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β-Amyloid-specific upregulation of stearoyl coenzyme A desaturase-1 in macrophages

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Abstract

β-Amyloid peptide (Aβ), a major component of senile plaques, the formation of which is characteristic of Alzheimer's disease (AD), is believed to induce inflammation of the brain mediated by microglia, leading to neuronal cell loss. In this study, we performed an oligonucleotide microarray analysis to investigate the molecular events underlying the Aβ-induced activation of macrophages and its specific suppression by the Aβ-specific-macrophage-activation inhibitor, RS-1178. Of the approximately 36,000 genes and expressed sequence tags analyzed, eight genes were specifically and significantly upregulated by a treatment with interferon γ (IFNγ) and Aβ compared to a treatment with IFNγ alone ($p < 0.002$). We found that the gene for a well-characterized lipogenetic enzyme, stearoyl coenzyme A desaturase-1 (SCD-1), was specifically upregulated by Aβ treatment and was suppressed to basal levels by RS-1178. Although the underlying mechanisms remain unknown, our results suggest the presence of a link between AD and SCD-1.

Keywords: Alzheimer's disease; β-Amyloid; Microglia; Inflammation; Microarray; Stearoyl coenzyme A desaturase

β-Amyloid peptide (Aβ), a major component of senile plaques, the formation of which is characteristic of Alzheimer's disease (AD), is believed to induce inflammation in the brain leading to neuronal cell loss and cognitive decline [1–4]. Accumulating evidence shows Aβ activates microglia, brain macrophages that carry out immune functions and mediate inflammatory responses in the brain [5–12]. Although several reports show involvement of tyrosine kinases such as Lyn and Fyn, or involvement of mitogen-activated protein kinase pathways, the downstream components of the Aβ-stimulated inflammatory signal transduction pathway have not been well characterized [13,14].

We have previously reported that RS-1178, a specific inhibitor of Aβ-induced macrophage activation, inhibited Aβ-induced macrophage activation but did not inhibit zymosan A-nor lipopolysaccharide (LPS)-induced macrophage activation [15]. Our results support the idea that stimulants such as LPS, zymosan A, and Aβ trigger distinct pathways.

In this study, we performed an oligonucleotide microarray analysis to investigate the molecular events underlying the activation of macrophages by Aβ and the specific suppression by RS-1178.

Materials and methods

Mouse interferon γ (IFNγ) was obtained from Genzyme Techne. LPS, polymyxin B, penicillin, and streptomycin were purchased from Sigma Chemical. Zymosan A was from Molecular Probes and phosphate buffered saline (PBS) was from Dainippon Pharmaceutical. Dulbecco's modified Eagle's medium (DMEM) was obtained from Asahi Techno Glass Corporation. Fetal bovine serum (FBS) was obtained from Invitrogen. The Aβ used in the present study was a synthetic peptide of human Aβ 1–42, which was purchased from AnaSpec. RS-1178 was synthesized at Sankyo [15]. RS-1178 was dissolved and diluted in dimethyl sulfoxide and further diluted to the desired concentration with PBS. The final concentration of dimethyl sulfoxide was 0.1%. All other reagents used were of the highest grade available.

Preparation and treatment of mouse peritoneal macrophages. Cells were harvested from the peritoneal cavity of 8-week-old ddY mice (Charles River Japan) [15]. For GeneChip analysis, macrophages were

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plated in 6-well plates (Corning) at a density of 1×10^7 cells/well in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. For real-time PCR analysis, macrophages were plated in 24-well plates (Corning) at a density of 8×10^6 cells/well in the medium. Twenty-four hours after macrophages were seeded, macrophage cultures in the medium were incubated with 4.4 μM Aβ, 7 ng/ml zymosan A or with 500 pg/ml LPS in the presence of 250 U/ml IFNγ. For experiments using Aβ and zymosan A, 10 μg/ml polymyxin B was added to the medium. RS-1178 (3.5 μM) was added simultaneously. The cultures were incubated for 5 h at 37 °C in 5% CO_2/95% air.

Isolation and analysis of RNA. Total RNA was extracted with Trizol (Invitrogen) and poly(A)$^+$ mRNA was purified by OligoTex dT30 ⟨super⟩ mRNA purification kit (Takara Bio). The purity was determined by spectrophotometry. The procedures described in detail in the Affymetrix GeneChip Expression Analysis Manual (Affymetrix) were essentially followed. RNA was converted to double-stranded cDNA using an oligo(dT) primer containing the T7 promoter (Gibco BRL Superscript Choice System, Life Technologies). The cDNA was purified using PLG tubes (Prime), extracted with phenol/chloroform, precipitated with ethanol, and used for biotinylated cRNA preparation (Bioarray High Yield RNA Transcription Labeling Kit, Enzo Diagnostics). The biotinylated cRNA was purified (RNeasy spin columns, Qiagen), quantified, and fragmented, and the purity was verified by agarose gel electrophoresis. Ten micrograms of cRNA was hybridized to Affymetrix Murine Genome U34Av2 GeneChip in Affymetrix Fluidics Station 400. The chip was washed and stained with streptavidin–phycoerythrin. The GeneChip was scanned with a probe array scanner and the data were analyzed using an Affymetrix GeneChip expression analysis software. Methods for data analysis, sensitivity evaluation, and quantification followed are described in the Affymetrix GeneChip Analysis Technical Manual, including statistical acceptance criteria (e.g., $p < 0.02$).

Real-time PCR. GeneChip results were confirmed by real-time PCR for SCD-1 and 18S rRNA (used as internal control). Total RNA (500 ng) from each sample was reverse transcribed using M-MuLV reverse transcriptase (Life Technologies). Real-time PCR was performed using the Taqman 5700 Sequence Detection System (Applied Biosystems). In brief, cDNA and gene-specific primers were added to SYBR Green PCR Master Mix (SYBR Green I Dye, AmpliTaq DNA polymerase, dNTPs with dUTP, and optimal buffer components; Applied Biosystems) and subjected to PCR amplification (40 cycles at 50 °C for 2 min, 95 °C for 10 min, and 60 °C for 1 min). The amplified transcripts were quantified using the comparative C_T method. The primers used for SCD-1 mRNA detection were 5′-TAGCTCCAGTG AGGTGGTGTG-3′ and 5′-GTGGGTTTGTTACAAGAGAAAGG ATA-3′, custom synthesized by Sigma Genosis.

Results and discussion

Numerous studies show that Aβ activates monocytic cells at micromolar concentrations in the presence of IFNγ in vitro [8,9,11]. This is consistent with our results that Aβ induced the production of nitric oxide and TNF α from macrophages in a dose-dependent manner in the presence of IFNγ [15].

In this study, we performed an oligonucleotide microarray analysis to investigate the molecular events underlying the activation of macrophages by Aβ and the specific suppression by RS-1178, a specific inhibitor of Aβ-induced macrophage activation. Mouse peritoneal macrophage cultures were treated with PBS, 4.4 μM Aβ, 7 ng/ml zymosan A, 500 pg/ml LPS or with 4.4 μM Aβ + 3.5 μM RS-1178 in the presence of 250 U/ml IFNγ. Dexamethasone was used to normalize the degree of macrophage activation by the various stimulants since dexamethasone suppresses macrophage activation via a common mechanism (data not shown, [15]). Thus, experiments in this study were carried out under conditions at which the degrees of activation by the stimulants are similar.

Using Affymetrix GeneChip expression analysis software, we performed comparison analyses to derive biologically meaningful results from the raw probe cell intensity data of expression arrays. As a result, we obtained discrete "change calls" (Increase (I), Marginal Increase (MI), No Change (NC), Marginal Decrease (MD), or Decrease (D)) representing significant changes in mRNA levels. Macrophage mRNA levels in groups treated with IFNγ plus Aβ, IFNγ plus LPS, IFNγ plus zymosan A, and IFNγ, Aβ plus RS-1178 were compared with the mRNA level in the group treated with IFNγ alone to exclude the effects of IFNγ.

Of the approximately 36,000 genes and expressed sequence tags analyzed, the "change calls" and the fold change of 21 genes that were significantly upregulated by the IFNγ plus Aβ treatment compared to the IFNγ-only treatment (indicated by "I; Increase" according to Affymetrix Signal Call algorithm based on Wilcoxon signed-rank test) are shown in Table 1. The selected genes were classified as genes involved in immune response, genes involved in cytokine and chemokine production, and genes expressing metabolic enzyme, signaling molecule or other protein with an unknown or miscellaneous function. Most of the genes expressing immune-response molecules, cytokines, and chemokines were upregulated by the IFNγ plus LPS or by the IFNγ plus zymosan A treatment as well, suggesting that they consist common inflammatory pathways in macrophages. In contrast, eight genes were specifically and significantly upregulated by the IFNγ plus Aβ treatment ($p < 0.002$).

Of particular interest, we found that the gene for SCD-1, a well-characterized lipogenetic enzyme, was specifically and significantly upregulated by the IFNγ plus Aβ treatment ($p = 0.00028$). Moreover, the upregulation of SCD-1 was suppressed to a level similar to that seen after an IFNγ-only treatment of macrophages by the Aβ-specific-macrophage-activation inhibitor, RS-1178. Although the exact mechanism by which RS-1178 exerts this suppressive effect remains unknown, RS-1178 seems to interact with the Aβ-induced signal transduction cascade in macrophages.

To confirm our results obtained by microarray hybridization, we performed real-time quantitative PCR. The specific upregulation of SCD-1 mRNA by the IFNγ plus Aβ treatment in macrophages was suppressed to a level similar to that after an IFNγ-only treatment by RS-1178 (Table 2). These results were

Table 1
Macrophage gene expression induced after 5 h of IFN and Aβ exposure

	Accession No.	IFN + Aβ vs. IFN	IFN + zym vs. IFN	IFN + LPS vs. IFN	IFN + Aβ + RS vs. IFN
Genes involved in immune response					
H2-Aa	X52643	I (1.3)	NC (1.0)	I (1.9)	NC (1.4)
BOB.1	Z54283	I (9.9)	NC (5.7)	NC (2.0)	NC (6.1)
L-CA	M23158	I (1.4)	NC (1.1)	NC (1.2)	NC (1.2)
Igk-V28	U48716	I (2.1)	NC (2.0)	NC (2.3)	I (3.0)
IgM	M80423	I (2.6)	I (1.7)	I (2.3)	I (2.3)
Iga	NM_007655	I (1.7)	I (1.9)	I (2.8)	I (2.8)
Igh-6	X03690	I (3.1)	I (1.8)	I (2.1)	I (2.7)
Genes involved in cytokine and chemokine production and regulation					
TNFα	M13049	I (1.3)	I (3.5)	I (9.9)	NC (1.0)
Il1β	M15131	I (1.2)	I (2.3)	I (5.7)	I (2.1)
Ptges	NM_022415	I (1.3)	I (2.3)	I (3.5)	NC (1.5)
MIP2	X53798	I (1.2)	I (4.9)	I (13.0)	NC (1.2)
Cish3	AF314501	I (1.4)	I (1.7)	I (3.6)	I (1.3)
Others					
Scd1	M21285	I (1.8)	NC (1.5)	NC (1.6)	NC (0.8)
Ms4a2	NM_007641	I (2.0)	NC (1.4)	I (2.4)	NC (1.1)
VSAG3	X63025	I (2.4)	NC (1.7)	I (3.1)	NC (1.9)
Kdap	D88899	I (2.0)	NC (1.7)	NC (2.1)	NC (2.1)
Tex292	XM_134389	I (2.6)	NC (1.7)	NC (1.7)	NC (1.0)
Taf9	AF305839	I (2.8)	NC (1.5)	NC (1.6)	NC (1.5)
Onzin	AF263458	I (2.3)	I (1.3)	I (2.0)	I (1.7)
Nap1l1	NM_015781	I (1.6)	NC (1.3)	I (1.6)	NC (2.0)
Rnac-pending	NM_021525	I (2.1)	I (2.6)	I (3.3)	NC (0.7)

Biotinylated RNA from macrophages treated with 250 U/ml IFNγ (IFN), 250 U/ml IFNγ plus 4.4 μM Aβ (IFN + Aβ), 250 U/ml IFNγ plus 7 ng/ml zymosan A (IFN + zym), 250 U/ml IFNγ plus 500 pg/ml of LPS (IFN + LPS), or with 250 U/ml IFN, 4.4 μM Aβ plus 3.5 μM RS-1178 (IFN + Aβ + RS) for 5 h were hybridized onto Affymetrix Murine Genome U34Av2 GeneChip and quantified as indicated under "Materials and methods." I, increase (according to Affymetrix Signal Call algorithm); NC, no change. Numbers in parentheses indicate the fold change relative to IFNγ-treated control. H2-Aa, histocompatibility 2 class II antigen A alpha; BOB.1, B cell Oct-binding protein 1; L-CA, leukocyte common antigen; Igk-V28, anti-HIV-1 reverse transcriptase single-chain variable fragment; IgM, immunoglobulin kappa-chain; Iga, immunoglobulin-associated α; Igh-6, immunoglobulin heavy chain 6; TNFα, tumor necrosis factor alpha; Il1β, interleukin 1-β; Ptges, prostaglandin E synthase; MIP2, macrophage inflammatory protein-2; Cish3, cytokine inducible SH2-containing protein 3; Scd1, stearoyl coenzyme A desaturase 1; Ms4a2, membrane-spanning 4-domain subfamily A member 2; VSAG3, viral superantigen; Kdap, kidney-derived aspartic protease-like protein; Tex292, testis expressed gene 292; Taf9, RNA polymerase II TATA box binding protein-associated factor G; and Nap1l1, nucleosome assembly protein 1-like 1.

Table 2
Real-time PCR analysis of SCD-1 mRNA

Treatment	Fold change
IFN	1.0
Aβ	1.5
IFN + Aβ	2.7
IFN + zym	1.1
IFN + LPS	1.8
IFN + Aβ + RS	1.3

Total RNA from macrophages treated with 250 U/ml IFNγ (IFN), 250 U/ml IFNγ plus 4.4 μM Aβ (IFN + Aβ), 250 U/ml IFNγ plus 7 ng/ml zymosan A (IFN + zym), 250 U/ml IFNγ plus 500 pg/ml LPS (IFN + LPS), or with 250 U/ml IFNγ plus 4.4 μM Aβ plus 3.5 μM RS-1178 (IFN + Aβ + RS) for 5 h was reverse transcribed and subjected to PCR amplification. The amplified transcripts were quantified using the comparative C_T method. Data indicate the mean fold change in SCD-1 mRNA levels relative to IFNγ-treated controls from duplicate determinations.

consistent with those obtained by microarray hybridization assay.

SCD-1 is the rate-limiting enzyme in the production of mono-unsaturated fatty acids (MUFAs), palmitoleoyl-CoA, and oleoyl-CoA in liver adipocytes. MUFAs play important roles in many processes, including energy metabolism, membrane fluidity, anti-oxidation, signal transduction, apoptosis, and senescence [16]. There have been reports stating that the induction of SCD-1 activity leads to an increase in cholesterol, plasma lipids, and lipoproteins [17–19]. However, the function of SCD-1 in macrophages remains unknown. Moreover, there have been no studies showing a direct relationship between SCD-1 and AD. Only several in vitro and epidemiological studies suggest a molecular link between AD, cholesterol, and/or lipids [20–26]. Future experiments clarifying the unknown mechanisms by which SCD-1 is upregulated by Aβ in macrophages may shed light on the relationship between AD, cholesterol, and/or lipids.

Although the underlying mechanisms remain unknown, based on our results that Aβ, an AD-related molecule, upregulated SCD-1 in macrophages, it can be considered that there is a link between SCD-1 and AD.

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