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Effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interluekin-4 in Toll-like receptor-4-mutant glial cells

Yoshihisa Kitamura [a,*], Jun-ichi Kakimura [a], Hideyasu Koike [a], Masaaki Umeki [a], Peter J. Gebicke-Haerter [b], Yasuyuki Nomura [c], Takashi Taniguchi [a]

[a] *Department of Neurobiology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8412, Japan*
[b] *Department of Psychiatry and Psychotherapy, Albert-Ludwigs-University of Freiburg, Freiburg D-79104, Germany*
[c] *Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*

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Abstract

15-Deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 are endogenous anti-inflammatory substances. In this study, we examined the effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 in glial cells from the Toll-like receptor-4-mutant (C3H/HeJ) and wild-type (C3H/HeN) mouse brains. The lipopolysaccharide-induced expression of inducible nitric oxide (NO) synthase and cyclooxygenase-2 in the Toll-like receptor-4-mutant glial cells have significantly lower levels (about half and quarter, respectively) than those in the wild-type cells. Treatment with both interleukin-4 (at 10 ng/ml, for 48 h) and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (at 3 μM, for 30 min) completely inhibited the lipopolysaccharide-induced expression of inducible NO synthase and cyclooxygenase-2. In contrast, heme oxygenase-1 was induced by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 alone, but was not changed by interleukin-4 or lipopolysaccharide. The inhibitory protein of nuclear factor-κB was degraded by lipopolysaccharide in both mutant and wild-type glial cells, and this degradation was not inhibited by either 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 or interleukin-4. These results suggest that the response to lipopolysaccharide is partially dependent on Toll-like receptor-4 in mouse glial cells, and that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 differently regulate the expression of inducible NO synthase and cyclooxygenase-2, and heme oxygenase-1.

Keywords: Toll-like receptor-4; 15-Deoxy-$\Delta^{12,14}$ prostaglandin J_2; Interleukin-4; Nitric oxide (NO) synthase, inducible; Cyclooxygenase-2; Heme oxygenase-1

1. Introduction

Lipopolysaccharide is primarily recognized by lipopolysaccharide-binding protein and the cell-surface glycoprotein CD14 (Ulevitch and Tobias, 1995; Perera et al., 1997). However, since CD14 lacks a cytoplasmic domain, the existence of a co-receptor, which initiates signal transduction, has been suggested. Recently, mammalian homologs of *Drosophila* Toll, designated Toll-like receptors, have been cloned (Medzhitov et al., 1997; Rock et al., 1998). The family of Toll-like receptors has an extracellular domain containing leucine-rich repeats and a cytoplasmic domain similar to those of interleukin-1 receptor. Among this family, Toll-like receptor-4 and -2 have been identified as receptors for lipopolysaccharide (Yang et al., 1998; Kirschning et al., 1998; Hoshino et al., 1999; Takeuchi et al., 1999). Interestingly, lipopolysaccharide-tolerant mouse C3H/HeJ has a single point mutation in the cytoplasmic domain of the Toll-like receptor-4 gene and exhibits loss-of-function in the response to lipopolysaccharide (Poltorak et al., 1998; Hoshino et al., 1999; Qureshi et al., 1999). The signaling pathway of Toll-like receptors and interleukin-1 receptor leads to activation of nuclear factor-κB (NF-κB) through an adapter protein MyD88 and interleukin-1 receptor-associated kinase (Kawai et al., 1999). NF-κB is a critical activator of genes involved in inflammation and immunity (Karin and Delhase, 1998; Ninomiya-Tsuji et al., 1999).

15-Deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 are endogenous anti-inflammatory substances (Hart et al., 1989; Jiang et al., 1998; Ricote et al., 1998). Since prostaglandin D_2 and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 are produced in the delayed stage of inflammatory episodes, they

* Corresponding author. Tel.: +81-75-595-4706; fax: +81-75-595-4796.
E-mail address: yo-kita@mb.kyoto-phu.ac.jp (Y. Kitamura).

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may play a role in the resolution of inflammation (Gilroy et al., 1999). Recent studies suggest that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 is an activator of a transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ) (Forman et al., 1995; Kliewer et al., 1995), and that PPARγ expression is induced by treatment with interleukin-4 in macrophages (Huang et al., 1999).

It is well known that inflammatory insult induces NF-κB activation and the expression of inducible nitric oxide (NO) synthase and cyclooxygenase-2, which catalyze the synthesis of pro-inflammatory NO and prostaglandins (Appleton et al., 1996). On the other hand, heme oxygenase-1 may modulate the inflammatory response (Willis et al., 1996; Kitamura et al., 1998), since biliverdin and bilirubin, which are produced by the activation of heme oxygenase, may act as physiological antioxidants and potent scavengers of oxygen radicals (Stocker et al., 1987; Llesuy and Tomaro, 1994). Previously, we determined the effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 on the expression of inducible NO synthase, cyclooxygenase-2 and heme oxygenase-1 in mixed glial and pure microglial cells from rat brains (Kitamura et al., 1999a,b, 2000). In the present study, we examined the effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 in glial cells from the wild-type and Toll-like receptor-4-mutant mouse brains.

2. Materials and methods

2.1. Materials

15-Deoxy-$\Delta^{12,14}$ prostaglandin J_2 was from Cayman Chemical (Ann Arbor, MI), rat interleukin-4 was from Life Technologies (Rockville, MD), lipopolysaccharide (*E. coli* Serotype 055:B5) was from Sigma (St. Louis, MO). Primary antibodies included mouse monoclonal antibodies against cyclooxygenase-2 and heat-shock protein-70 (HSP70) (Transduction, Lexington, KY), and heme oxygenase-1 (StressGen, Victoria, Canada); rabbit polyclonal antibodies against inducible NO synthase and heat-shock factor-1 (HSF1) (Affinity BioReagents, Golden, CO), and α-subunit of NF-κB inhibitor (IκB-α) (New England BioLabs, Beverly, MA); goat polyclonal anti-interferon-inducible p202 (IFI-202) antibody (Santa Cruz Biotechnology, Santa Cruz, CA). An enhanced chemiluminescent detection kit (ECL kit) from Amersham Pharmacia Biotech (Buckinghamshire, England) and a Bradford protein assay kit from BioRad Laboratories (Hercules, CA) were used.

2.2. Culture of glial cells and cell treatment

C3H/HeJ (Toll-like receptor-4-mutant) and C3H/HeN (wild-type) mice were purchased from SLC (Shizuoka, Japan). Glial cells (mixture of astrocytes and microglia) were cultured from whole brains of newborn mice and allowed to grow to confluency (10–14 days) in Dulbecco's modified Eagle's medium and 10% fetal calf serum with 50 μg/ml of penicillin and 100 μg/ml of streptomycin. Mixed glial cells were kept at 37°C in 5% CO_2/95% air and further cultured for 7–10 days in six-well plates and 100-mm dishes. Glial cells were pretreated for 48 h with vehicle or interleukin-4 (at 10 ng/ml), and further treated for 30 min with vehicle or 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (at 3 μM). After these treatments, glial cells were incubated with vehicle or lipopolysaccharide (at 10 μg/ml) for 24 h or 0–60 min. Subsequently, cell lysates or cytosolic fractions were prepared.

2.3. Immunoblot assay

Treated cell lysates or cytosolic fractions were dissolved in Laemmli's sample buffer, and equal protein amounts were subjected to immunoblotting using antibodies against inducible NO synthase (diluted 1:2000), cyclooxygenase-2 (1:500), heme oxygenase-1 (1:2000), IκB-α (1:1000), HSFI (1:3000), HSP70 (1:1000) and IFI-202 (1:500). For quantitative analysis, the bands of these proteins on radiographic films were scanned with a CCD color scanner (DuoScan, AGFA), and then analyzed. Results of the densitometric analysis of immunoblots are given as mean ± standard error of mean (S.E.M.). The statistical significance of differences was determined by an analysis of variance (ANOVA). Further statistical analysis for post hoc comparisons was done using the Bonferroni/Dunn test.

3. Results

3.1. Inhibitory effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 on lipopolysaccharide-induced expression of inducible NO synthase and cyclooxygenase-2 in Toll-like receptor-4-mutant and wild-type glial cells

We previously found that PPARγ mRNA was expressed by treatment with 10 ng/ml of interleukin-4 for over 24 h (Kitamura et al., 2000), and that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 at 3 μM inhibited the lipopolysaccharide-induced expression of inducible NO synthase and cyclooxgenase-2 (Kitamura et al., 1999a,b) in rat glial cells. In this study, therefore, we pretreated the Toll-like receptor-4-mutant and wild-type mouse glial cells for 48 h with vehicle or 10 ng/ml of interleukin-4, and then further treated them for 30 min with vehicle or 3 μM 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2. Subsequently, these treated cells were stimulated by 10 μg/ml of lipopolysaccharide.

A 130-kDa protein of inducible NO synthase was slightly detected in the control treatment in the wild-type and Toll-like receptor-4-mutant glial cells, but the level of inducible NO synthase in cells pretreated with interleukin-4

was undetectable in both glial cells (Fig. 1A). In contrast, lipopolysaccharide induced marked expression of inducible NO synthase, but this expression in the Toll-like receptor-4-mutant glial cells has significantly lower level (about half) than that in the wild-type cells. In addition, the lipopolysaccharide-induced expression of inducible NO synthase was significantly inhibited by treatment with 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 alone or inteleukin-4 alone. Treatment with both interleukin-4 and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 completely inhibited the lipopolysaccharide-induced expression of inducible NO synthase in the wild-type and Toll-like receptor-4-mutant glial cells.

Similarly, the lipopolysaccharide-induced expression of 70-kDa protein of cyclooxygenase-2 in the Toll-like receptor-4-mutant glial cells has significantly lower level than that in the wild-type cells (Fig. 1B). However, the lipopolysaccharide-induced expression of cyclooxygenase-2 was markedly lower level (about quarter) than that of inducible NO synthase (about half) in the mutant cells compared to those in the wild-type cells. Therefore, the inhibition of cyclooxygenase-2 expression by treatment with 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 alone or interleukin-4 alone might be not observed. Treatment with both 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 almost completely inhibited the lipopolysaccharide-induced expression of cyclooxygenase-2 in both mutant and wild-type glial cells (Fig. 1B).

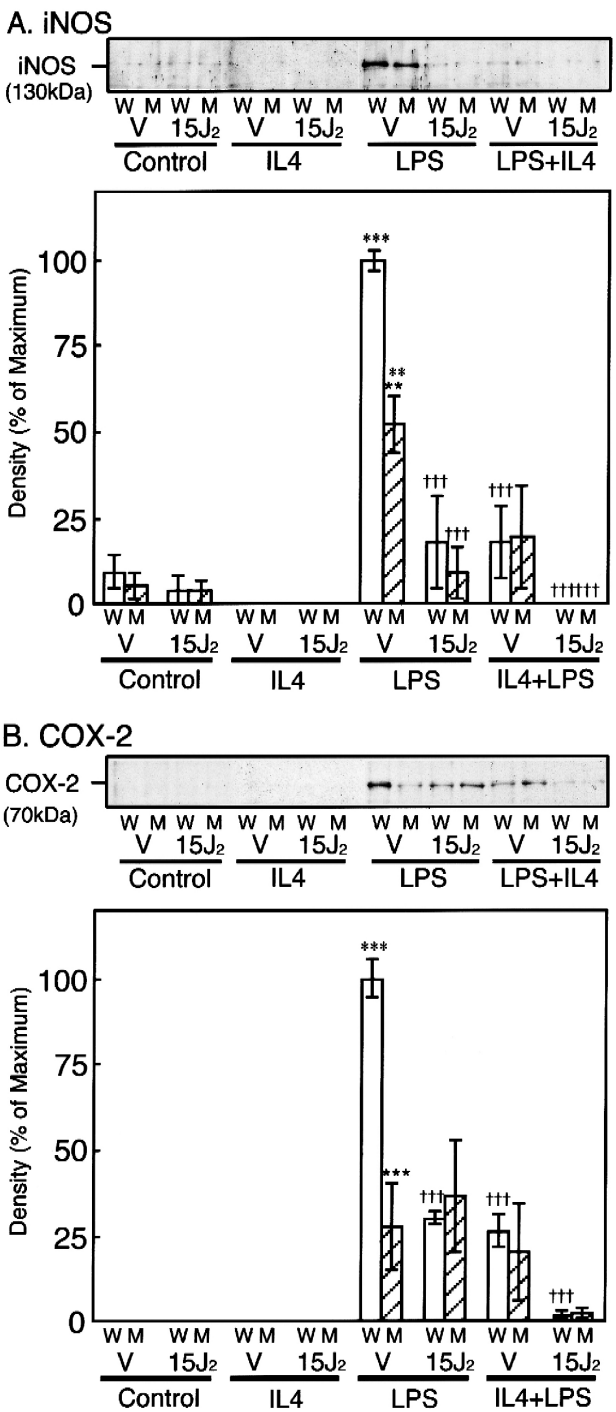

Fig. 1. Inhibitory effects of interleukin-4 and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 on expression of inducible NO synthase and cyclooxygenase-2 in wild-type and Toll-like receptor-4-mutant glial cells. Glial cells prepared from newborn C3H/HeN (wild-type, W) and C3H/HeJ (Toll-like receptor-4-mutant, M) mouse brains were pretreated with vehicle (control), 10 ng/ml of interleukin-4 for 48 h (IL4), or 3 μM 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 for 30 min ($15J_2$). Subsequently, these treated glial cells were further incubated for 24 h with vehicle or 10 μg/ml of lipopolysaccharide (LPS), and then cells were scraped and lysed. Each sample (10 μg protein/lane) was then subjected to immunoblot analysis (upper panel) of antibodies against inducible NO synthase (iNOS, A) and cyclooxygenase-2 (COX-2, B), and then the protein bands of 130-kDa inducible NO synthase and 70-kDa cyclooxygenase-2 were assessed (lower panel). The density of the protein band in the vehicle of lipopolysaccharide-treatment in the wild-type cells was taken as 100%. Each value is the mean ± S.E.M. of three determinations. The F values for inducible NO synthase and cyclooxygenase-2 levels by ANOVA were $F(15,32) = 16.069$ ($P < 0.0001$) and 16.132 ($P < 0.0001$), respectively. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the vehicle in the control-treatment. ††† $P < 0.001$ vs. the vehicle in lipopolysaccharide-treatment. ★★ $P < 0.01$, ★★★ $P < 0.001$ vs. the corresponding vehicle in the wild-type cells (post hoc Bonferroni/Dunn test).

3.2. Induction of heme oxygenase-1 expression by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2

A 32-kDa protein of heme oxygenase-1 was slightly expressed, and this level was similar to that in the control treatment in both wild-type and Toll-like receptor-4-mutant glial cells (Fig. 2). Although lipopolysaccharide did not induce expression of heme oxygenase-1, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 markedly induced expression of heme oxygenase-1 (Fig. 2). In cells pretreated with interleukin-4, the level of heme oxygenase-1 was slightly greater than that in the control-pretreatment, but this difference was not significant. In addition, the 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2-induced expression of heme oxygenase-1 was not enhanced by pretreatment with interleukin-4 and also did not change in the mutant and wild-type glial cells. Thus,

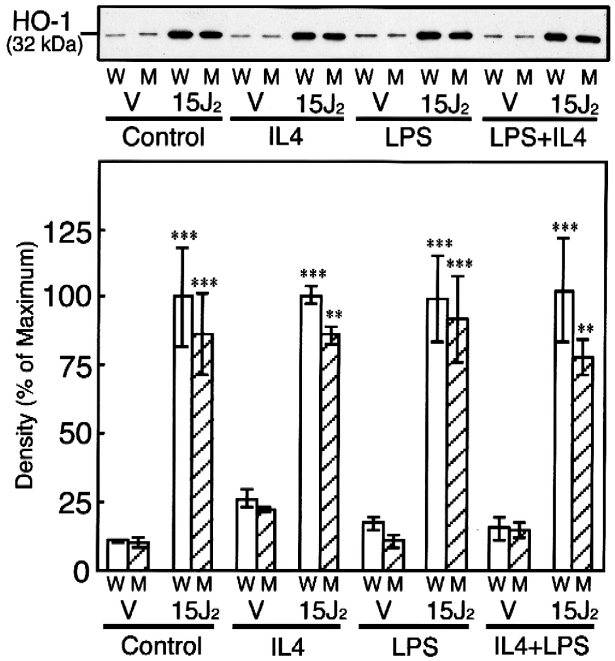

Fig. 2. Expression of heme oxygenase-1 induced by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 in wild-type and Toll-like receptor-4-mutant glial cells. Glial cultures were prepared and treated as described in Fig. 1. Each sample was subjected to immunoblot analysis (upper panel) of anti-heme oxygenase-1 antibody, and the protein band of 32-kDa heme oxygenase-1 (HO-1) was assessed (lower panel). The density of the protein band of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 ($15J_2$) for control-treatment in the wild-type cells was taken as 100%. Each value is the mean ± S.E.M. of three determinations. The F value by ANOVA was $F(15,32) = 18.072$ ($P < 0.0001$). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the corresponding vehicle in each group (post hoc Bonferroni/Dunn test).

marked expression of heme oxygenase-1 was induced only by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (Fig. 2).

3.3. Changes in IκB-α, HSF1, HSP70 and IFI-202 proteins in wild-type and mutant glial cells

We further examined the effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 on the regulation of transcription factors. Cytosolic IκB-α protein was degraded by an addition of lipopolysaccharide after 15 min in both wild-type and Toll-like receptor-4-mutant glial cells (Fig. 3A). The IκB-α protein level in the mutant glial cells was slightly lower than that in the wild-type cells, but this difference was not significant. At 60 min after the addition of lipopolysaccharide, IκB-α protein was significantly degraded in both wild-type and mutant cells (Fig. 3B). However, the lipopolysaccharide-induced degradation of IκB-α was not markedly inhibited by treatment with interleukin-4 for 48 h, or 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 for 30 min.

It is well known that the activation of HSF1 induces HSP70 expression (Massa et al., 1996). Therefore, we examined protein levels of 83-kDa HSF1 and 70-kDa HSP70 under the same treatment condition as in Figs. 1 and 2. Protein levels of HSF1 and HSP70 were slightly increased by treatment with interleukin-4 and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2, but those increases were not significant (Table 1). On the other hand, lipopolysaccharide significantly enhanced HSF1 and HSP70 levels, but such enhancements were not significantly inhibited by either 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 or interleukin-4 in the

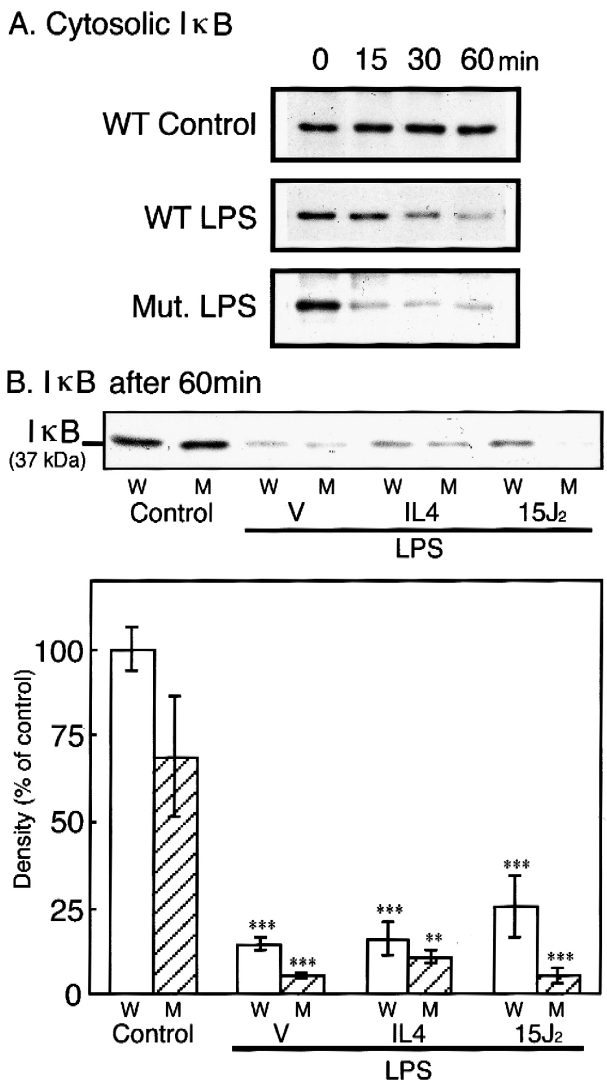

Fig. 3. Neither interleukin-4 nor 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 significantly affected lipopolysaccharide-induced degradation of IκB-α in either wild-type or Toll-like receptor-4-mutant glial cells. After wild-type (WT, W) and Toll-like receptor-4-mutant (Mut., M) glial cells were pretreated with vehicle (V), 10 ng/ml of interleukin-4 for 48 h (IL4) or 3 μM 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 for 30 min ($15J_2$), glial cells were further treated for 0–60 min (A) or 60 min (B) with vehicle (control) or 10 μg/ml of lipopolysaccharide (LPS). After these treatments, cells were scraped and cytosolic fractions were prepared. Each sample was then subjected to immunoblot analysis of anti-IκB-α antibody, and the protein band of 37-kDa IκB-α was assessed. The density of the protein band in the vehicle control in the wild-type cells was taken as 100%. Each value is the mean ± S.E.M. of three determinations. The F value by ANOVA (in B) was $F(7,16) = 21.315$ ($P < 0.0001$). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the corresponding vehicle (control) (post hoc Bonferroni/Dunn test).

Table 1
Changes in levels of HSF1, HSP70 and IFI-202 in wild-type and Toll-like receptor-4-mutant glial cells

Treatment and cells		Percent of maximum		
		HSF1	HSP70	IFI-202
A. Control treatment				
Vehicle	Wild-type	39 ± 4	38 ± 6	90 ± 8
	TLR4-mutant	37 ± 5	43 ± 9	93 ± 3
15d-PGJ_2 (3 μM)	Wild-type	71 ± 7	56 ± 7	94 ± 6
	TLR4-mutant	62 ± 4	65 ± 8	89 ± 9
B. Pretreatment of IL-4 (10 ng / ml, 48 h)				
Vehicle	Wild-type	60 ± 5	56 ± 12	100 ± 8
	TLR4-mutant	28 ± 4	57 ± 2	100 ± 8
15d-PGJ_2 (3 μM)	Wild-type	64 ± 15	54 ± 11	100 ± 3
	TLR4-mutant	51 ± 12	56 ± 17	92 ± 5
C. LPS treatment (10 μg / ml)				
Vehicle	Wild-type	100 ± 12***	100 ± 12**	100 ± 8
	TLR4-mutant	69 ± 21	100 ± 10**	93 ± 13
15d-PGJ_2 (3 μM)	Wild-type	80 ± 6	97 ± 11**	85 ± 9
	TLR4-mutant	46 ± 9	85 ± 3**	93 ± 14
D. IL-4 pretreatment (10 ng / ml, 48 h) plus LPS treatment (10 μg / ml)				
Vehicle	Wild-type	59 ± 3	73 ± 10	94 ± 6
	TLR4-mutant	38 ± 4	79 ± 11	98 ± 7
15d-PGJ_2 (3 μM)	Wild-type	51 ± 2	71 ± 6	98 ± 10
	TLR4-mutant	42 ± 6	61 ± 9	76 ± 9

TLR4 = Toll-like receptor-4; 15d-PGJ_2 = 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2; IL-4 = interleukin-4; LPS = lipopolysaccharide.
Glial cultures were prepared and treated as described in Fig. 1. Protein bands for 83-kDa HSF1, 70-kDa HSP70 and 52-kDa IFI-202 were assessed. The density of the protein band in the vehicle of lipopolysaccharide-treatment in the wild-type cells was taken as 100%. Each value is the mean ± S.E.M. of three determinations. The F values for the HSF1, HSP70 and IFI-202 levels by ANOVA were $F(15,32) = 4.098$ ($P = 0.0004$), 4.228 ($P = 0.0003$) and 0.720 ($P = 0.7469$, N.S.), respectively.
* $P < 0.05$ vs. the vehicle in the control-treatment (post hoc Bonferroni/Dunn test).
** $P < 0.01$ vs. the vehicle in the control-treatment (post hoc Bonferroni/Dunn test).

wild-type or mutant glial cells. Further, we measured 52-kDa protein of IFI-202 in the same cultures. However, IFI-202 protein levels were not changed by either treatments or mutant cells (Table 1).

4. Discussion

Recent studies have suggested that Toll-like receptor-4 and -2 are receptors for lipopolysaccharide and activate NF-κB signaling (Kirschning et al., 1998; Yang et al., 1998; Hoshino et al., 1999; Takeuchi et al., 1999). Takeuchi et al. (1999) reported that Toll-like receptor-4 and -2 recognized lipopolysaccharide (as well as Gram-negative bacterial cell wall components) and peptidoglycan (as well as Gram-positive bacterial cell wall components), respectively. However, Kirschning et al. (1998) obtained opposite results. On the other hand, a missense mutation in the cytoplasmic domain of the Toll-like receptor-4 gene which results in a substitution of proline at amino acid position 712 by histidine (P712H) was identified in C3H/HeJ mouse which exhibits lipopolysaccharide-hyporesponsiveness (Poltorak et al., 1998; Qureshi et al., 1999; Hoshino et al., 1999). In the present study, expression levels of inducible NO synthase and cyclooxygenase-2 induced by lipopolysaccharide were significantly lower in the Toll-like receptor-4-mutant glial cells (from C3H/HeJ mouse brains) than those in the wild-type cells (from C3H/HeN mice), but those induction were detected in the Toll-like receptor-4-mutant glial cells. In contrast, the lipopolysaccharide-induced degradation of IκB-α was similar between the Toll-like receptor-4-mutant and wild-type glial cells. Thus, the lipopolysaccharide-induced responses in glial cells were partially Toll-like receptor-4-dependent manner. However, Toll-like receptor-2 also may participate in the response to lipopolysaccharide in mouse glial cells.

Previous studies have suggested that anti-inflammatory cyclopentenone prostaglandins, such as prostaglandin A_1, prostaglandin A_2 and Δ^{12}-prostaglandin J_2, activated HSFI and induced HSP70 (Amici et al., 1992; Holbrook et al., 1992; Koizumi et al., 1993; Rossi et al., 1997). Recent studies have suggested that prostaglandin D_2 and its metabolites such as prostaglandin J_2, Δ^{12}-prostaglandin J_2 and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 activated PPARγ (Forman et al., 1995; Kliewer et al., 1995), and that PPARγ inhibited the transcriptional activities of NF-κB, AP-1 and GAS (Ricote et al., 1998). However, more recent papers have indicated that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2, prostaglandin A_1 and prostaglandin A_2 induced inhibition of the transcriptional activity of NF-κB in a PPARγ-independent manner (Petrova et al., 1999; Rossi et al., 2000). Rossi et al. (2000) showed that relatively low concentrations (less than 10 μM) of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 inhibited the DNA-binding activity of NF-κB, compared to those which inhibited IκB kinase (IKK) activity (over 10 μM), but they concluded that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 inhibited IKK activity and then inhibited NF-κB signaling. On the other hand, Petrova et al. (1999) indicated that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 at less than 5 μM did not inhibit the nuclear translocation and DNA-binding activity of NF-κB, suggesting that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 affected distinct mechanisms and probably interfered directly with NF-κB transcriptional activity. Concerning the anti-inflammatory cytokine interleukin-4, a previous study suggested that interleukin-4 activated Janus kinases (JAK1 and JAK3) and the signal transducer and activator of transcription-6 (STAT6, also known as IL4-STAT), and then activated transcriptional activity (Takeda et al., 1997). Recent studies have suggested that interleukin-4 inhibited lipopolysaccharide-induced activation of the mitogen-activated protein (MAP) kinase ERK2 (Niiro et al., 1998), and that pretreatment with interleukin-4 markedly induced

PPARγ expression (Huang et al., 1999). In addition, recent related studies have suggested that (1) HSF1 acted as a transcriptional repressor at interleukin-1β promoter and then inhibited the lipopolysaccharide-induced production of interleukin-1β (Cahill et al., 1996), (2) HSP70 inhibited the nuclear translocation of NF-κB and then inhibited the lipopolysaccharide-induced expression of inducible NO synthase (Feinstein et al., 1996), (3) PPARs were associated with the HSP70 family (Huang et al., 1994), and (4) IFI-202 protein inhibited the transcriptional activities of NF-κB and AP-1 (Min et al., 1996). Thus, the regulatory mechanisms of transcriptional activities by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 are very confusing.

We have previously found that the PPARγ mRNA, which was undetectable in the control rat glial cells, were markedly expressed by interleukin-4 but not by lipopolysaccharide (Kitamura et al., 2000). In the present study, the lipopolysaccharide-induced expression of inducible NO synthase and cyclooxygenase-2 was inhibited by treatment with 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 alone and interleukin-4 alone, and completely inhibited by both treatments in the wild-type mouse glial cells. Thus, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2-induced inhibition on the lipopolysaccharide-induced responses may be mediated by PPARγ-independent and -dependent pathways in glial cells (Fig. 4). However, the lipopolysaccharide-induced degradation of IκB-α was inhibited by neither 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 nor interleukin-4, and the expression of neither inducible NO synthase nor cyclooxygenase-2 was correlated with the expression of HSF1, HSP70 or IFI-202. These results suggest that 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and PPARγ directly may inhibit the transcriptional activity of NF-κB (Fig. 4).

On the other hand, neither interleukin-4 nor lipopolysaccharide influenced an expression of heme oxygenase-1 in the wild-type or mutant glial cells. This expression was not correlated with HSF1, HSP70 or IFI-202. Heme oxygenase-1 expression was induced only by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (Fig. 4). Thus, heme oxygenase-1 expression is not regulated by PPARγ, NF-κB, HSF1, HSP70 and IFI-202. The expression of heme oxygenase-1 may be induced directly by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2, or mediated through 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2-induced activation of unknown transcription factors. Thus, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 may each regulate transcriptional activities, but the details of these mechanisms will require further study. We previously found that several nonsteroidal anti-inflammatory drugs

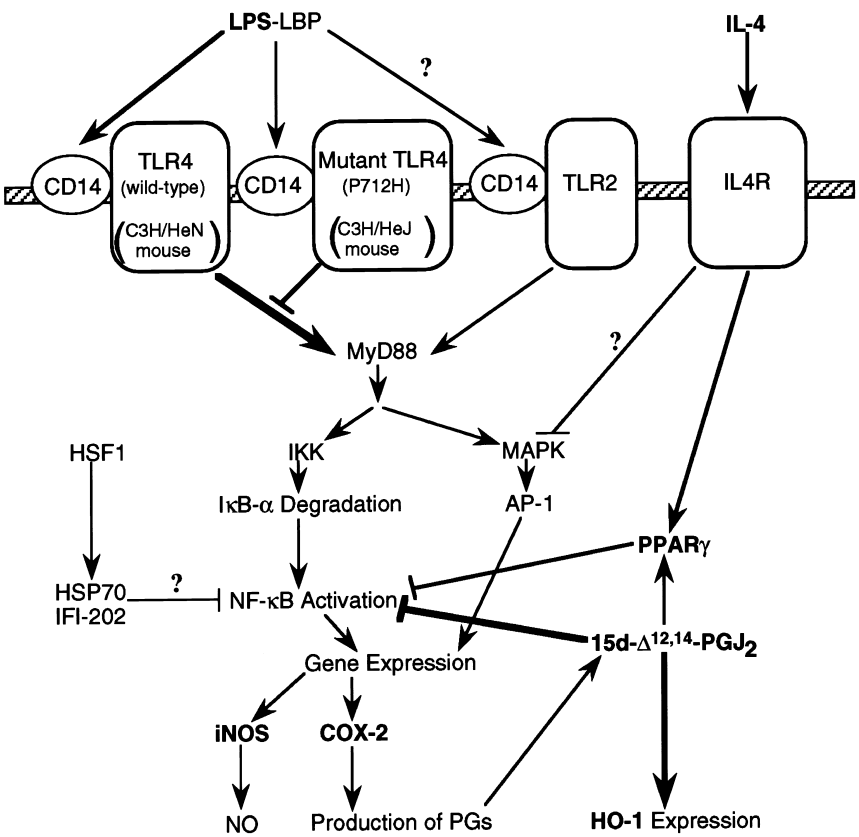

Fig. 4. Hypothetical model of signaling interactions between lipopolysaccharide, interleukin-4 and 15-deoxy-$\Delta^{12,14}$ prostagladin J_2 in mouse glial cells. LPS = lipopolysaccharide, LBP = lipopolysaccharide-binding protein; TLR4/2 = Toll-like receptor-4/2; P712H = a substitution of proline at amino acid position 712 by histidine in TLR4; IL-4 = interleukin-4; IL4R = interleukin-4 receptor; IKK = IκB kinase; iNOS = inducible NO synthase; COX-2 = cyclooxygenase-2; HO-1 = heme oxygenase-1; PPARγ = peroxisome proliferator-activated receptor-γ, PGs = prostaglandins; 15 d-$\Delta^{12,14}$-PGJ_2 = 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2.

(NSAIDs) inhibited the lipopolysaccharide-induced expression of inducible NO synthase and cyclooxygenase-2 but induced an expression of heme oxygenase-1 in rat glial cells (Kitamura et al., 1999a,b), and that the PPARγ protein level was increased in Alzheimer's disease brains (Kitamura et al., 1999a). More recently, it has been reported that β-amyloid-induced neuronal death mediated by the activation of microglia was inhibited by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and NSAIDs (Combs et al., 2000). Thus, several kinds of anti-inflammatory substances such as cytokines, cyclopentenone prostaglandins and NSAIDs inhibit the activation of glial cells and may prevent neuronal death in Alzheimer's disease.

In conclusion, the expression levels of inducible NO synthase and cyclooxygenase-2 induced by lipopolysaccharide in the Toll-like receptor-4-mutant glial cells were significantly lower than those in the wild-type cells. In addition, treatment with 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 inhibited the lipopolysaccharide-induced expression of inducible NO synthase and cyclooxygenase-2. In contrast, heme oxygenase-1 was induced by 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2, but was not affected by other treatments. The lipopolysaccharide-induced degradation of IκB-α was inhibited by neither 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 nor interleukin-4. In addition, protein level of HSF1, HSF70 or IFI-202 did not correlate with the expression of either inducible NO synthase, cyclooxygenase-2 or heme oxygenase-1. These results suggest that (1) the response to lipopolysaccharide is partially dependent on the Toll-like receptor-4 in mouse glial cells, (2) 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 differently regulate the expression of inducible NO synthase and cyclooxygenase-2, and heme oxygenase-1, and (3) 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 may directly inhibit the transcriptional activity of NF-κB in a PPARγ-dependent and/or-independent manner. Thus, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 and interleukin-4 may regulate transcriptional activities for anti-inflammation in glial cells.

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