Amyloid-β peptides induce cell proliferation and macrophage colony-stimulating factor expression via the PI3-kinase/Akt pathway in cultured Ra2 microglial cells

Sachiko Ito[a], Makoto Sawada[b], Masataka Haneda[a,1], Satoshi Fujii[c], Kentaro Oh-hashi[c], Kazutoshi Kiuchi[c], Masahide Takahashi[d], Ken-ichi Isobe[a,*,1]

[a] *Department of Basic Gerontology, National Institute for Longevity Sciences, 36-3 Gengo, Morioka-cho, Obu, Aichi 474-8522, Japan*
[b] *Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan*
[c] *Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido, Gifu 501-1193, Japan*
[d] *Department of Pathology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan*

Received 28 December 2004; revised 8 February 2005; accepted 21 February 2005

Available online 5 March 2005

Edited by Jesus Avila

Abstract Alzheimer's disease is characterized by numerous amyloid-β peptide (Aβ) plaques surrounded by microglia. Here we report that Aβ induces the proliferation of the mouse microglial cell line Ra2 by increasing the expression of macrophage colony-stimulating factor (M-CSF). We examined signal cascades for Aβ-induced M-CSF mRNA expression. The induction of M-CSF was blocked by a phosphatidylinositol 3 kinase (PI3-kinase) inhibitor (LY294002), a Src family tyrosine kinase inhibitor (PP1) and an Akt inhibitor. Electrophoretic mobility shift assays showed that Aβ enhanced NF-κB binding activity to the NF-κB site of the mouse M-CSF promoter, which was blocked by LY294002. These results indicate that Aβ induces M-CSF mRNA expression via the PI3-kinase/Akt/NF-κB pathway.

Keywords: Microglia; Alzheimer's disease; Amyloid-β; Akt; NF-κB; Macrophage colony-stimulating factor

1. Introduction

Alzheimer's disease (AD) is characterized by the presence of senile plaques in the brain composed primarily of amyloid-β peptide (Aβ). Microglia have been reported to surround the Aβ plaques, which provokes a microglia-mediated inflammatory response that contributes to neuronal cell loss [1]. On the other hand, microglia play an important role in the clearance of Aβ by phagocytosis, primarily through scavenger receptor class A (SR-A, CD204), scavenger receptor-BI (SR-BI) and CD36 [2–4]. Recently, it has been reported that microglia isolated from CD36-deficient mice had marked reductions in Aβ-induced cytokine/chemokine secretion [5]. CD36 binds to Aβ in vitro [6], and is physically associated with members of the Src family tyrosine kinase [7,8], which transduce signals from this receptor [9]. Another receptors such as receptor for advanced glycosylation end-products (RAGE), integrins and heparan sulfate proteoglycans, also have been reported to bind with Aβ [10].

There are many reports that microglia are activated by Aβ, but it has been unclear whether Aβ is associated with the proliferation of microglia. Here we report that Aβ induces proliferation of the microglial cell line Ra2 by increasing macrophage colony-stimulating factor (M-CSF) expression. We also elucidated signal transduction pathways from Aβ-treatment to M-CSF mRNA expression in microglia.

2. Materials and methods

2.1. Materials

Synthetic human Aβ25–35, Aβ1–42 and Aβ1–16 were obtained from Peptide Institute Inc. Aβ35–25 was from AnaSpec Inc. Aβ25–35, Aβ1–16 and Aβ35–25 were dissolved in H_2O and Aβ 1–42 was dissolved in 0.1% NH_3 according to the manufacturer's instructions. Anti-phospho-Akt (Serine 473), anti-Akt, anti-phospho-IκBα (Serine 32), and anti-IκBα antibodies were from Cell Signaling. PP1 was from Biomol. Wortmannin, LY294002 and Akt inhibitor [1L-6-hydroxymethyl-chiro-inositol 2-(*R*)-2-*O*-methyl-3-*O*-octadecylcarbonate] were from Calbiochem. Piceatannol was from Sigma–Aldrich. Mouse recombinant granulocyte–macrophage colony-stimulating factor (mrGM-CSF) was from Pharmingen. Mouse recombinant M-CSF (mrM-CSF) was from Techne. Aβ25-35 and Aβ1–42 were used at 50 and 10 μM, respectively, in all studies unless otherwise stated.

2.2. Cell culture

Microglial cell line Ra2 was cultured in MGI medium [Eagle's MEM supplemented with 0.2% glucose, 5 μg/ml bovine Insulin (Sigma–Aldrich), and 10% fetal bovine serum (FBS, Invitrogen)] and 0.8 ng/ml mrGM-CSF (Pharmingen) [11]. Before Aβ-treatment, Ra2 cells were cultured in MGI medium without mrGM-CSF for 16 h. Primary microglia and primary astrocytes were prepared using newborn C57BL/6 mice as described previously [12], and cultured in MGI medium containing 0.8 ng/ml mrGM-CSF. The neuroblastoma cell line Neuro2a was cultured in DMEM supplemented with 10% FBS. Primary neurons were obtained from the cortex of 14-day-old C57BL/6 mouse embryos as described previously [13] with some modifications. Neural cells cultured in DMEM supplemented with TIS (5 μg/ml

*Corresponding author. Fax: +81 52 744 2972.
E-mail address: kisobe@med.nagoya-u.ac.jp (K. Isobe).

[1] Present address. Department of Immunology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-β; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; GM-CSF, granulocyte–macrophage colony stimulating factor; M-CSF, macrophage colony-stimulating factor; PBS, phosphate-buffered saline; PI3-kinase, phosphatidylinositol 3 kinase; RAGE, receptor for advanced glycation end-products

0014-5793/$30.00
doi:10.1016/j.febslet.2005.02.048

transferrin, 5 μg/ml insulin, and 5 ng/ml selenite, Sigma), 10% FBS and 5 μM cytosine arabinoside (Ara-C, Sigma). Before Aβ-treatment, primary neurons were cultured in MGI medium for 16 h.

2.3. Cell proliferation (WST-1) assay

Cell proliferation was determined by analyzing the conversion of WST-1 (light red) to its formazan derivate (dark red) using a WST-1 Cell Counting Kit (Dotite). For neutralization of M-CSF, anti-mouse M-CSF antibody (Techne) was added to the culture medium. At the end of the experiments, the media were replaced, and cells were incubated with 10 μl of the WST-1 reagent for 1 h at 37 °C in 5% CO_2. The absorbance at 450 nm was measured by using a microplate reader (Bio-Rad).

2.4. Immunoblotting

Cells were lysed in sample buffer (62.5 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 5% bromophenol blue). Then 50 μg of total protein was resolved by SDS–PAGE and transferred to PVDF membranes (Millipore). Immunoblotting was performed with the appropriate antibody using the enhanced chemiluminescence (ECL) system (Amersham Pharmacia).

2.5. RT-PCR and real-time quantitative RT-PCR

Total RNA was isolated using an RNeasy mini kit (Qiagen) according to the manufacturer's instructions. Two micrograms of total RNA was reverse transcribed to cDNA using SuperScript II Reverse Transcriptase (Invitrogen). For RT-PCR and real-time quantitative PCR, the primers for mouse M-CSF and β-actin genes were as follows (5′ to 3′): M-CSF sense, CCATCGAGACCCTCAGACAT; M-CSF antisense1 for RT-PCR, CCTAAGGGAAAGGGTCCTGA; M-CSF antisense2 for real-time PCR, GATGAGGACAGACAGGTGGA; β-actin sense, AGTGTGACGTTGACATCCGT; and β-actin antisense, GCAGCTCAGTAACAGTCCGC. Conventional RT-PCR was performed using 0.5 μl cDNA, and 30 cycles of amplification for M-CSF or 23 cycles for β-actin at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. Quantitative real-time PCR was performed on the Smart Cycler system (Takara) using the following program: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 1 min at 60 °C, and 8 s at 72 °C. The reactions were carried out using 0.5 μl cDNA with Smart Kit for Sybr Green I (Eurogentec). To check the specificity of reactions, a single band of the correct size was visualized by running out on 2% agarose gels. Values were expressed as relative expression of M-CSF mRNA normalized to the β-actin mRNA.

2.6. Nuclear extracts and electrophoretic mobility shift assays (EMSAs)

Nuclear extracts of Ra2 cells were prepared as previously described [14]. Three micrograms of nuclear extract was incubated with 5 fmol of ^{32}P end-labeled double-stranded oligonucleotides derived from M-CSF promoter in binding buffer [10 mM Tris, pH 7.5, 4% glycerol, 1 mM $MgCl_2$, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, and 0.05 μg/μl poly(dI–dC)] for 20 min at room temperature. For competition assays, 1 pmol of unlabeled probe was incubated in the reaction mix before the addition of the ^{32}P-labeled probe. The oligonucleotides used in these experiments were as follows: NF-κB probe, 5′-GCCTTGAGGGAAAGTCCCTAGGGGC-3′; AP1 probe, 5′-GTAGTATGTGTCAGTGCC-3′. For supershift assays, nuclear extracts were preincubated with anti-NF-κB p50 or p65 antibodies (Santa Cruz) for 1 h at 4 °C. The DNA–protein complex was separated on 5% native polyacrylamide gels. The dried gels were visualized using an Image Reader (Fujifilm).

2.7. Statistical analysis

Results are expressed as means ± S.D. Statistical analysis was done by a two-tailed Student's *t* test. A *P* value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. Aβ promotes microglial cell proliferation

To investigate the possible role of Aβ in the activation of microglia, we examined if Aβ could sustain the cell proliferation of microglial cell line Ra2. Ra2 cells proliferate in MGI medium containing GM-CSF and stop proliferating without GM-CSF [11]. Under MGI medium without GM-CSF, the effects of M-CSF or Aβ on the proliferation of Ra2 cells were analyzed by the WST-1 assay. The addition of M-CSF induced cell proliferation dose-dependently (Fig. 1A). Aβ25–35 increased Ra2 cell proliferation dose-dependently (Fig. 1B). Aβ25–35 does not occur naturally but has shown to mimic the effects of Aβ1–42 [15–17]. Aβ1–42, which occurs in a brain affected by AD, was more effective in cell proliferation than Aβ25–35 (Fig. 1C). It has been reported that Aβ stimulates the proliferation of microglia to enclose Aβ plaque [18,19]. We examined if M-CSF provoked the cell proliferation with Aβ-treatment. The effect of Aβ on the proliferation was blocked by anti-M-CSF antibody ($P < 0.05$) (Fig. 1D). The treatment with M-CSF was performed as a control. The effect of M-CSF was blocked by anti-M-CSF antibody (Fig. 1D). We found that Aβ induces microglial cell proliferation by M-CSF production.

3.2. Aβ induces M-CSF mRNA expression in microglia

To examine whether Aβ could induce M-CSF mRNA expression in microglia, Ra2 cells were stimulated with Aβ25–35 for 16 h at various concentrations. Aβ25–35 induced M-CSF mRNA expression dose-dependently (Fig. 2A). As a result of real-time quantitative RT-PCR (Fig. 2A, right), M-CSF mRNA induction by 50 μM Aβ25–35 was about sevenfold of non-treated control. Aβ25–35 induced time-dependent increases in M-CSF mRNA expression (Fig. 2B). Aβ1–42 also induced M-CSF expression (Fig. 2A and B). GM-CSF mRNA, on the other hand, was not induced by Aβ25–35 or Aβ1–42 (data not shown). Aβ1–16 did not induce M-CSF mRNA expression (Fig. 2C), nor

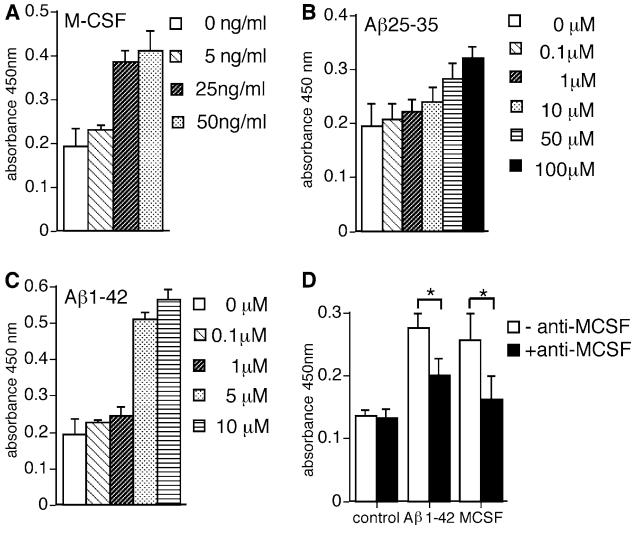

Fig. 1. Aβ promotes Ra2 cell proliferation. Cellular proliferation was measured by WST-1 assay. (A, B and C) Ra2 cells were incubated with the medium containing M-CSF, Aβ25–35 or Aβ1–42 at indicated concentrations for 48 h. (D) Ra2 cells were preincubated with 1 μg/ml anti-M-CSF antibody for 1 h before treatment with 5 μM Aβ1–42 or 25 ng/ml M-CSF for 24 h. Mean ± S.D. values from a single experiment were performed in triplicate. Similar results were obtained in each of two separate experiments ($^{*}P < 0.05$).

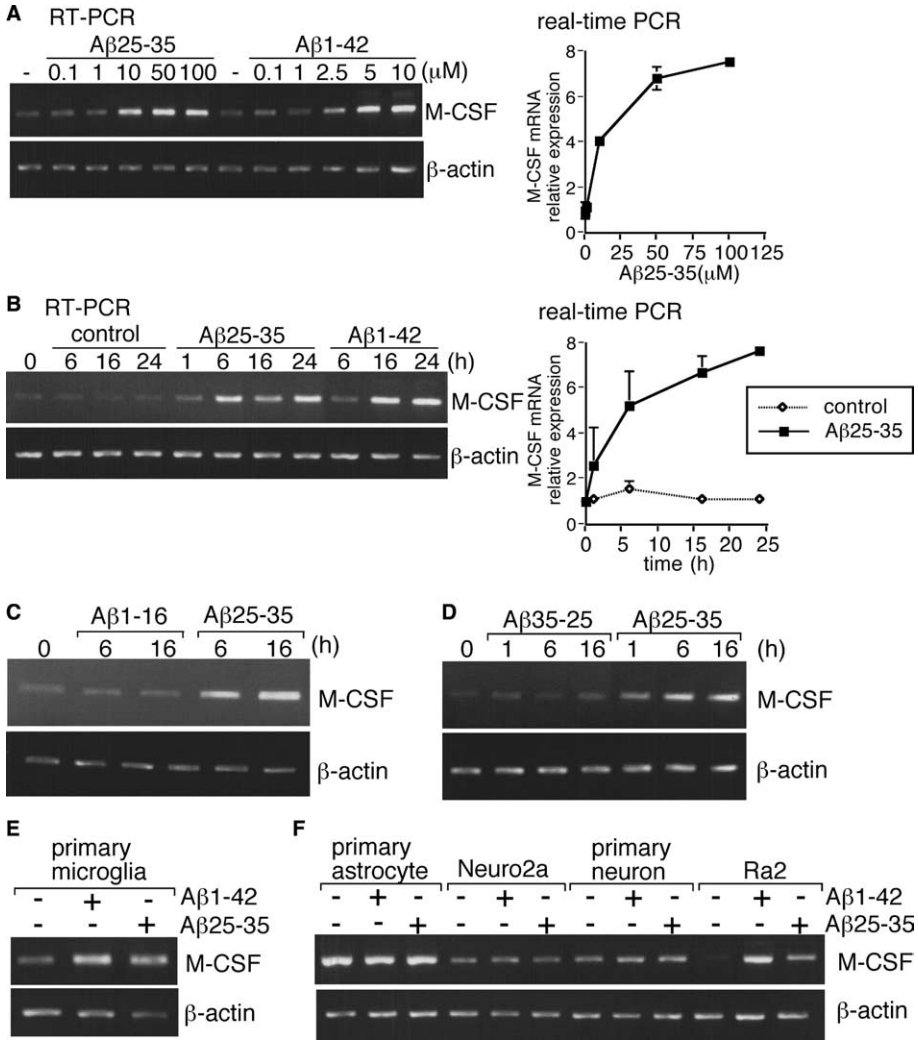

Fig. 2. Aβ stimulates M-CSF mRNA expression in microglia. (A and B) M-CSF and β-actin mRNA were determined by RT-PCR (left) and quantified by real-time PCR (right). Data represent means ± S.D. of three separate determinations. Ra2 cells were treated with Aβ25–35 or Aβ1–42 at indicated concentrations for 16 h (A). Time course of M-CSF relative expression of Ra2 cells treated with 50 μM Aβ25–35 and 10 μM Aβ1–42 (B). (C and D) Ra2 cells were treated with 50 μM Aβ1–16 (C) or 50 μM Aβ35–25 (D) for indicated times. (E and F) Primary microglia (E), primary neurons, primary astrocytes and neuroblastoma Neuro2a (F) were treated with 50 μM Aβ25–35 or 10 μM Aβ1–42 for 16 h.

did Aβ 35–25, which was a reverse sequence of Aβ25–35 (Fig. 2D). In primary microglia, as well as in Ra2 cells, Aβ25–35 and Aβ1–42 increased M-CSF mRNA expression (Fig. 2E). We also examined whether Aβ induced increases in M-CSF mRNA expression in primary astrocytes, primary neurons, and neuroblastoma cells Neuro2a. These cells constitutively expressed M-CSF mRNA, but Aβ25–35 and Aβ1–42 did not induce further expression of M-CSF mRNA (Fig. 2F). These results demonstrate that Aβ induced M-CSF mRNA expression in only microglia.

3.3. Aβ induces M-CSF mRNA via Src family tyrosine kinase and PI3-kinase signal cascade

Because our studies showed that Aβ25–35 had induced M-CSF expression in Ra2 cells, we examined signal cascades for Aβ-induced M-CSF mRNA expression by using several chemical inhibitors. The Src family tyrosine kinase is associated with CD36, which transduces signal cascades by Aβ [7,8,20]. In addition, Syk tyrosine kinase is activated by Aβ [21]. First, we examined if M-CSF mRNA expression was induced by Aβ via tyrosine kinase, Src family and Syk. A specific inhibitor of Src family kinase, PP1, prevented the increase in M-CSF mRNA induced by Aβ (Fig. 3A). A Syk-selective inhibitor, piceatannol, also blocked the increase in M-CSF mRNA expression (Fig. 3B). Next, to investigate whether the PI3-kinase pathway regulates Aβ-induced M-CSF expression, Ra2 cells were pretreated with the PI3-kinase inhibitors, wortmannin or LY294002. Wortmannin and LY294002 inhibited the increase in M-CSF mRNA expression dose-dependently (Fig. 3C and D). Fig. 3E shows the result of quantitative amounts of mRNA by real-time PCR. It has been reported that Aβ stimulates tyrosine kinase, PI3-kinase and Akt activation in neural and macrophage cells [21–24]. However, analysis of these signal transductions in microglia has not been reported. This is the first report that Aβ induces M-CSF expression through

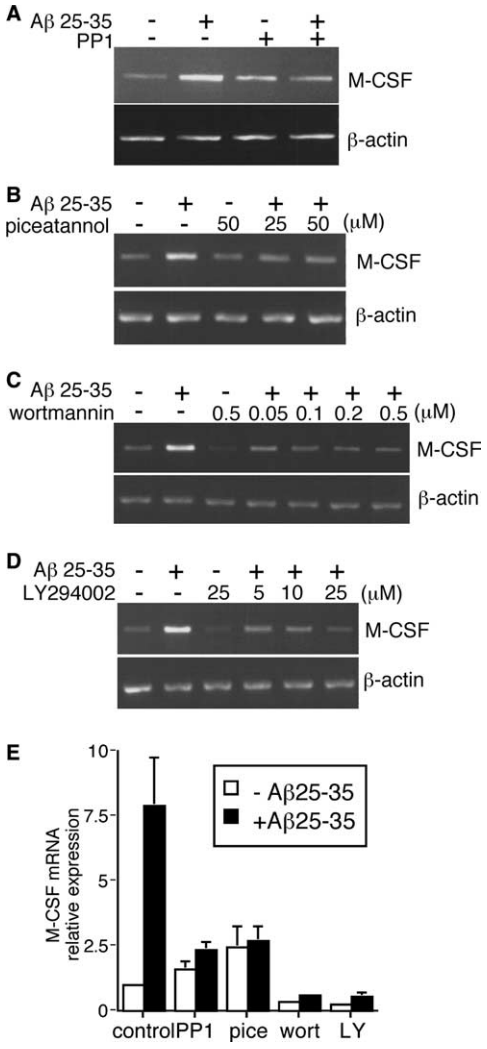

Fig. 3. Signal transduction for M-CSF mRNA expression induced by Aβ. (A–D) M-CSF and β-actin mRNA expression were determined by RT-PCR. Ra2 cells were preincubated with 10 μM PP1 (A), wortmannin (C), LY294002 (D) for 30 min or piceatannol (B) for 1 h before treatment of 50 μM Aβ25–35 for 6 h. Beacuse all inhibitors were dissolved in DMSO, control cells were treated with DMSO. (E) M-CSF mRNA expressions were measured by real-time PCR (pice, piceatannol; wort, wortmannin; LY, LY294002). Data represent means ± S.D. values of three separate determinations.

the Src family and Syk tyrosine kinases and the PI3-kinase in microglia.

3.4. Aβ activates Akt signaling pathway in microglia

We examined whether Akt was involved in the Aβ-induced M-CSF expression in Ra2 cells. Akt inhibitor blocked the increase of M-CSF mRNA expression (Fig. 4A). Immunoblotting analysis revealed that Akt was transiently phoshorylated at serine 473 by Aβ (Fig. 4B). LY294002 and PP1 suppressed the phosphorylation of Akt induced by Aβ (Fig. 4D). Piceatannol also blocked the phosphorylation of Akt (Fig. 4E). Because tyrosine kinases and PI3-kinase activate MEK/Erk/Elk [25,26], we examined whether these signal pathways were related to M-CSF mRNA expression induced by Aβ. Aβ induced MEK and Erk1/2 phosphorylation in Ra2 cells. However, specific inhibitors of MEK, U0126 and PD98059 did not inhibit Aβ-induced M-CSF mRNA expression (data not shown). These results indicate that the tyrosine kinases, Src family and Syk, and the PI3-kinase activate Akt for Aβ-induced M-CSF expression.

3.5. Aβ activates NF-κB via PI3-kinase signal cascade

Because NF-κB is a target of Akt [27], next we examined if IκBα phosphorylation was induced by Aβ. The phosphorylation of IκBα on serine 32 results in the release and nuclear translocation of active NF-κB [28]. IκBα was phosphorylated time-dependently, the phosphorylation peaked at 60 min and then declined (Fig. 4C). The phosphorylation was inhibited by LY294002 and PP1 (Fig. 4D). Piceatannol also blocked the phosphorylation of IκBα (Fig. 4E).

The M-CSF promoter region has a putative NF-κB binding site at −369–378 bp from the transcriptional start site [29]. To investigate whether this NF-κB binding site is associated with Aβ-induced M-CSF expression, EMSA was carried out with nuclear extracts prepared from untreated and Aβ-treated Ra2 cells. The amount of protein binding to the NF-κB probe was increased by Aβ-treatment (Fig. 5A, compare lanes 2 and 3). NF-κB binding activity was almost completely eliminated by adding an excess of the unlabeled NF-κB probe but not by the unlabeled AP1 probe (Fig. 5A, lanes 6 and 7). Anti-p50 antibody supershifted the complexes (Fig. 5A, lane 4) and anti-p65 antibody partially disrupted the DNA binding of the complexes (Fig. 5A, lane 5). To examine whether the Aβ-induced increase in nuclear NF-κB binding activity correlated with tyrosine kinase and PI3-kinase, Ra2 cells were preincubated with chemical inhibitors before treatment with Aβ. LY294002 reduced Aβ-induced binding to the NF-κB probe and piceatannol blocked the DNA-binding complex (Fig. 5B, lanes 4–7). These results indicate that Aβ enhances the binding of NF-κB to M-CSF promoter via the Syk tyrosine kinase and the PI3-kinase.

We have shown in the present study that Aβ proliferates microglia and induces M-CSF via the PI3-kinase/Akt/NFκB signal pathways. It has been reported that Aβ binds to CD36, which transduces signals via tyrosine kinase [6,20]. CD36 may participate in the initiation of intracellular signaling to M-CSF expression. RAGE also has been reported to induce NF-κB activation to M-CSF production [30]. Further works are needed to prove the receptors of Aβ, which induces PI3-kinase/Akt/NF-κB signal pathways to M-CSF mRNA expression. Aβ increases production of reactive oxygen species (ROS) and activates Akt in neural cells [23]. And in microglia CD36 mediates production of ROS in response to Aβ [31]. We found that antioxidants such as reduced glutathione and α-tocopherol slightly blocked M-CSF mRNA expression (data not shown). Also in microglia, ROS may partly participate in activating the signal cascade to M-CSF expression. It is important to reveal the relation among the receptors of Aβ, production of ROS and signal cascades.

Monsonego et al. [32] showed that activated microglia migrated outside the brain and could present Aβ peptide to T lymphocytes. Further analysis of microglial activation may reveal the immunological mechanism of AD, and may enhance the prospects of immune manipulation to prevent AD.

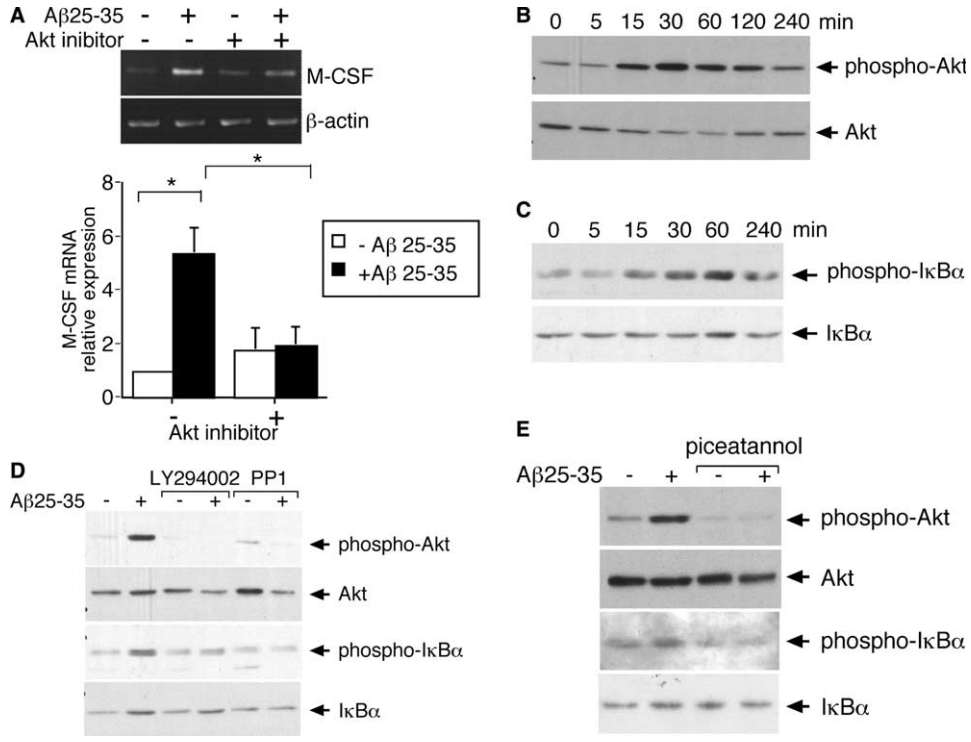

Fig. 4. Aβ-induced Akt and IκB phosphorylation through tyrosine kinase and PI3-kinase. (A) RT-PCR (top) and real-time PCR (bottom) of M-CSF mRNA. Ra2 cells were preincubated with or without 20 μM Akt inhibitor before treatment with 25 μM Aβ25–35 for 6 h. Data represent means ± S.D. values of three separate determinations. ($^{*}P < 0.01$) (B–E) Immunoblotting analysis using anti-phospho Akt (Ser 473) or anti-phospho IκBα (Ser 32) antibody. The same blots were reprobed with anti-Akt or anti-IκB antibody. Ra2 cells were treated with Aβ25–35 for indicated times (B and C). Ra2 cells were preincubated with 25 μM LY294002 or 10 μM PP1 for 30 min or 50 μM piceatannol for 1 h before treatment with 50 μM Aβ25–35 for 30 min (D and E).

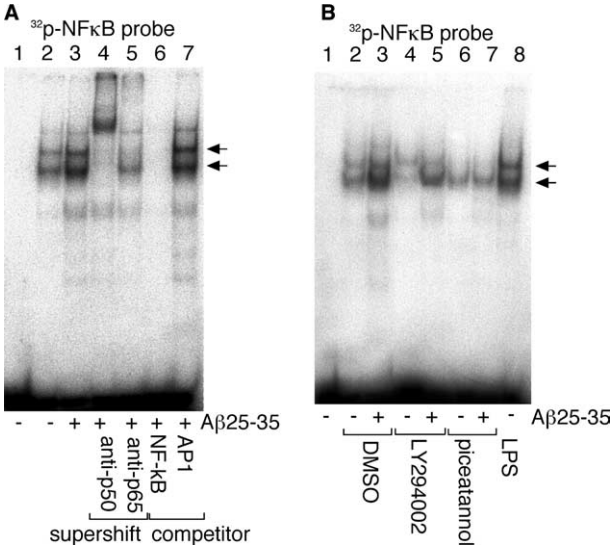

Fig. 5. NF-κB binding activity in EMSA. ^{32}P-labeled probe without nuclear extracts are showed in lane 1. (A) Ra2 cells were treated with 50 μM Aβ25–35 for 4 h (lanes 3–7). Anti-p50 or anti-p65 antibody was added to the extracts for supershift assay (lanes 4 and 5). Unlabeled competitor of NF-κB or AP1 probe was added to the extract (lanes 6 and 7). (B) Ra2 cells were preincubated with 25 μM LY294002 for 30 min (lanes 4 and 5) or 50 μM piceatannol for 1 h (lanes 6 and 7) before treatment with or without 50 μM Aβ25–35 for 4 h (lanes 2–7). Control cells were preincubated with DMSO (lanes 2 and 3). Nuclear extract of Ra2 cells treated with 1 μg/ml LPS for 4 h were used as a positive control (lane 8).

Acknowledgements: This study was supported by the Program for the Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research (of Japan), and in part by grants-in-aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

[1] Akiyama, H., et al. (2000) Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383–421.
[2] Husemann, J., Loike, J.D., Anankov, R., Febbraio, M. and Silverstein, S.C. (2002) Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia 40, 195–205.
[3] El Khoury, J., Hickman, S.E., Thomas, C.A., Cao, L., Silverstein, S.C. and Loike, J.D. (1996) Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature 382, 716–719.
[4] Paresce, D.M., Ghosh, R.N. and Maxfield, F.R. (1996) Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. Neuron 17, 553–565.
[5] El Khoury, J.B., Moore, K.J., Means, T.K., Leung, J., Terada, K., Toft, M., Freeman, M.W. and Luster, A.D. (2003) CD36 mediates the innate host response to beta-amyloid. J. Exp. Med. 197, 1657–1666, Epub 2003 Jun 1609.
[6] Bamberger, M.E., Harris, M.E., McDonald, D.R., Husemann, J. and Landreth, G.E. (2003) A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. J. Neurosci. 23, 2665–2674.
[7] Huang, M.M., Bolen, J.B., Barnwell, J.W., Shattil, S.J. and Brugge, J.S. (1991) Membrane glycoprotein IV (CD36) is physically associated with the Fyn, Lyn, and Yes protein-tyrosine kinases in human platelets. Proc. Natl. Acad. Sci. USA 88, 7844–7848.

[8] Bull, H.A., Brickell, P.M. and Dowd, P.M. (1994) Src-related protein tyrosine kinases are physically associated with the surface antigen CD36 in human dermal microvascular endothelial cells. FEBS Lett. 351, 41–44.
[9] Jimenez, B., Volpert, O.V., Crawford, S.E., Febbraio, M., Silverstein, R.L. and Bouck, N. (2000) Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat. Med. 6, 41–48.
[10] Verdier, Y. and Penke, B. (2004) Binding sites of amyloid beta-peptide in cell plasma membrane and implications for Alzheimer's disease. Curr. Protein Pept. Sci. 5, 19–31.
[11] Sawada, M., Imai, F., Suzuki, H., Hayakawa, M., Kanno, T. and Nagatsu, T. (1998) Brain-specific gene expression by immortalized microglial cell-mediated gene transfer in the mammalian brain. FEBS Lett. 433, 37–40.
[12] Sawada, M., Suzumura, A., Hosoya, H., Marunouchi, T. and Nagatsu, T. (1999) Interleukin-10 inhibits both production of cytokines and expression of cytokine receptors in microglia. J. Neurochem. 72, 1466–1471.
[13] Hattori, T., Takei, N., Mizuno, Y., Kato, K. and Kohsaka, S. (1995) Neurotrophic and neuroprotective effects of neuron-specific enolase on cultured neurons from embryonic rat brain. Neurosci. Res. 21, 191–198.
[14] Maehara, K., Hasegawa, T., Xiao, H., Takeuchi, A., Abe, R. and Isobe, K. (1999) Cooperative interaction of NF-kappaB and C/EBP binding sites is necessary for manganese superoxide dismutase gene transcription mediated by lipopolysaccharide and interferon-gamma. FEBS Lett. 449, 115–119.
[15] Terzi, E., Holzemann, G. and Seelig, J. (1994) Reversible random coil-beta-sheet transition of the Alzheimer beta-amyloid fragment (25–35). Biochemistry 33, 1345–1350.
[16] Shearman, M.S., Ragan, C.I. and Iversen, L.L. (1994) Inhibition of PC12 cell redox activity is a specific, early indicator of the mechanism of beta-amyloid-mediated cell death. Proc. Natl. Acad. Sci. USA 91, 1470–1474.
[17] Pike, C.J., Walencewicz-Wasserman, A.J., Kosmoski, J., Cribbs, D.H., Glabe, C.G. and Cotman, C.W. (1995) Structure-activity analyses of beta-amyloid peptides: contributions of the beta 25–35 region to aggregation and neurotoxicity. J. Neurochem. 64, 253–265.
[18] Araujo, D.M. and Cotman, C.W. (1992) Beta-amyloid stimulates glial cells in vitro to produce growth factors that accumulate in senile plaques in Alzheimer's disease. Brain Res. 569, 141–145.
[19] Nagele, R.G., Wegiel, J., Venkataraman, V., Imaki, H. and Wang, K.C. (2004) Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol. Aging 25, 663–674.
[20] Moore, K.J., El Khoury, J., Medeiros, L.A., Terada, K., Geula, C., Luster, A.D. and Freeman, M.W. (2002) A CD36-initiated signaling cascade mediates inflammatory effects of beta-amyloid. J. Biol. Chem. 277, 47373–47379.
[21] Combs, C.K., Johnson, D.E., Cannady, S.B., Lehman, T.M. and Landreth, G.E. (1999) Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of beta-amyloid and prion proteins. J. Neurosci. 19, 928–939.
[22] Zhang, C., Qiu, H.E., Krafft, G.A. and Klein, W.L. (1996) A beta peptide enhances focal adhesion kinase/Fyn association in a rat CNS nerve cell line. Neurosci. Lett. 211, 187–190.
[23] Martin, D., Salinas, M., Lopez-Valdaliso, R., Serrano, E., Recuero, M. and Cuadrado, A. (2001) Effect of the Alzheimer amyloid fragment Abeta(25–35) on Akt/PKB kinase and survival of PC12 cells. J. Neurochem. 78, 1000–1008.
[24] Luo, Y., Sunderland, T. and Wolozin, B. (1996) Physiologic levels of beta-amyloid activate phosphatidylinositol 3-kinase with the involvement of tyrosine phosphorylation. J. Neurochem. 67, 978–987.
[25] Tang, Y., Yu, J. and Field, J. (1999) Signals from the Ras, Rac, and Rho GTPases converge on the Pak protein kinase in Rat-1 fibroblasts. Mol. Cell. Biol. 19, 1881–1891.
[26] Jiang, K., Zhong, B., Gilvary, D.L., Corliss, B.C., Hong-Geller, E., Wei, S. and Djeu, J.Y. (2000) Pivotal role of phosphoinositide-3 kinase in regulation of cytotoxicity in natural killer cells. Nat. Immunol. 1, 419–425.
[27] Romashkova, J.A. and Makarov, S.S. (1999) NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature 401, 86–90.
[28] Traenckner, E.B., Pahl, H.L., Henkel, T., Schmidt, K.N., Wilk, S. and Baeuerle, P.A. (1995) Phosphorylation of human I kappa B-alpha on serines 32 and 36 controls I kappa B-alpha proteolysis and NF-kappa B activation in response to diverse stimuli. Embo J. 14, 2876–2883.
[29] Harrington, M.A., Edenberg, H.J., Saxman, S., Pedigo, L.M., Daub, R. and Broxmeyer, H.E. (1991) Cloning and characterization of the murine promoter for the colony-stimulating factor-1-encoding gene. Gene 102, 165–170.
[30] Lue, L.F., Walker, D.G., Brachova, L., Beach, T.G., Rogers, J., Schmidt, A.M., Stern, D.M. and Yan, S.D. (2001) Involvement of microglial receptor for advanced glycation endproducts (RAGE) in Alzheimer's disease: identification of a cellular activation mechanism. Exp. Neurol. 171, 29–45.
[31] Coraci, I.S., et al. (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. Am. J. Pathol. 160, 101–112.
[32] Monsonego, A., Imitola, J., Zota, V., Oida, T. and Weiner, H.L. (2003) Microglia-mediated nitric oxide cytotoxicity of T cells following amyloid beta-peptide presentation to Th1 cells. J. Immunol. 171, 2216–2224.