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.

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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

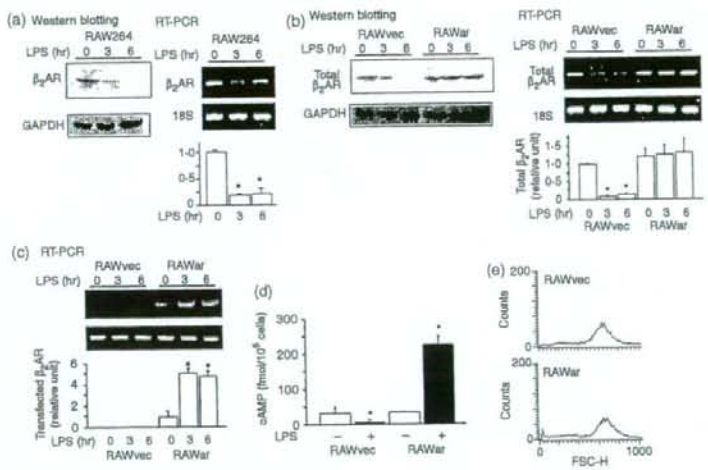


Figure 1. Lipopolysaccharide (LPS) stimulation down-regulates β_2-adrenergic receptor (β_2AR) expression. (a) RAW264 cells were stimulated with LPS. The protein levels of β_2AR and GAPDH (loading control) in the plasma membrane were analysed by Western blotting (left panel). The β_2AR messenger RNA (mRNA) 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).

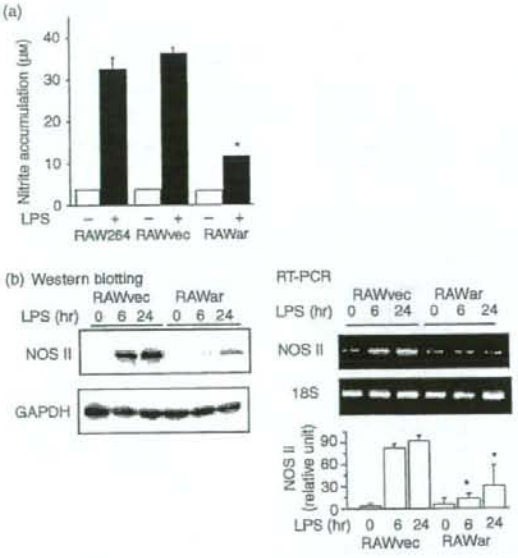


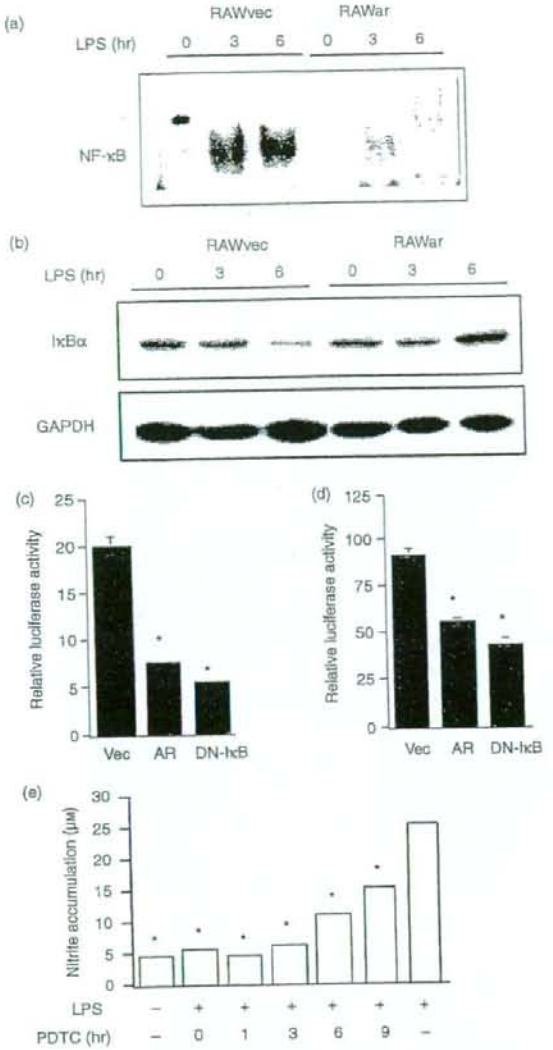
Figure 2. Forced β_2-adenergic receptor (β_2AR) expression suppresses nitric oxide (NO) production and nitric oxide synthase II (NOS II) expression. (a) Cells were stimulated with lipopolysaccharide (LPS) for 24 hr, and nitrite accumulation in the supernatants was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. *P < 0·001 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 < 0·01 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



established (Fig. 4, right panels). Since transfection with the vector did not influence NO production (Fig. 1c), cells transfected with β-arrestin 2 were compared with RAW264 cells. As shown in the RAWar cells (Fig. 2), NO production (Fig. 5a) and NOS II protein and mRNA expressions (Fig. 5b) were definitely decreased in the RAWarr2 cells.

Anti-β-arrestin 2 antibodies coimmunoprecipitated IκBα in RAW264 cells before, but not after, LPS stimulation for 6 hr (Fig. 6). On the other hand, the amount of IκBα coprecipitated by anti-β-arrestin 2 antibodies was not reduced but rather was increased in the RAWar and RAWarr2 cells after LPS stimulation, indicating that the LPS-stimulated down-regulation of β_2AR and β-arrestin 2 is essential for IκBα degradation.

Figure 3. Forced β_2-adrenergic receptor (β_2AR) expression suppresses nuclear factor-κB (NF-κB) activation. (a) The vector control cells and β_2AR transfectant were stimulated with lipopolysaccharide (LPS), and NF-κB activation was analysed by electrophoretic mobility shift assay. (b) The vector control cells and β_2AR transfectant were stimulated with LPS, and cytoplasmic inhibitor of NF-κB (IκBα) and GAPDH (loading control) were analysed by Western blotting. (c, d) RAW264 cells were cotransfected with the pNF-κB-Luc vector (c) or the NOS II promoter–luciferase construct (d) and vector (Vec), pcDNA4-β_2AR (AR) or pCMV-IκBαM (DN-IκB). The cells were cultured with LPS for 24 hr, and luciferase activities were determined. The results are expressed as means ± SEM from six-well cultures. *$P < 0.001$ versus cells cotransfected with Vec. (e) Pyrrolidine dithiocarbamate (PDTC) was added to the cultures at the indicated time-points after addition of LPS. Nitrite accumulation in the supernatants at 24 hr of culture was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. The error bars are too small to be distinguishable in the figure (numeric data from the left bar: 3·75 ± 0·18, 5·07 ± 0·22, 4·22 ± 0·07, 5·69 ± 0·12, 10·38 ± 0·06, 15·00 ± 0·05, and 25·20 ± 0·28). *$P < 0.001$ versus LPS-stimulated cells without PDTC. Data shown are representative of two or three separate experiments.

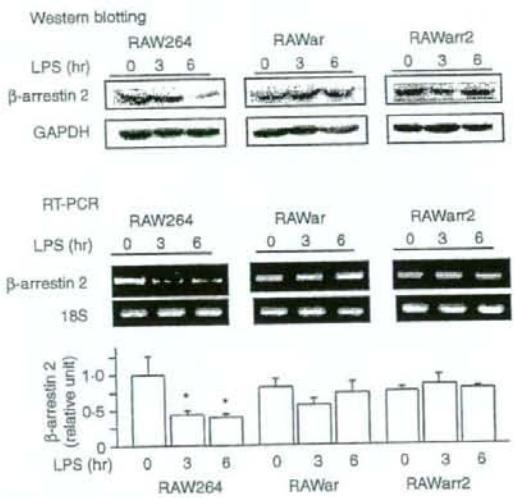


Figure 4. Lipopolysaccharide (LPS) stimulation down-regulates β-arrestin 2 expression. RAW264, RAWar, and RAWarr2 cells were stimulated with LPS, and the protein levels of β-arrestin 2 and GAPDH (upper panel) and messenger RNA expressions of β-arrestin 2 and 18S ribosomal RNA (middle panel) were analysed as in Fig. 1(a). Bar graphs show the relative intensity of the band from three separate experiments (mean ± SEM) (lower panel). *$P < 0.01$ versus 0 hr.

Discussion

In this study, we investigated the role played by β_2AR in the antimicrobial responses of macrophages. First, we demonstrated that β_2AR expression is decreased by LPS

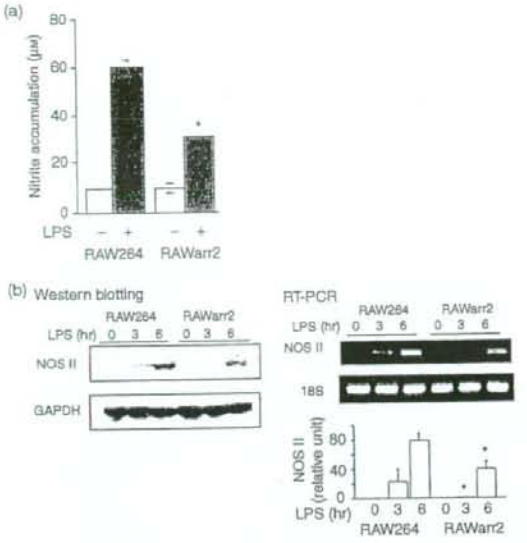


Figure 5. Forced β-arrestin 2 expression suppresses nitric oxide (NO) production and nitric oxide synthase II (NOS II) expression. (a) Cells were stimulated with lipopolysaccharide (LPS) for 24 hr, and nitrite accumulation in the supernatants was measured using the Griess reagent. The results are expressed as means ± SEM from three-well cultures. *$P < 0.001$ versus LPS-stimulated RAW264 cells. (b) The protein levels of NOS II and GAPDH (left panel) and messenger RNA expressions of NOS II and 18S ribosomal RNA (light upper panel) were analysed as in Fig. 1(a). Bar graphs show the relative intensity of the polymerase chain reaction bands from three separate experiments (mean ± SEM) (right lower panel). *$P < 0.01$ versus corresponding RAW264 cells. Data shown are representative of three to four separate experiments.

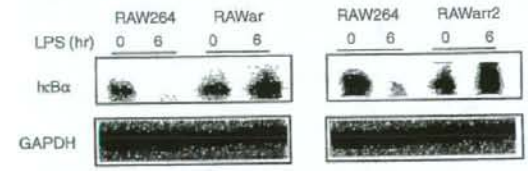


Figure 6. β-arrestin 2 interacts with cytosolic inhibitor of NF-κB (IκBα). Before and after stimulation with lipopolysaccharide (LPS) for 6 hr, cells were lysed and immunoprecipitated with anti-β-arrestin 2 antibodies. Western blotting analysis was performed using anti-IκBα antibodies (upper panel). The protein levels of GAPDH in equal amounts of lysates were used for control (lower panel).

stimulation. To investigate the role of β_2AR down-regulation in response to LPS directly, we established a macrophage cell line, RAWar. Prevention of the down-regulation of β_2AR expression in RAWar cells resulted in reduced NO production, suggesting that the LPS-associated down-regulation of β_2AR expression plays an important role in NO production in macrophages.

Decreases in NOS II mRNA expression were observed in the RAWar cells, indicating that NOS II expression was transcriptionally down-regulated by forced β_2AR expression. Prevention of the down-regulation of β_2AR expression in the RAWar cells resulted in a marked decrease in NF-κB activation and inhibited cytosolic IκBα degradation, indicating that the forced β_2AR expression inhibited LPS-induced NF-κB activation by IκBα stabilization.

On the other hand, β-arrestins, which are universally expressed members of the arrestin family, are the major regulators of GPCR signalling and bind to activated GPCRs, causing receptor desensitization and internalization.[14] Recently, β-arrestins have been shown to play functional roles in the regulation of a variety of signalling pathways and in the mediation of cross-talk between signalling pathways. Moreover, there is accumulating evidence that β-arrestin 2, which is expressed abundantly in the spleen, is functionally involved in some important immune responses.[23–26] We have demonstrated that β-arrestin 2 is down-regulated in LPS-stimulated RAW264 cells. Down-regulation of β-arrestin 2 was abolished in RAWar cells, suggesting that β-arrestin 2 expression is regulated by β_2AR. These findings suggest that β_2AR participates in signal transduction pathways from TLR4 by regulating the level of β-arrestin 2 expression. Meanwhile, the amount of IκBα coimmunoprecipitated by anti-β-arrestin 2 antibodies was decreased in the RAW264 cells after their stimulation with LPS but not in the RAWar or RAWarr2 cells, suggesting that β_2AR inhibited LPS-induced NF-κB activation by stabilizing IκBα through β-arrestin 2. The release of NF-κB following the degradation of IκBα proteins is an essential step in the generation of transcriptionally competent NF-κB. In addition, NF-κB activity following stimulation is dependent on the level of cytoplasmic NF-κB/IκBα complexes free from stabilizing factors. Therefore, the following appear likely: (1) LPS-stimulated signals suppress β_2AR expression, (2) the reduction of β_2AR results in the down-regulation of β-arrestin 2 expression, (3) β-arrestin 2 stabilizes cytoplasmic IκBα and inhibits NF-κB activation (so reduction in the level of β-arrestin 2 accelerates IκBα degradation and NF-κB activation in LPS-stimulated cells) and (4) nuclear translocation of NF-κB enhances NOS II expression.

The cross-talk between β_2AR and the TLR signalling pathways is schematically summarized in Fig. 7.

Catecholamines increase cAMP via β_2AR activation, and PKA activation inhibits NF-κB-induced transcription by phosphorylating cAMP responsive element binding protein (CREB), which competes with p65 for the limited amounts of CREB-binding protein (CBP) (Fig. 7a(ii)).[27] However, β_2AR agonists did not suppress NO production (unpublished observation). In the present study, we demonstrated that LPS stimulation suppressed the cAMP accumulation in RAWvec cells stimulated with a β_2AR

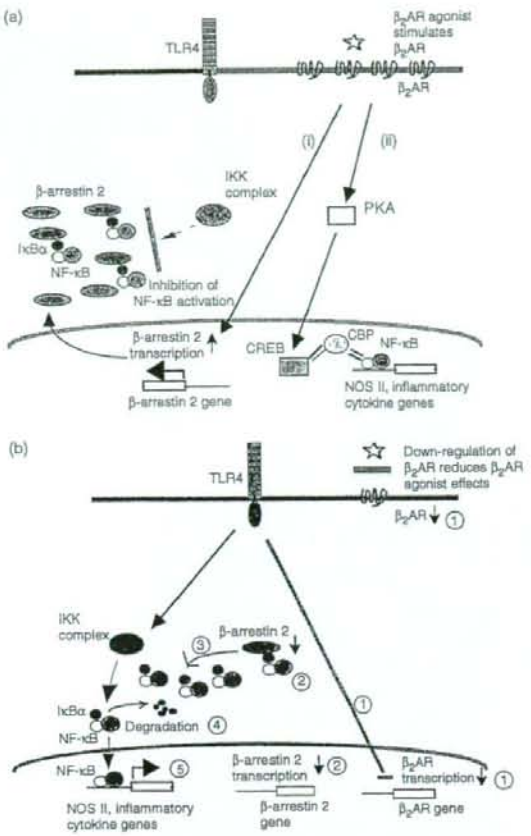


Figure 7. Cross-talk between β_2-adrenergic receptor (β_2AR) and Toll-like receptor (TLR) signalling pathways. (a) β_2AR agonists suppress nuclear factor-κB (NF-κB) activation by increasing cytoplasmic β-arrestin 2, which stabilizes the NF-κB/inhibitor of NF-κB (IκBα) complexes in cytoplasm (i) or by activating cAMP response element binding protein (CREB), which then produces competition between CREB-binding protein (CBP) and NF-κB in the nucleus (ii). (b) TLR4-dependent signals lead to the following steps both in the presence or absence of β_2AR agonists: ① TLR4-dependent down-regulation of β_2AR expression, ② down-regulation of β-arrestin 2, ③ release of NF-κB/IκBα complexes in the cytoplasm, ④ degradation of IκBα, and ⑤ translocation of NF-κB to the nucleus and transcription of its target genes.

agonist. In addition, we showed that prevention of the down-regulation of β_2AR inhibits the degradation of IκBα through β-arrestin 2, which stabilizes IκBα in the steady state (Fig. 7a(ii)). Therefore, the down-regulation of expression of both β_2AR and β-arrestin 2 by the TLR4-dependent pathway might provide a mechanism for 'escaping' anti-proinflammatory signals, such as the β_2AR–cAMP–PKA pathway[27] or the β_2AR–β-arrestin 2–IκBα pathway. As the levels of β_2AR ligands vary under different conditions, understanding the cross-talk between TLRs and β_2AR pathways may have both physiological and pathophysiological importance. Taken together, the observations of the present study regarding the regulation of TLR4 signalling through β_2AR appear to provide another therapeutic target for the regulation of inflammatory disease conditions.

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骨粗鬆症治療

別　刷

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特集：転倒と骨粗鬆症

転倒リスク評価とリスクを高める薬剤

鳥羽研二* 菊地令子* 岩田安希子*

転倒は，心身などの内的要因と居住環境や履物などによる外的要因に起因する複雑な老年症候群の1つである．多様な危険因子を個人評価する場合には，時間と人手がかかる．これを解決するため，簡便な「転倒スコア」および「転倒スコア短縮版」を開発した．後者は高齢者健診の1次スクリーニングに，前者はリスク別のケアプラン策定に有用である．数種類以上の薬剤処方は，転倒のリスクを高める．なかでも精神神経用薬は，おおむね50％以上転倒危険率を増す．転倒にかぎっていえば，短期間作動型の睡眠薬でも，非定形抗精神病薬，新しい抗うつ薬のいずれも転倒の危険を除外できていない．精神神経用薬の減量や中止は，転倒防止の第一級のエビデンスである．

Key words 転倒予測技術，簡便性と実用性，多剤服用，精神神経用薬，薬物中止介入

はじめに

転倒・骨折は高齢者における寝たきり要因の第3位に位置づけられ，骨粗鬆症性骨折のなかで最も重い骨折である大腿骨頸部骨折は，その90％以上が転倒によって生ずるとされている[1]．転倒の際に骨折を生じなくとも，数度の転倒を経験すると，意欲や日常生活動作能力（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倍のリスクであり[2]，転倒予防は寝たきり予防にきわめて重要である．従来の転倒リスク評価研究の問題点を克服し，そこから得られた「多剤服用が転倒リスク」である結果を踏まえ，最近の薬剤と転倒のレビューを紹介する．

転倒リスク，これまでの研究

従来，転倒危険因子は，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きたが，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，めまいや認知症などは成績が一致していない[2]．転倒は，内的要因である身体的側面と外的要因である環境要因による複合的症候群と捉えられるが，後者は地域や文化的，生活習慣的側面により大きく異なる可能性もある．

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

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表❶ 測定方法の難易度で分けた，転倒の危険因子

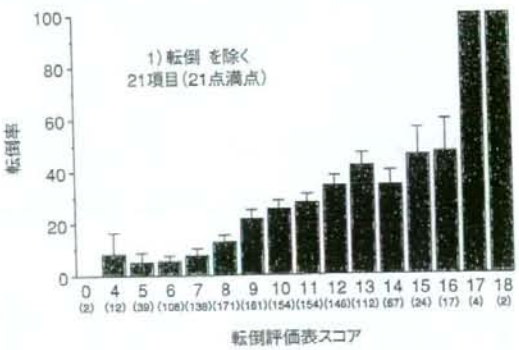
特殊機器，医師の問診などが必要な専門検査
・歩行運動系（関節症，ミオパチーなど）
　歩行速度遅延
　バランス低下
　下肢筋力低下
・心血管系障害（不整脈，起立性低血圧など）
・神経系障害（認知症，パーキンソンニズムなど）
・薬剤（鎮静薬，睡眠薬など）
問診表などで可能な簡易な方法
・老研式活動能力指標低下
　（手段的ADL，知的能動性，社会的役割の13項目で構成）
・過去の転倒歴
・環境要因　照明不良，障害物，段差，不適切な履物など

（鈴木隆雄，2002[1]より改変引用）

表❷ 質問項目と陽性頻度

1）転倒：解答数2,439名で708例（4.7±1.0回/年）	28.8%
2）つまずくことがある	56.5%
3）手摺につかまらず，階段ののぼり降りができない	50.6%
4）歩く速度が遅くなってきた	65.2%
5）横断歩道を青のうちに渡りきれない	17.0%
6）1キロメートルくらいつづけてあるけない	35.8%
7）片足で5秒くらい立てない	38.6%
8）杖をつかっている	28.3%
9）タオルを固く絞れない	16.8%
10）めまい，ふらつきがある	32.4%
11）背中が丸くなってきた	44.9%
12）膝が痛む	47.3%
13）目が見えにくい	53.1%
14）耳が聞こえにくい	42.5%
15）物忘れが気になる	63.7%
16）転ばないかと不安になる	45.8%
17）毎日薬を5種類以上飲んでいる	31.2%
18）家のなかで歩くとき暗く感ずる	11.4%
19）廊下，居間，玄関に障害物	20.8%
20）家のなかに段差がある	69.1%
21）階段を使わなくてはならない	27.7%
22）生活上家の近くの急な坂道を歩く	33.3%

（鳥羽研二ら，2005[3]より引用）



図❶ 転倒評価表スコアと転倒率（過去1年）
（鳥羽研二ら，2005[3]より引用）

た（表❶）[1]．本研究では，国内外の文献的レビューをもとに，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の研究班によって簡易な「転倒リスク評価表」（表❷）[3]を作成し，その妥当性，有効性を検証した．

方法

2003年度厚生労働科学研究費効果的医療技術の確立推進臨床研究事業，転倒骨折班の合同討議，国内外のレビュー[1]から，筋力低下，バランス欠如，歩行障害，視力障害，移動障害，認知機能障害，ADL障害，起立性低血圧，加齢，転倒の既往，慢性疾患，薬剤，段差が必須項目としてあげられた（表❶）[1]．これらの項目を具体的に質問表のみで被験者が内容を理解し，かつ因子のもつ意味が変容しないよう議論を重ね，問診表を完成させた（表❷）．くり返し再現性，季節変動などの基本的検討はすでになされ，良好な結果を得ている[3]．

調査対象

全国7地域（浦臼町，仙台市，塩尻市，多摩地区，香北町，相良村）の住民2,439名（男性932名，女性1,507名：76.3±7.4歳）に対し，問診表の意味を説明し調査の同意を得た後，自記式にて回答，自記不可能な場合は調査員が聞き取り調査をおこなった．

解析

1）過去の転倒歴を従属変数として，多変量解析をおこなった．

2）観察期間中の転倒歴を従属変数として，過去の転倒歴を含む，調査票の項目を独立とし重回帰分析をおこなった．

年齢，性は強制注入した．p値が0.05未満を統計学的に有意とした．なお有意な傾向として，$p<0.1$の項目も記載した．

結果

各項目の陽性頻度：過去1年の転倒歴は708名（男性229名，女性479名，平均年齢77.5±7.4歳），転倒率は28.8%，観察期間中は25%であり，骨折は1.8%にみられた．質問項目と陽性頻度を表❷に，転倒評価表スコアと転倒率を図❶に示す．スコアが大きくなるほど転倒率が高くなることが示された．

ほかの検査方法との比較

本スコアの実用性に関し，松林らは，この問診表を用い，北海道浦臼町で転倒調査を施行した．転倒の従属変数として，転倒危険を察知するカットオフポイントは，転倒スコア10点以上で，感度，特異度とも70％以上の結果であった．また，従来のUp&Goテストや，歩行速度，Functional Reachより，転倒予測の感度，特異度にすぐれている結果を得ている[4]．

より多数の症例で前向き研究による観察期間中の転倒を従属変数とする多変量解析によって，独立した有意な項目は，5項目に絞られた．オッズ比を加味した，簡易式「転倒チェック」シートを示す(表❸)[5]．簡易式「転倒チェック」シートの転倒予測の感度，特異度は70％以上である．

表❸ 簡易式の「転倒チェック」シート(該当項目に✓をつける)

□ 過去１年間に転んだことがある	5点
□ 背中が丸くなってきた	2点
□ 歩く速度が遅くなってきたと思う	2点
□ つえを使っている	2点
□ 毎日５種類以上の薬を飲んでいる	2点
合計	点

※６点以上は「要注意」

(Okochi J *et al*, 2006[6] より引用)

薬剤と転倒

精神神経用薬

地域住民における薬剤，とくに精神神経用薬の転倒リスクに関する研究は多数なされている．最近の母集団が大きい研究では，イタリアの2,854名の在宅ケアプログラムを受給している高齢者において，いずれかの精神神経用薬を服用している者の転倒危険率(オッズ比)は1.47(95％CI：1.24～1.74)でほぼ5割り増しであった．非定形精神病薬でもオッズ比は1.45倍と有意であった[6]．

従来，短期間作動型のベンゾジアゼピン系睡眠薬は，長期間作動型にくらべ安全と考えられてきた．しかし転倒にかぎっては，この研究では，長期間作動型1.45倍に対し，短期間作動型1.32倍で有意差はなかった．アメリカボルチモアの女性8,127名参加の多施設コホート研究[7]でも，長期間作動型が1.56倍，短期間作動型は1.42倍で有意差を認めなかった．高齢者の肝臓での代謝に起因するか，腎排泄遅延が影響するかなど機序の不明な点は多いが，従来考えられてきたように「短期間作動型睡眠導入薬なら安全」の神話は壊れたといってよい．

抗うつ薬に関しては，2つの研究では異った結果を得ている．イタリアにおいてはオッズ比0.92だったのに対し，ボルチモアでは1.54倍と有意であった．さらにこの研究では，SSRIは3.45倍の転倒率を示し，高齢者に副作用が多いと批判されている3環系抗うつ薬の1.28倍より有意に高頻度で転倒をおこした．従来，抗うつ薬は口渇，排尿障害などが副作用として重視されてきたが，今後は転倒を含めた冷静な比較が求められよう．

抗痙攣薬に関しては，従来より高齢者の生命予後にエビデンスがないことが知られてきた．今回ボルチモアの研究では，抗痙攣薬の転倒危険率は2.56倍と高く，より適応を慎重に選ぶ必要がある．この研究で麻薬は転倒危険率に影響がなかったことも重要である(オッズ比＝0.99)．

循環器用薬と鎮痛薬

Tinetti ら[8] は1999年に，60歳以上の症例に関して循環器用薬や鎮痛薬と転倒に関連があった29の研究のメタアナリシスを発表している．精神神経用薬にくらべ，危険度が増す率は低いが，高齢者には好んで処方されるため参考までに表❹に記した．

この研究では，75歳以上と未満で，結果に差がなかった．また，転倒率の多い集団と少ない集団でも薬剤の作用に差がなく，ジゴキシン，Ia型抗不整脈薬，利尿薬は転倒の危険を増すことに注意を喚起した．いずれの疾患集団でも3または4薬剤以上の処方で転倒が増すことが示されており，単一薬剤より，重層的な危険を増す「多剤服用は転倒の危険がある」ということがメタアナリシスでも示されている．われわれの結果と一致するものであり，高齢者の処方にあたっては，転倒リスクを考慮し，患者・家族に説明したうえで処方しなくてはならない．

表❹ 各種循環器用薬，鎮痛薬の転倒危険率

薬剤	オッズ比	95%CI	有意*
利尿薬	1.08	1.02～1.16	*
サイアザイド	1.06	0.97～1.16	
ループ	0.90	0.73～1.12	
βブロッカー	0.93	0.77～1.14	
中枢性交感神経抑制薬	1.16	0.87～1.55	
ACE阻害薬	1.20	0.92～1.58	
Ca 拮抗薬	0.94	0.77～1.14	
亜硝酸薬	1.13	0.78～1.36	
抗不整脈薬(Ia)	1.59	1.02～2.48	*
ジゴキシン	1.22	1.05～1.42	*
鎮痛薬			
麻薬	0.97	0.78～1.20	
非麻薬	1.09	0.88～1.34	
NSAIDs	1.16	0.97～1.38	
アスピリン	1.12	0.80～1.57	

(Tinetti ME *et al*, 1999[8]より引用)

表❺ 向精神薬の使用と転倒リスク
(N=1,845； n=204 Fallers)

Medication	Multivariable-Adjusted Analysis (ハザード比95%CI)	P-value
オランザピン(yes/no)	1.74(1.04～2.90)	p=0.04
リスペリドン(yes/no)	1.32(0.57～3.06)	p=0.52
定型抗精神病薬(yes/no)	1.35(0.87～2.09)	p=0.19
抗うつ薬(yes/no)	1.45(1.09～1.93)	p=0.01
抗不安薬(yes/no)	1.19(0.94～1.50)	p=0.15

(Hien le TT *et al*, 2005[9]より引用)

認知症薬と転倒

認知症の周辺症状に対する「非定形抗精神病薬」に関しては，死亡率が1.7倍になることから，FDA(米国食品医薬品局)では長期処方を原則禁止している．Hienら[9]は，これらに先立ち，非定形抗精神病薬と転倒リスクを大規模研究でまとめている(表❺)．

薬剤中止の介入と転倒予防

精神神経用薬の中止は転倒を70%減少させることが知られている[10]．米国の「転倒ガイドライン」でも，4種類以上の投薬を受けている患者の投薬数を減らすことは，地域においても，長期介護施設においても第一級のエビデンスと位置づけられている[11]．

おわりに

転倒予防事業で，今後の転倒危険者を抽出する検査を考える場合，従来のように，環境因子の問診表と下肢筋力検査(歩行速度，かた足立ち時間)などに時間を費やすより，過去の転倒回数を十分聴取し，身体的側面(骨粗鬆症，認知症，膝関節症)の情報を得るため，「転倒スコア」を活用することが簡易で，有用であると示唆された．

転倒予防には，まず多剤処方の見直しが最も安価な治療法であり，とくに，睡眠薬の安易な処方は慎むべきであり，代替手段を考えてからという処方態度を心がけたい．

本研究は，厚生労働科学研究長寿科学総合研究事業の助成によっておこなわれた．

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医学と医療の最前線

介護予防のエビデンス

鳥羽　研二

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介護予防のエビデンス

鳥羽　研二

要　旨

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がスタートである．介護予防は，慢性疾患ケアから，障害には段階的構造があることの理解を経て，社会サービス受給への概念に変化した．虚弱（Frailty）の進展因子として，疾患，液性因子（ホルモンや生化学物質），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．簡便な虚弱者のスクリーニング方法が開発されている．我が国の介護保険は概ね順調に推移したが，介護予防が失敗したのは，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，内外のエビデンスに基づき，科学的アプローチを行うことが必要である．

〔日内会誌　97：2566～2574，2008〕

Key words：介護予防，障害の構造，虚弱，虚弱予防のグランドデザイン

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．介護保険の開始前に，介護予防に関して異なった二つの見通しがあった．岡本は，「要支援に対する予防給付は画期的で，介護予防がなされる」という明るい見通しを述べているが，同時に「寝たきり進行のプロセスは殆ど研究されていない」とも述べ，地道なプロスペクティブな研究の必要性を指摘した[1]．一方松林は，地域で予防介入を長く実践してきた立場から，介護に偏し，予防の比重が低くなる介護保険に危機感を表明している[2]．既に筆者も予測していたところであるが[3]，残念ながら，危惧が現実のものとなった．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1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，2007年4月の「介護予防」の概念の導入と，「介護予防事業」の介護保険からの一部切り離しに関係していることは言うまでもない．

昨年の改正の要点は，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のぞき，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点が最も危惧される点である．

とば　けんじ：杏林大学高齢医学

表 1. 総合的日常生活活動度測定法：ADL20

生活支援	身体支援	
独居高齢者の生活自立要因	最低限のセルフケア（sADL），移動の介護（mADL）	
＝手段的 ADL	sADL	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	歩行屋内
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　日本老年医学会雑誌 29（11）：841-848，1992）

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，介護保険制度によって一般的となった用語についても改めて歴史的な概念の変遷を整理し，「どの様な状態をどうやって予防するのか」という基本的な疑問に答えるよう配慮した．

1．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わけられる．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ADL」（表 2）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ADL」（表 1）の介助と，とこずれ処置，オムツ交換，摂食介助などといった，褥瘡，尿失禁，嚥下障害などの「老年症候群のケア」が含まれる．

従って，介護予防という概念は，手段的ADL依存の予防，基本的ADL低下予防，及び老年症候群の発症・悪化予防という極めて幅がひろ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分かりにくくし，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基本的ADL低下予防を主体に念頭におきがちである．

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，前者は手段的ADLを手助けをうけながら共同で行うことによって機能を維持し，後者は基本的ADLの改善，維持を主な目的としているが，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構造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Majory Wallen

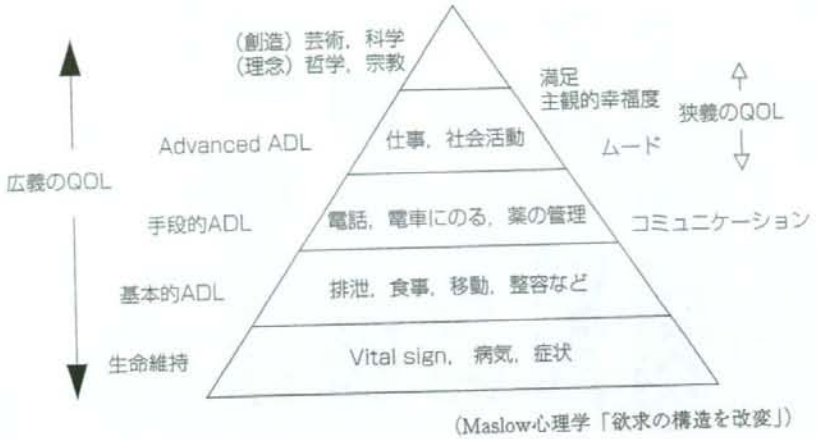

図 1．QOL の構造

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[4]．その後，虚弱や要介護者という概念は，長期入院や入所者と同義語と考えられたり[5]，疾患―障害―能力低下―不利というリハビリテーションの基本的概念の中で，能力の低下した対象が虚弱や要介護者という捉え方が広まり[6]，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[7]．

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（Quality of life）構造が理解されるようになった（図 1）．

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[8]．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．

2．予防：悪化因子（リスクファクター）の解析

1）遺伝子要因

下等動物レベルを別にして，生活機能低下や寝たきりの危険遺伝子に対する研究は非常に少ない．

一部の研究では，アポリポタンパク質E4 遺伝子型が虚弱の危険因子と考えられることが確認され，E4 対立遺伝子を持つ高齢女性に，より大きな機能低下が確認された[9]が否定的成績もある[10]．動脈硬化の危険因子として確認された多くの遺伝子多型に関しても，機能低下に関する研究は今後の課題であろう．

2）ホルモン，液性因子

高齢患者の虚弱や障害，有害な結果の血清マーカーとして，テストステロン値の低下[11]，DHEA 値の低下[12]，朝のコルチゾール・DHEA硫酸塩比の上昇[13]，高感度CRP，IL-6 上昇[14]，総コレステロールの減少[15]，血清アルブミン値の低下[16]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持ち[17]，テストステロン補充によっ