

Fig. 1 The kynurenine pathway (KP) of tryptophan metabolism. IDO and tryptophan 2,3-dioxygenase (TDO) represent the first and rate-limiting enzymes in the KP.

Alzheimer's disease (AD) is the leading cause of dementia (Selkoe 2001). The pathological hallmarks of AD are extracellular amyloid plaques, intracellular neurofibrillary tangles, and dystrophic neurites (Selkoe 2001; Morgan *et al.* 2004). Amyloid β (A β) peptides consisting of 40 or 42 amino acid residues represent a primary component of amyloid plaques. A β (1–42) is considered to be the most neurotoxic form of the peptides (Drouet *et al.* 2000). A β (1–42) can activate primary cultured human microglia and induce IDO expression (Guillemin *et al.* 2003; Walker *et al.* 2006). Furthermore, IDO over-expression and increased production of QUIN have been observed in microglia associated with amyloid plaques in the brains of AD patients (Guillemin *et al.* 2005b). Thus, over-expression of IDO and over-activation of the KP in microglia are likely to be involved in the pathogenesis of AD.

We have previously studied the potential role of the KP in the pathogenesis of AD using a Tg2576 mouse model of AD. These mice express the Swedish mutant of amyloid precursor protein in the brains and have amyloid plaques consisting of A β increasing in an age-dependent manner (Hsiao *et al.* 1996; Kawarabayashi *et al.* 2001). We found that in the Tg2576 mouse brain the amyloid plaques could activate microglia but not sufficiently to induce IDO expression. We also found that an additional brain inflammation generated systemically, such as a peritoneal challenge with lipopolysaccharide, was required for IDO induction in the activated microglia infiltrating into amyloid plaques (Akimoto *et al.* 2007). In this sense, the microglial cells are 'primed' by A β which activates IDO and KP in response to a subsequent

brain inflammation. The secondary brain inflammation is likely to be mediated by proinflammatory cytokines including interleukin-1 β (IL-1 β), IL-6, tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ), all able to activate microglia (Basu *et al.* 2002). Increased levels of these cytokines have been found in the brain of Tg2576 mice during such systemic inflammation (Sly *et al.* 2001). We hypothesized here that proinflammatory cytokines might further stimulate the microglia primed by A β for the expression of IDO. In this study, we used THP-1 cells (a human monocytic cell line) and human peripheral blood mononuclear cells (PBMC), both of which are commonly used as model for the microglial response to A β and proinflammatory cytokines (Giri *et al.* 2003; Wilkinson *et al.* 2006).

Materials and methods

Materials

Synthetic A β peptides (1–40, 1–42, 42–1, and 25–35) were purchased from Peptide Institute Inc. (Osaka, Japan), and prepared as stock solution in dimethyl sulfoxide at a concentration of 4 mM. Human recombinant IL-1 β , IL-6, and TNF- α were purchased from Roche Diagnostics, Tokyo, Japan, and human recombinant IFN- γ was purchased from Sigma (St Louis, MO, USA). One unit of the biological activity of each cytokine was defined according to the manufacture's instruction. All cytokines were dissolved in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin and stored at -80°C until use. Anti-human IDO monoclonal antibody (mAb) was purified from culture medium of a hybridoma clone established by Takikawa *et al.* (1988). Histofine[®] Simple Stain MAX PO, a horseradish peroxidase-conjugated secondary antibody for immunoblotting, was purchased from NICHIREI (Tokyo, Japan). Anti-TNF- α mAb (IgG₁) was obtained from R & D Systems (Tokyo, Japan). Control mouse IgG₁ was a product of Sigma. THP-1 cells, a human acute monocytic leukemia cell line (Tsuchiya *et al.* 1980), were obtained from Health Science Research Resources Bank (Osaka, Japan).

Cell culture and cellular assay

THP-1 cells were maintained in RPMI-1640 (Sigma, St Louis, MO, USA) containing 10% heat-inactivated fetal calf serum at 37°C in 5% CO₂ and 95% air. The cells used in this study were between passages 12 and 15. Human PBMC were isolated from blood of healthy volunteers with Ficoll-paque PLUS solution (GE Healthcare Life Sciences, Tokyo, Japan), and separated from lymphocytes on the basis of plastic adherence as described by Guillemin *et al.* (2003). PBMC were cultured in Dulbecco's modified Eagle's medium (Sigma) containing 20% heat-inactivated fetal calf serum and 10% heat-inactivated human AB serum at 37°C in 5% CO₂ and 95% air. Before cellular assay, cells were washed once with a serum-free AIM-V medium (Invitrogen, Carlsbad, CA, USA), re-suspended in the AIM-V, and plated in culture plate. Kynurenine (Kyn) concentration in culture medium was determined after deproteinization with trichloroacetic acid at a final concentration of 5% with a Shimadzu Prominence[®] HPLC system (Kyoto, Japan) equipped with an Inertsil[®] reverse phased column (4.6 mm x 15 cm; GL Science,

Tokyo, Japan). The mobile phase was 10% methanol/10 mM ammonium acetate and Kyn was detected by absorbance at 360 nm. Phase contrast photomicrographs of THP-1 cells (Fig. 2) were taken using an Olympus IX70 inverted microscope (Tokyo, Japan) equipped with a Leica DFC290 digital camera (Wetzlar, Germany). The study with PBMC from blood of healthy volunteers was approved by the human ethical committee of the National Center for Geriatrics and Gerontology and all participants signed informed consent.

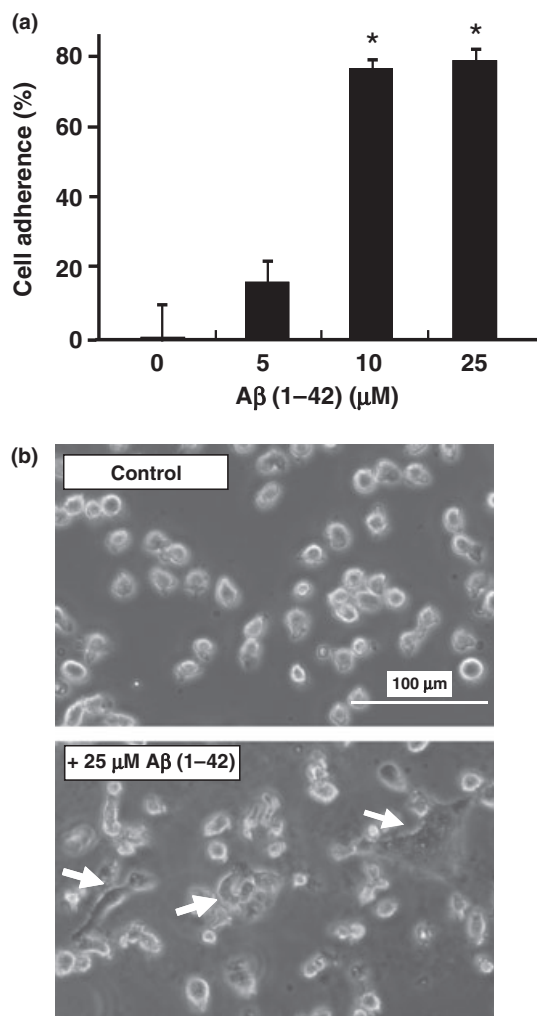


Fig. 2 Increase in adherent activity and change in morphology of THP-1 cells treated with A β (1–42). (a) Adherent activity of THP-1 cells treated with A β (1–42). THP-1 cells (1×10^5 cells) suspended in 1.0 mL of the AIM-V medium in a 24-well plate were treated with indicated concentrations of A β . After 24 h, non-adherent cells were gently collected from the culture plate by pipetting and counted with a hemocytometer under a microscope. * $p < 0.01$ compared with the control cells treated with the peptide vehicle (0.63% of dimethylsulfoxide at a final concentration). (b) Phase contrast microphotographs of THP-1 cells treated with the peptide vehicle (upper) or 25 μ M A β (1–42) (lower) for 24 h. Arrows indicate typical amoeboid spread cells. Scale bar: 100 μ m. Magnification: 200 \times .

Assay of IDO activity

Indoleamine 2,3-dioxygenase activity in cells was assayed as described previously (Takikawa *et al.* 1988). Cultured cells were collected by trypsinization or pipetting, washed with twice with cold PBS, and disrupted by sonication for 30 s in cold PBS containing Complete proteinase inhibitor cocktail (Roche Diagnostics). The cellular homogenate was then centrifuged at 15 000 g for 10 min for 5 min at 4 $^{\circ}$ C and the supernatant (cellular extract) was used as the enzyme source. The reaction mixture (200 μ L) contained 50 mM potassium phosphate buffer (pH 6.5), 20 mM ascorbate, 10 mM methylene blue, 100 mg/mL catalase, 400 mM tryptophan, and the cellular extract. The reaction at 37 $^{\circ}$ C was started by the addition of tryptophan and terminated after 60 min with trichloroacetic acid at a final concentration of 5% and further incubated at 50 $^{\circ}$ C to hydrolyze *N*-formylkynurenine to Kyn. After centrifugation at 15 000 g for 5 min at 25 $^{\circ}$ C, Kyn in the supernatant was measured by HPLC as described above. Under this condition, the production of Kyn from tryptophan increased linearly for up to 90 min. Protein of the cellular extract was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA) with bovine serum albumin as standard.

Immunoblotting

Cells were lysed in the lysis buffer (1% Triton X-100 in PBS containing Complete proteinase inhibitor cocktail) for 30 min on ice. After insolubles were removed from the lysate by centrifugation for 10 min at 10 000 g at 4 $^{\circ}$ C, protein concentration of the cell lysate was measured by bicinchoninic acid protein assay kit (Pierce). Aliquots containing 10 μ g of protein were resolved by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto Immobilon-P transfer membranes (Millipore Corporation, Bedford, MA, USA). The membranes were sequentially reacted with blocking solution, primary antibody (1 : 10000), and the secondary antibody (1 : 500) for 1 h at 25 $^{\circ}$ C. Blots were detected by ECL plus (GE Healthcare). Band intensities were quantified using Scion image software (Scion Corp., Frederick, MD, USA).

RNA isolation and quantitative RT-PCR

Total RNA were isolated from THP-1 cells using a standard method with Trizol[®] reagent (Invitrogen), and reverse-transcribed by SuperScript[®] First-Strand Synthesis System for RT-PCR (Invitrogen). Quantitative RT-PCR (qRT-PCR) was performed using SYBER premix EX *taq* (Takara Bio Inc., Otsu, Japan) with a thermal cycler Dice RealTime System TP800 (Takara Bio Inc) according to the manufacturer's instruction. Comparison of IDO expression among the different experimental groups was performed with relative amount of IDO mRNA normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA as an internal standard. The sequences of primers were 5'-TCTTCTCA- TTTCGTGATGGAG-ACTG-3' and 5'-AAAGTGTCCCGTCTTGCATTTG-3' for IDO, and 5'-GCACCGTCAAGGCTGAGAAC-3' and 5'-TGGTGAA-GACGCCAGTGGG- 3' for glyceraldehyde-3-phosphate dehydrogenase.

Antibody neutralization assay

Anti-human TNF- α mAb or control isotype mouse IgG (IgG₁) was added into THP-1 cell culture at 1 μ g/mL with A β (1–42). After

culture for 24 h, IFN- γ was added to the culture and further incubated for 24 h. The concentration of Kyn in culture medium was determined by HPLC as described above.

Statistical analysis

Each value in all figures represents the mean \pm SD of the three separate experiments. All data were analyzed by ANOVA. If a significant difference was identified, multiple comparisons were adjusted using the Scheffé's test. $p < 0.01$ was regarded as statistically significant.

Results

Activation of THP-1 cells by A β (1–42)

To mimic the *in vivo* situation in which microglia surrounding amyloid plaques consisting of A β in Tg2576 mice are activated by continuous contact with A β , we added A β to culture of THP-1 cells and examined the cell adherence to plastic plate as cell adherence is a hallmark of monocyte/macrophage activation (Kamal and Harold 1998). After treatment with A β (1–42) for 24 h, THP-1 cells became adherent in a dose-dependent manner; at 5 μ M of A β (1–42), about 20% of the cells attached to the plate and at over 10 μ M, most of the cells were adherent (Fig. 2a). This effect was observed only for A β (1–42) but not A β (1–40) or A β 25–35 even at 25 μ M (data not shown). The negative control peptide A β (42–1) at 25 μ M was also totally inactive (data not shown). The activation of THP-1 cells by A β (1–42) was associated with marked changes in morphology from round shape to ameboid spread shape (Fig. 2b) which is a characteristic morphological feature of reactive monocytes/macrophages (Kamal and Harold 1998).

Effect of various proinflammatory cytokines on the Kyn production in THP-1 cells pre-treated with A β (1–42)

To examine if the adherence of the THP-1 cells generated by A β (1–42) induces IDO and activate the KP, we measured the level of tryptophan metabolite Kyn in the culture medium using HPLC. We found that the increase in the level of Kyn after stimulation with A β (1–42) for 24 h was very low (0.1–0.2 μ M), indicating that IDO was hardly activated. This was confirmed by immunoblotting of IDO protein and qRT-PCR for IDO mRNA as described below. Then we tested our hypothesis that a secondary stimulation by proinflammatory cytokines may induce the activation of KP in the reactive THP-1 cells. To this end, we first pre-treated the THP-1 cells with A β (1–42) at 25 μ M for 24 h, then added proinflammatory cytokines, IL-1 β , IL-6, TNF- α , or IFN- γ to the culture at 100 U/mL, and determined the levels of Kyn in the culture medium 24 h after the addition of cytokine. Of these cytokines, only IFN- γ enhanced markedly the production of Kyn from the reactive THP-1 cells (Fig. 3). This enhancement was more prominent at higher concentrations of IFN- γ and was also dependent on the concentration of A β (1–42)

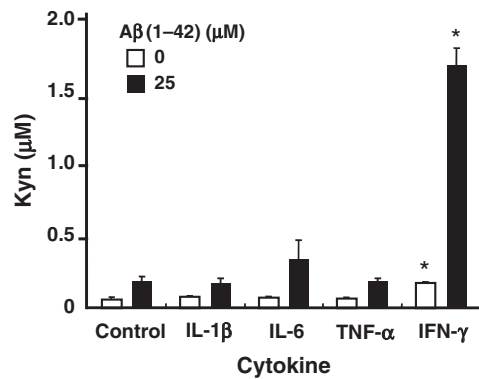


Fig. 3 Production of Kyn in THP-1 cells treated with A β (1–42) and proinflammatory cytokines. THP-1 cells (5×10^4 cells) suspended in 0.2 mL of the AIM-V medium in a 96-well plate were treated with the peptide vehicle or 25 μ M A β (1–42) for 24 h, and then further cultured for 24 h with proinflammatory cytokines at 100 U/mL or the cytokine vehicle (0.1% bovine serum albumin in PBS) (control). The levels of Kyn in the culture medium were measured after additional 24 h culture. \square , the peptide vehicle; \blacksquare , 25 μ M A β (1–42). * $p < 0.01$ compared with each control treated with or without the A β .

(Fig. 4). It is worth noting that the dose-dependency of A β (1–42) for the Kyn production almost coincided with that for the conversion of THP-1 cells to the adherent reactive cells (Fig. 2). Pre-treatment with A β (1–40) or A β (25–35) at 5–50 μ M neither induced THP-1 cells to become adherent reactive cells nor activate the KP by the secondary stimulation with IFN- γ (10–1000 U/mL) (data not shown). Taken together these results indicated that changes in cell morphology induced by A β (1–42) were closely associated with the activation of the KP by IFN- γ .

Optimization of pre-treatment with A β (1–42) for the IFN- γ -mediated activation of KP in THP-1 cells

We determined the optimal duration of the pre-treatment with A β (1–42) required for the IFN- γ -mediated activation of KP in THP-1 cells. Lack of pre-treatment and pre-treatment for 12 h with the addition of A β did not induce any KP activation by IFN- γ . A minimum of 24 h pre-treatment was necessary to lead to a significant activation by IFN- γ (Fig. 5a). We also found that the pre-treatment with A β (1–42) for 24 h caused a long-term sustained activation of KP by IFN- γ for up to 72 h (Fig. 5b). These results demonstrated that pre-treatment of THP-1 cells with A β (1–42) for more than 24 h was required to the subsequent higher KP activation by IFN- γ .

Analysis of IDO expression in THP-1 cells stimulated by A β (1–42) and IFN- γ

The levels of Kyn in culture medium of various cells are closely related to those of IDO expressed by the cells (Takikawa *et al.* 1988). Therefore, we analyzed the changes in IDO expression in THP-1 cells stimulated with the

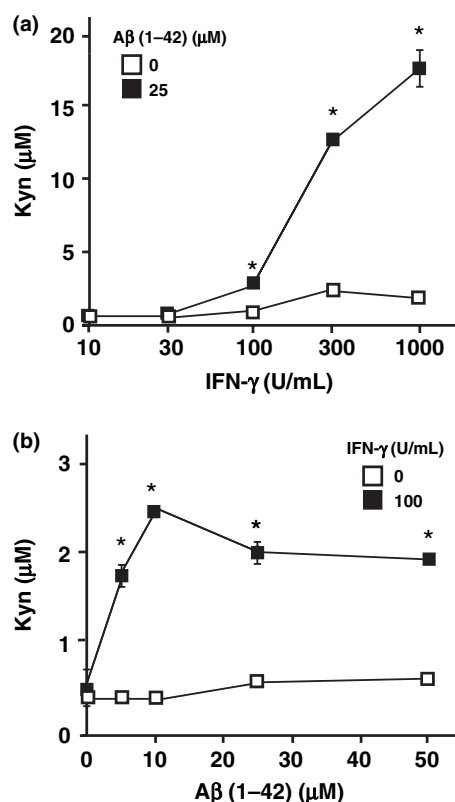


Fig. 4 Production of Kyn in THP-1 cells stimulated with the combination of A β and IFN- γ . (a) The dose-dependency of IFN- γ . THP-1 cells (5×10^4 cells) suspended in 0.2 mL of the AIM-V medium in a 96-well plate were first treated with (■) or without (□) 25 μ M A β (1-42) for 24 h and stimulated with indicated concentrations of IFN- γ for additional 24 h. The levels of Kyn in the culture medium were measured after the stimulation with IFN- γ . □, peptide vehicle; ■, +A β 1-42 (25 μ M). * $p < 0.01$ compared with the values obtained with the peptide vehicle. (b) The dose-dependency of A β (1-42). THP-1 cells (5×10^4 cells) suspended in 0.2 mL of the AIM-V medium in a 96-well plate were first treated with indicated concentrations of A β (1-42) for 24 h and further stimulated with 100 U/mL of IFN- γ (■) or the cytokine vehicle (0.1% BSA in PBS) (□) for additional 24 h. The levels of Kyn in the culture medium were measured after the secondary stimulation. □, cytokine vehicle; ■, IFN- γ (100 U/mL). * $p < 0.01$ compared with the values obtained with the cytokine vehicle.

combination of A β (1-42) and IFN- γ . IDO mRNA was very weakly expressed in THP-1 cells when cultured without any stimulation. This expression increased by 7.2 ± 1.7 -fold upon stimulation with 25 μ M A β (1-42) alone and was strongly elevated by 3156 ± 488.8 -fold in combination with IFN- γ at 100 U/mL (Fig. 6a). Stimulation with IFN- γ alone without pre-treatment with A β (1-42) resulted in small increase of 65 ± 5.2 -fold in mRNA levels; (Fig. 6a). In accordance with these qRT-PCR results, both IDO enzyme activity (Fig. 6b) and the level of IDO protein (Fig. 6c) were markedly elevated by the sequential stimulation with A β and IFN- γ . Taken together, these results showed that KP

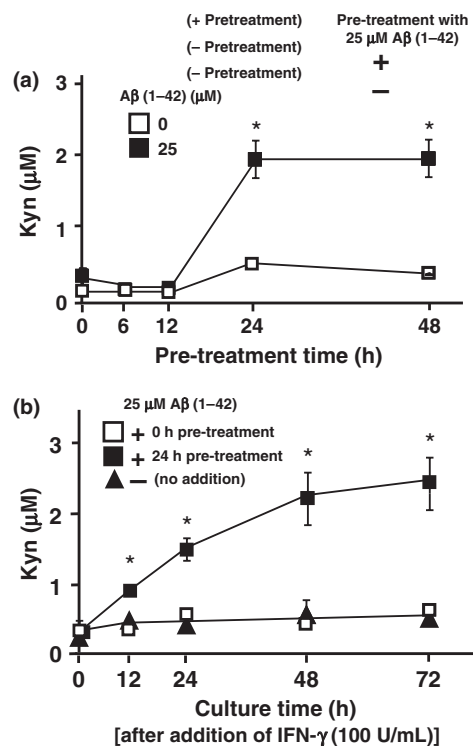


Fig. 5 Optimum duration of the A β (1-42) pre-treatment for the activation of KP by IFN- γ in THP-1 cells and the time course of the production of Kyn in the reactive THP-1 cells stimulated with IFN- γ . (a) Effect of pre-treatment time with A β (1-42) for the production of Kyn by the subsequent stimulation with IFN- γ in THP-1 cells. THP-1 cells (5×10^4 cells) suspended in 0.2 mL of the AIM-V medium in a 96-well plate were cultured with 25 μ M A β (1-42) (■) or without the peptide (i.e., with the peptide vehicle) (□). At indicated culture periods (6, 12, 24, and 48 h), IFN- γ (100 U/mL) was added to the cultures and the levels of Kyn in culture medium were measured after additional 24 h culture. □, peptide vehicle; ■, +25 μ M A β (1-42). * $p < 0.01$ compared with the values obtained without the pre-treatment with the A β . (b) The time course of the production of Kyn in the THP-1 cells stimulated with the combination of A β (1-42) and IFN- γ . THP-1 cells (5×10^4 cells) suspended in 0.2 mL of the AIM-V medium in a 96-well plate were pre-treated with 25 μ M A β (1-42) for 24 h and further cultured with 100 U/mL IFN- γ for up to 72 h. The time course of Kyn production in the cultures after the addition of IFN- γ was depicted with ■. Those of the cultures stimulated simultaneously with 25 μ M A β (1-42) and 100 U/mL IFN- γ or stimulated with 100 U/mL IFN- γ alone after pre-treatment of the peptide vehicle for 24 h were indicated with □ and ▲, respectively. * $p < 0.01$ compared with the values obtained without culture with IFN- γ .

activation in THP-1 cells by A β (1-42) and IFN- γ was associated with an increase in IDO transcript and functional IDO protein.

Analysis of IDO expression in PBMC stimulated with A β (1-42) and IFN- γ

It could be possible that the above-mentioned KP activation may be the unique property of THP-1 cells acquired during immortalization. Therefore, we tested if the similar

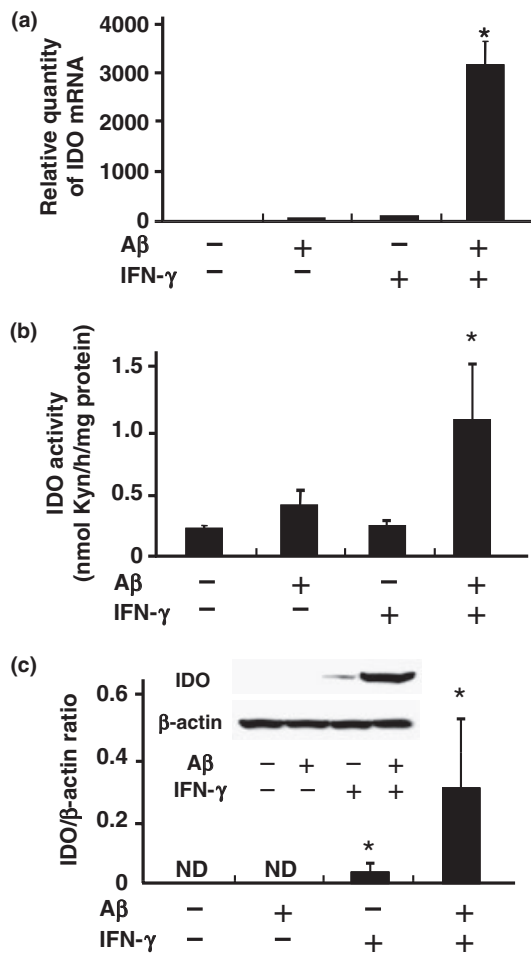


Fig. 6 Analysis of the expression of IDO in THP-1 treated with the combination of Aβ (1–42) and IFN-γ. THP-1 cells (1×10^5 cells) suspended in 1.0 mL of the AIM-V medium in a 24-well plate were analyzed after pre-treatment with or without 25 μM Aβ (1–42) for 24 h and further stimulation with or without 100 U/mL IFN-γ for 24 h. (a) qRT-PCR of IDO mRNA. The levels of IDO mRNA were normalized with those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and expressed as relative quantity to that of the control (the control value: 1) without stimulation with 25 μM Aβ (1–42) nor 100 U/mL IFN-γ. (b) IDO enzyme activity. (c) Immunoblot analysis of IDO protein. The band intensities of IDO were normalized with those of β-actin. * $p < 0.01$ compared with others. ND, not detectable.

combinational effects of Aβ (1–42) and IFN-γ could also induce IDO in primary cultures of human peripheral blood mononuclear cells (PBMC). PBMC were first pre-treated with 25 μM Aβ (1–42) for 24 h, then stimulated with different concentrations of IFN-γ for another 24 h, and analyzed by immunoblotting. Similar to THP-1 cells, IDO expression in PBMC was undetectable without any stimulation but weakly induced by IFN-γ alone (Fig. 7a). However, with the Aβ (1–42) pre-treatment, IDO expression was greatly enhanced by IFN-γ in a dose-dependent manner (Fig. 7a). In fact, the pre-treatment increased IDO protein

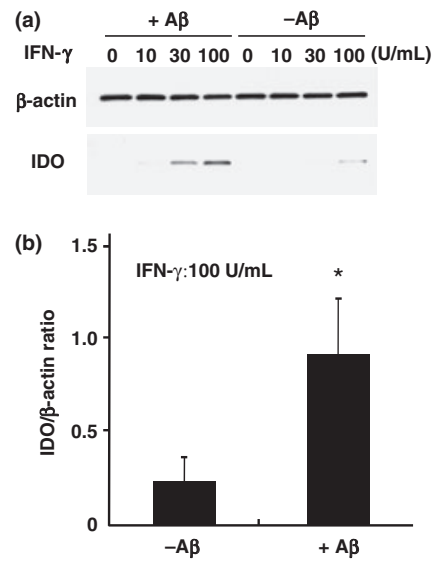


Fig. 7 Analysis of IDO expression in PBMC treated with Aβ (1–42) and IFN-γ. (a) Immunoblotting of IDO in PBMC. PBMC (1×10^5 cells) suspended in 1.0 mL of AIM-V medium and cultured in a 24-well plate with the peptide vehicle or 25 μM Aβ (1–42) for 24 h and further stimulated with indicated concentrations of IFN-γ for 24 h. IDO expression in PBMC was analyzed by immunoblotting after stimulation with IFN-γ. (b) Quantitative analysis of the immunoblots. The band intensities obtained with 100 U/mL IFN-γ were normalized with those of β-actin. * $p < 0.01$ compared with the value obtained with the peptide vehicle (-Aβ).

level by about fourfold with 100 U/mL IFN-γ (Fig. 7b), which was comparable with results with THP-1 cells (Fig. 6c). Thus, the high IDO induction by the sequential stimulation with Aβ (1–42) and IFN-γ was well similar between human monocytic cell line THP-1 cells and human primary PBMC.

Involvement of TNF-α in KP activation by Aβ (1–42) and IFN-γ

We previously demonstrated that IDO is induced by IFN-γ in many different cell types (Takikawa *et al.* 1988), and other showed that this induction could be enhanced by TNF-α in macrophages and epithelial cells (Currier *et al.* 2000; Robinson *et al.* 2003). Other studies also reported that TNF-α secretion by microglial cells was induced by a combination of Aβ (1–42) and IFN-γ (Meda *et al.* 1995; Klegeris *et al.* 1997). We therefore hypothesized that an autocrine stimulation by TNF-α may be involved in KP activation in THP-1 cells by the sequential treatment with Aβ (1–42) and IFN-γ. To test this hypothesis, we stimulated THP-1 cells with Aβ (1–42) and IFN-γ in the presence of anti-TNF-α mAb at 1 μg/mL, which was able to neutralize as much as 100 U/mL of TNF-α. The anti-TNF-α mAb inhibited the Kyn production by more than 60% ($39.1 \pm 8.7\%$) whereas the control isotype IgG₁ at 1 μg/mL

Table 1 Effect of anti-TNF- α mAb on the production of Kyn in THP-1 cells treated with the combination of A β (1–42) and IFN- γ

	Kyn (μ M)	Percent of control
Control	2.3 \pm 0.2	100.0
+Anti-TNF- α (1 μ g/mL)	0.9 \pm 0.2	39.1 \pm 8.7*
+IgG1 (1 μ g/mL)	2.2 \pm 0.1	95.6 \pm 4.3

TNF- α , tumor necrosis factor α ; Kyn, kynurenine.

THP-1 cells (5×10^4 cells) suspended in 0.2 mL of AIM-V medium were cultured in a 96-well plate with 25 μ M A β (1–42) in the presence of anti-human TNF- α mAb (IgG $_1$) or control isotype IgG $_1$ at 1 μ g/mL for 24 h. Control cells were cultured with 25 μ M A β (1–42) alone. Then IFN- γ was added to the cultures at 100 U/mL and cultured for another 24 h. Kyn was measured in culture supernatants after the additional 24 h culture.

* $p < 0.01$ compared with others.

was totally ineffective (Table 1). These results indicated that an autocrine TNF- α was significantly involved in the KP activation in THP-1 cells.

Discussion

In this study, we tested our hypothesis that activated microglia surrounding amyloid plaques consisting of A β are ‘primed’ for the activation of KP in response to the additional stimulation by proinflammatory cytokines. We used THP-1 cells and human primary PBMC as models for microglia, and found that both monocytic cell types pre-treated with A β (1–42), one of the main components of amyloid plaques, become highly responsive to a secondary stimulation with the proinflammatory cytokine IFN- γ and markedly activate the KP through induction of IDO. Only the combination of A β (1–42) and IFN- γ was able to activate the KP whereas A β (1–40) or A β (25–35) and other major proinflammatory cytokines including IL-1 β , TNF- α , or IL-6 were not (Fig. 3), although an autocrine TNF- α was partly involved in the induction of IDO (Table 1). However, the combined concomitant stimulation of THP-1 cells with A β (1–42) and IFN- γ did not activate the KP. We showed that a pre-treatment with A β (1–42) for at least 24 h was prerequisite for the secondary stimulation with IFN- γ to be effective on the KP activation (Fig. 5). This pre-treatment with A β lead to a cellular activation as shown by the morphological change and enhanced adhesiveness (Fig. 2). This activation by A β (1–42) alone, however, was not sufficient to switch on the cellular machinery involved in IDO induction (Fig. 4). Thus, our *in vitro* model further supported our hypothesis that the microglia receiving a chronic stimulation by amyloid plaques consisting of A β (1–42) are ‘primed’ for the activation of KP by the induction of IDO in a response to the proinflammatory cytokine IFN- γ .

Both THP-1 cells primed with A β (1–42) and PBMC differentiated by granulocyte macrophage-colony stimulating

factor (GM-CSF) have a similar response in term of KP activation by IFN- γ (Jansen and Reinhard 1999). However, PBMC required longer time of culture (3 to 7 days) with GM-CSF to adopt a reactive state in response to the IFN. These differentiated cells did not exhibit such strong morphological changes and adhesiveness as we found with THP-1 cells after a short (24 h) pre-treatment with A β (1–42) (Fig. 2). Therefore, the cellular changes induced by GM-CSF appear to be different from those with A β ‘priming’.

What is the relevance of these present findings to the pathogenesis of AD? It is known that systemic inflammatory infection is the risk factor for AD progression (Holmes *et al.* 2003) and that similar systemic inflammation caused by an intraperitoneal injection of lipopolysaccharide increased proinflammatory cytokine production including IFN- γ in the mouse brain (Pitossi *et al.* 1997). Level of IFN- γ in the mouse brain increased with aging (Frank *et al.* 2006; Kumagai *et al.* 2007), which represented the major risk factor for AD (Evans *et al.* 1989; Hebert *et al.* 2003). On the other hand, it has been recently reported that IFN- γ can be produced by mouse microglia when stimulated with IL-18 (Kawanokuchi *et al.* 2006) and that expression of IL-18 is elevated in microglia, astrocytes, and neurons within the AD brains (Ojala *et al.* 2009). Under stress conditions, IL-18 was also involved in the activation of murine microglia (Sugama *et al.* 2007), which accelerated learning and memory impairment and worsened the amyloid pathology in the mouse models of AD (Dong *et al.* 2004; Jeong *et al.* 2006). Therefore, under neuroinflammatory conditions (infection, aging, and stress) associated with the accelerating progression in both human AD or the AD-like pathology of the mouse models, ‘primed’ microglia surrounding amyloid plaques appear to be activated by IFN- γ to induce IDO and to produce neurotoxic QUIN, thereby promoting the neurodegeneration. In the rat brain QUIN neurotoxicity was greatly enhanced by proinflammatory cytokine IL-1 β (Stone and Behan 2007). This neurotoxic combination is likely to reach neurons around amyloid plaques in human AD brains, as production of both QUIN and IL-1 β was increased in activated microglia attacking senile plaques (Griffin *et al.* 1989; Guillemin *et al.* 2005b). Thus, our findings may explain some part of the molecular mechanisms underlying the accelerated neurodegeneration by risk factors known to enhance inflammation in the AD brain.

A β (1–40) and A β (1–42) are the major components of amyloid plaques (Gravina *et al.* 1995; Kawarabayashi *et al.* 2001). However, considerable circumstantial evidence suggests that A β (1–42) rather than A β (1–40) is the critical molecule involved in the pathogenesis of AD (Jarrett *et al.* 1993; Iwatsubo *et al.* 1994; Gravina *et al.* 1995; McGowan *et al.* 2005). In fact, even at lower concentrations A β (1–42) was significantly more toxic to cultured neurons compared with A β (1–40) (Drouet *et al.* 2000; Dahlgren *et al.* 2002). A β (1–42) can form soluble oligomeric structures, insoluble

fibrils, and highly aggregated form of the fibrils at lower concentrations and higher rates compared with A β (1–40) or any other A β variants (Burdick *et al.* 1992; Jarrett *et al.* 1993). These unique properties of A β (1–42) may be responsible for the specificity for the priming effect on the induction of IDO in THP-1 cells.

Several surface receptors expressed in monocytic cells including microglia have been reported to interact with A β fibrils; among them the scavenger receptor complex consisting of class A scavenger receptor, CD36, α 6/ β 1-integrin, and CD47 (El Khoury *et al.* 1996; Wilkinson *et al.* 2006), and the receptor for advanced glycation end products (Yan *et al.* 1996), and Toll-like receptors 2 and 4 (Chen *et al.* 2006; Richard *et al.* 2008; Udan *et al.* 2008). Our preliminary data (not shown) suggested that the scavenger receptor complex was not involved in the priming effect of A β (1–42) because the antagonist for the receptor complex, 4N1K peptide at up to 300 μ M (Wilkinson *et al.* 2006) did not suppress the induction of IDO. Moreover, the phagocytosis of A β fibrils was not required for the effect of A β (1–42) as treatment with an inhibitor of phagocytosis, cytochalasin D (3 μ M) (Sulahian *et al.* 2008) did not inhibit the enzyme induction (data not shown). The possible involvement of receptor for advanced glycation or Toll-like receptor 2/4 in the priming effect of A β (1–42) is currently under investigation.

It is generally accepted that inflammation-mediated neurotoxicity in neurodegenerative disease including AD can occur as a consequence of microglial overactivation (Perry *et al.* 2003; Block *et al.* 2007). This concept is based on the fact that such over-activated microglia can generate neurotoxic products including reactive oxygen species and proinflammatory cytokines (Cunningham *et al.* 2005; Block *et al.* 2007). In addition to these neurotoxic compounds, we previously demonstrated that activated microglia associated with amyloid plaques in human AD brains produced neurotoxic amounts of QUIN (Guillemin *et al.* 2005a, 2007). Based on our previous *in vivo* findings in Tg2576 mice and our present *in vitro* data with models of microglia, we concluded that the microglia producing QUIN in AD brains were over-activated by the combination of the chronic exposure to amyloid peptides, and more particularly A β (1–42), and the secondary inflammatory cytokine, IFN- γ . Several drugs that block the KP are currently under therapeutic investigation by our laboratory and others. Targeting IDO or other KP enzymes with specific inhibitors would lead to a decrease in QUIN production and may therefore bring new therapeutic strategies for AD.

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