficacy of AADC gene therapy. Future studies focusing on optimal vector dosing and defining the relationship between vector dose and clinical effects are necessary.[21]

In conclusion, these data indicate that AAV vector–mediated gene transfer of AADC is safe and may benefit advanced PD patients.

MATERIALS AND METHODS

Study design. The protocol and consent forms were approved by the institutional review board. The protocol was also reviewed by the committee of the Ministry of Health, Labour and Welfare of Japan. A data safety monitoring board reviewed the ongoing study. All subjects reviewed the consent form and provided their written, informed consent.

This 24-week, phase I, open-label study was primarily designed to evaluate the safety and tolerability of intraputaminal AAV-hAADC-2 infusion in idiopathic PD. Patients were evaluated preoperatively and monthly postoperatively for 6 months, using multiple measures, including the UPDRS, motor state diaries, the MMSE, the short form of the GDS, and laboratory tests. The UPDRS was done in the practically defined OFF state 12 hours after withdrawal of all antiparkinsonian medications, and in the ON state 1 hour after administration of the usual morning dose of medication. Motor scores for the UPDRS can range from 0 to 56, with higher scores indicating poorer function. Using diaries that separated the day into half-hour segments, the patients recorded their mobility during the 4 days before admission and for another 4 days at 6 months after admission. They were trained to rate their condition as sleeping, immobile, mobile without troublesome dyskinesias, or mobile with troublesome dyskinesias. The total number of hours spent in each of these categories was calculated, and the differences between the baseline and the 6-month scores were compared between the groups.

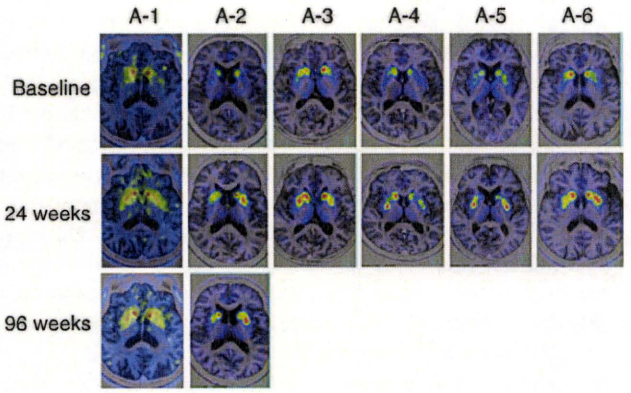

Figure 4 FMT-PET images. Axial images at the level of the putamen are shown before and 24 weeks after gene therapy for all six patients. Increased FMT uptake persisted until 96 weeks in two patients. The 4-week images are not shown because they are similar to the 24-week images. FMT, 6-[^{18}F]fluoro-L-*m*-tyrosine; PET, positron emission tomography.

The short-duration response to levodopa was evaluated in three patients (patients 4–6) at baseline and 6 months after gene transfer; they took 100 mg of levodopa orally with 25 mg benserazide after 20 hours without dopaminergic medication. Motor symptoms based on UPDRS motor (part III) and plasma levodopa concentrations were assessed at baseline and 30 minutes, 1, 2, 3, and 4 hours after levodopa intake.

Patients. The main entry criteria were: age 45–75 years; diagnosis of moderate to advanced PD, defined as Hoehn and Yahr Stage IV and UPDRS in the practically defined OFF condition of at least 20; at least

5 years of levodopa therapy; a minimum 8-point improvement in the UPDRS motor score after levodopa intake; and motor complications not satisfactorily controlled with medical therapy. The main exclusion criteria were atypical parkinsonism, dementia (MMSE score <20), and previous neurosurgical treatment for PD.

Vector and stereotaxic infusion. The vector used in this trial was a recombinant AAV2 with an expression cassette consisting of a human cytomegalovirus immediate-early promoter, followed by the human growth hormone first intron, complementary DNA of human AADC, and simian virus 40 polyadenylation signal sequence.[3–6] Clinical grade AAV-hAADC-2 was manufactured by Avigen (Alameda, CA) and provided by Genzyme (Boston, MA). The patients received AAV-hAADC-2 *via* bilateral intraputaminal infusions. Two target points were determined in the putamen that were sufficiently separated from each other in dorsolateral directions and identified on a magnetic resonance image. One burr hole was trepanned in each side of the cranial bone, through which the vector was injected into the two target points *via* the two-track insertion route. The vector-containing solution was prepared to a concentration of 1.5×10^{12} vector genome/ml, and 50 µl per point of the solution were injected at 1 µl/min; each patient received 3×10^{11} vector genome of AAV-hAADC-2.

Neutralizing antibody titers against AAV2 were determined by measuring β-galactosidase activities in HEK293 cells transduced with 5×10^3 vector genome/cell of AAV2 vectors expressing β-galactosidase in various dilutions of sera.[22]

PET. The AADC expression level in the putamen was assessed on PET imaging with FMT 6 days before surgery and 1 and 6 months after gene transfer. All patients stopped dopaminergic medications 18 hours before PET and took 2.5 mg/kg of carbidopa orally 1 hour before FMT injection. Subsequently, 0.12 mCi/kg of FMT in saline were infused into an antecubital vein, and a 90-minute dynamic acquisition sequence was obtained. The PET and magnetic resonance imaging data were co-registered with a fusion processing program (Syntegra; Philips, Amsterdam, The Netherlands) to produce the fusion images. Radioactivities within volumes of interest drawn in the putamen and occipital lobe were calculated between 80 and 90 minutes after tracer injection. A change in putaminal FMT uptake from baseline to 24 weeks was assessed using the putaminal–occipital ratio of radioactivities.

Statistical analysis. Values at baseline and 6 months after gene transfer were compared using Student's *t*-test (paired analyses). A two-sided *P* value <0.05 was taken to indicate significant differences. Two-way analysis of variance with Bonferroni correction of *P* values was used for the short-duration response to levodopa.

ACKNOWLEDGMENTS

This study was supported by grants from the Japanese Government: a grant-in-aid from the Research Committee of CNS Degenerative Diseases *via* the MHLW and grants from the Ministry of Education, Culture, Sports, Science and Technology. We thank Hiroshi Ichinose and Toshiharu Nagatsu for their helpful comments, and Naomi Takino, Hitomi Miyauchi, Keiko Ayabe, and Tetsuo Ito for their technical assistance. We also thank Avigen and Genzyme for providing clinical grade AAV vector.

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特集　脳深部刺激療法

Parkinson病に対する脳深部刺激の効果*

藤本健一**

Key Words：subthalamic nucleus, ventral intermediate nucleus, globus pallidus interna, pedunculopontine nucleus

はじめに

Parkinson病(PD)に対する脳深部刺激(DBS)療法が開始されて23年，わが国で保険適応となって10年が経過し，その効果に関する論文も多数発表されている．薬効の切れたoffでの症状がUPDRS(Unified Parkinson's Disease Rating Scale)で何％改善され，PD治療薬が1日当たりのL-dopa換算量(levodopa equivalent daily dose：LEDD)で何％減少したと，その効果を強調する報告が多い．その一方で，PDの患者から「知人がDBSの手術を受けたが，結果は期待はずれだった」という話を耳にすることも多い．この差はいったい何だろうか？PDは患者ごとに症状も異なれば進行の速さも異なる．DBSの実施時期も，電極の挿入部位もDBSに対する患者の期待度も異なる．このように背景が異なる事象を画一的に議論しても評価が一致しないのは当然である．病気の治療は患者・介護者の満足のため行う行為である．どのような症例にどのような治療法を選択するか，期待できる効果とリスクは何か，それを患者・介護者にどのように説明するか，治療満足度を高めるために主治医の果たす役割は大きい．本稿ではこれまでの報告と当施設での百余例のDBSの経験をもとに，PDに対するDBSの効果を論じる．DBSの治療満足度を高めるには，過去の経験から得られた事実を患者・介護者に正確に伝え，治療法の選択について十分に議論することが大切である．

脳深部刺激の効果と長期予後

DBSの標的はいくつかあるが，大きなインパクトを与えたのはLimousinらによる視床下核(subthalamic nucleus：STN)のDBSであった[1]．視床腹中間核(ventral intermediate nucleus：Vim)や淡蒼球内節(globus pallidus interna：GPi)は従来から凝固術の標的となっていたが，STNの凝固はバリズムを誘発する可能性がある．調節性のあるDBSの出現によって，STNはPD治療の新たな標的として注目を集めることになった．その後多くのSTN-DBSが実施され，現在ではその効果や副作用，長期予後についての評価がほぼ定まった．

まず基本的に，STN-DBSはPDのさまざまな症状のうち，L-dopaが有効な運動症状に対して効果を示す．長期臥床により筋肉が萎縮し関節が拘縮した症例にいくら大量のL-dopaを与えても歩けないのと同様に，このような症例にSTN-DBSを行っても歩けるようにはならない．PDでは最善の薬物療法を実施しても，5年後には約4割の

* Effects of deep brain stimulation for Parkinson's disease.

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患者がwearing-off現象を経験する．L-dopaを服薬すれば普通に動けるが，短時間で効果が切れて動けなくなる．このような症例でSTN-DBSは著しい効果を発揮する．すなわち，offの症状を改善してonの状態に近づけ，offでのUPDRS-II(ADLスコア)やUPDRS-III(運動スコア)を50％程度改善する[2]．その結果，1日のoff時間は約5時間短縮される[3)~5)]．薬物療法によるoff時間の短縮効果はせいぜい1日当たり1.5時間程度であるので，薬物療法と比較してSTN-DBSのoff時間短縮効果がいかに優れているかがわかる．

STN-DBSによってPD治療薬の必要量が減り，LEDDで50％以上の減量が可能である[2]．L-dopaの1回服薬量を減らすことによってdopa誘発性ジスキネジア(dopa induced dyskinesia：DID)を軽減することも可能で，DIDは70％近く軽減される[2]．さらにLEDDの減少に伴い，幻覚や妄想などの精神症状，消化器症状，起立性低血圧など，ドパミン補充に伴うさまざまな副作用を回避することができる．PD患者の中には副作用のため十分なドパミン補充(最善の薬物療法)が行えず，本来のonを体験できずに我慢を強いられている患者が存在する．STN-DBSの効果に関する従来の論文では，最善の薬物療法が行われている患者でのoff症状の改善効果が強調されているが，最善の薬物療法に耐えられない患者に対するDBSの効果はもっと注目されてよい．

DBSの長期予後に関して，治療開始5年後の効果が報告されている[6)7)]．Krackらは，平均55.0歳でLEDD 1,409±605mgの49例(5年フォローは42例)，Schüpbachらは，平均54.9歳でLEDD 1,468±811mgの37例(5年フォローは30例)に対する両側STN-DBS後5年間の経過を報告した．5年後の休薬状態(off)で，Krackらの報告[5]ではUPDRS-IIが49％低下，UPDRS-IIIが54％低下，DIDが58％低下，LEDDは63％低下，Schüpbachらの報告[7]ではUPDRS-IIが40％低下，UPDRS-IIIが54％低下，DIDが79％低下，LEDDが58％低下していた．

薬物療法と脳深部刺激療法の比較

最善の薬物療法を行った場合とDBSを行った場合とで，PDの症状はどの程度改善されるかを，患者を無作為割り付けして比較検討した研究がある．最初の研究はドイツとオーストリアの10施設で行われたもので，78ペア(156例)の進行期PD患者を対象として両側STN-DBSと最善の薬物療法とを比較した[3]．評価項目は6カ月後の生活の質と休薬状態(off)でのPDの重症度である．生活の質の評価にはPD患者への質問票であるPDQ-39(Parkinson's Disease Questionnaire-39)を，重症度の評価にはUPDRS-IIIを用いた．PDQ-39は78ペア中50ペアでSTN-DBS群の改善が優れていた($p=0.02$)．UPDRS-IIIは78ペア中55ペアでSTN-DBS群の改善が優れていた($p<0.001$)．重篤な有害事象はSTN-DBS群の13％，薬物療法群の4％に認められた($p<0.04$)．STN-DBS群のうち1例は脳内出血により死亡した．軽微なものも含めたすべての有害事象はSTN-DBS群の50％，薬物療法群の64％に認められ，薬物療法群で多かった($p=0.08$)．

DBSと最善の薬物療法の比較は米国の13施設でも行われた[4]．薬物療法で満足できる治療効果の得られなくなった進行期PD患者255例(70歳以上の高齢者25％を含む)を対象として，DBSと最善の薬物療法を継続した場合とを比較する研究である．対象患者を無作為に両側STN-DBS，両側GPi-DBS，薬物療法に割り付けることによって母集団のバイアスを排除した．治療開始6カ月後にDBS群では支障となるDIDを伴わないon時間が1日平均4.6時間増加したのに対して，薬物療法群では変化を認めなかった．運動機能もDBS群の方が薬物療法群と比べて有意に改善し($p<0.001$)，臨床的に意味のある運動機能の改善(UPDRS-IIIで5点以上改善)に達した患者の割合はDBS群の71％に対して薬物療法群は32％であった．DBS群は薬物療法群と比べてPDQ-39全般およびPDQ-39の8項目中7項目において有意な改善を示した($p<0.001$)．認知機能検査ではDBS群は薬物療法群と比較して情報処理能力の一部に軽度の低下を認めた．重篤な有害事象が1回以上発生したのはDBS群が49例，薬物療法群が15例で有意差を認めた($p<0.001$)．有害事象のうち39例は手術に起因するもので，脳出血による死亡が1例認められた．

同様の研究は英国においても実施された[5]．薬

物療法で満足できる症状コントロールのできなくなった366例の進行期PDを対象とした13施設での共同研究である．366例を手術群と薬物療法群それぞれ183例ずつに無作為に割り付けたが，実際に手術を受けたのは183例中178例(すべてDBS)であった．標的はSTNが174例，GPiが4例で，176例は両側に刺激電極を挿入した．1年後のPDQ-39に回答したのは162例であった．一方，183例の薬物療法群のうち12例は途中で気が変わって手術を受けるなどして，1年後に薬物療法を継続していてPDQ-39に回答したのは153例であった．153例中48例はアポモルフィンの持続皮下注射を受けていた．1年後のPDQ-39は手術群で5点改善したのに対して薬物療法群での改善は0.3点にとどまり統計的に有意差を認めた($p=0.001$)．PDQ-39の中で手術群と薬物療法群で差を認めたのは運動能力(−8.9, $p=0.0004$)，日常生活動作(−12.4, $p<0.0001$)，身体の不調(−7.5, $p=0.004$)の項目で，その他の項目では差を認めなかった．なお，手術群のうち36例(19%)は手術に関連した重篤な有害事象を経験し，手術時の脳内出血により1例が死亡した．

これらの無作為化対照試験に共通しているのは，DBSは最善の薬物療法に比べて運動症状やそれに伴う日常生活動作，患者満足度を改善したことである．通常の薬物療法のみならず，アポモルフィンの持続皮下注射を導入してもDBSには及ばなかった[5]．その一方で重篤な有害事象は明らかにDBS群に多く，その多くは手術に起因していた．各研究で手術に伴う脳内出血による死亡を1例ずつ認めた．DBSは成功すれば良好な結果が期待できる一方，手術に伴う危険を伴い，頻度は少ないが重篤な副作用を覚悟する必要があることが示された．

脳深部刺激のターゲット

PDに対するDBSでは，通常Vim, STN, GPiがターゲットとなる．最近は脚橋被蓋核(pedunculopontine nucleus：PPN)の刺激も研究されている．

PDに対するDBS治療の最初の標的はVimであった[8]．従来から行われていたVim凝固術の課程で，挿入した電極の先端を高頻度刺激するとPDの症状が軽快することからVim-DBSが試みられ，DBSが広まるきっかけとなった．Vim-DBSはPDにおける4～5 Hzの安静時振戦や一定の肢位をとり続けると出現するre-emergent tremorに対して著効する[9][10]．筋強剛やDIDに対しても効果を示すが，動作緩慢やすくみ現象，姿勢反射障害などには無効である[11]．したがって，振戦が主症状のPD症例では良い適応となる．両側Vim核に凝固巣を作製すると意欲低下や物忘れが出現することがある．一方，DBSは凝固と異なり調節性があるため，両側治療でもこれらの副作用の危険が少ない．両側に振戦を認めて最初から両側Vim核の治療が必要なときはVim-DBSを選択するのが一般的である．なお，振戦型のPDは一般に進行が遅く，片側だけに症状を認める状態で数年から10年以上経過することも稀でない．このような症例では当面は対側Vim核だけの治療でよいため，あえてDBSを選択せず，従来のVim凝固術を選択することもある．

PDにおける振戦以外の症状に対してはSTNかGPiを標的とする．STN-DBSの方がGPi-DBSよりも動作緩慢や歩行障害に対する効果が勝り，刺激強度が低くて済み，LEDDを減らすことができることからSTN-DBSが選択されることが多い[12]．

GPi-DBSの利点はDID抑制効果が高いことで，これはL-dopa減量によらない直接効果と考えられている[10][13]．これに対してSTN-DBSによるDID抑制は，PD治療薬の減量に負うところが大きい．GPi-DBS開始3～5年後の追跡調査では，振戦，筋強剛，DIDへの効果は持続するものの，動作緩慢，歩行障害，姿勢反射障害，日内変動などへの効果は減弱していた[13]．なお，GPiはSTNと比べて大きく，GPi内部での刺激部位によって効果を認める症状が異なる可能性がある．Bejjaniらは，GPiの腹側部の刺激は筋強剛やDIDに，背側部の刺激は動作緩慢や歩行障害に効果を示すとしている[14]．

STN-DBSとGPi-DBSを直接比較した無作為化対照試験は少ない．STN-DBSとGPi-DBS各10例ずつを対象としたパイロット試験によると，12カ月後の休薬時(off)のUPDRSはそれぞれ48%と39%改善した[15]．動作緩慢はGPiよりもSTNで改善率が高かったが，DIDの軽減率はそれぞれ62%と89%でGPiの方が高かった．L-dopaの減量はそ