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Strain differences regarding susceptibility to ursolic acid-induced interleukin-1β release in murine macrophages

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ABSTRACT

Interleukin (IL)-1β is a proinflammatory cytokine responsible for the onset of a broad range of diseases, such as inflammatory bowel disease and rheumatoid arthritis. We have recently found that aggregated ursolic acid (UA), a triterpene carboxylic acid, is recognized by CD36 for generating reactive oxygen species (ROS) via NADPH oxidase (NOX) activation, thereby releasing IL-1β protein from murine peritoneal macrophages (pMϕ) in female ICR mice. In the present study, we investigated the ability of UA for inducing IL-1β production in pMϕ from 4 different strains of female mice (C57BL/6J, C3H/He, DDY, and ICR), as well as an established macrophage line (RAW264.7 cells). The levels of IL-1β released from UA-treated pMϕ of C57BL/6J and DDY mice were significantly lower than from those of ICR mice, whereas IL-1β was not released from the pMϕ of C3H/He mice or RAW264.7 cells. Of paramount importance, CD36 as well as the NOX components gp91^{phox} and p47^{phox} (C3H/He mice) and gp91^{phox} (RAW264.7 cells) were scarcely detected. In addition, the different susceptibilities to UA-induced IL-1β release were suggested to be correlated with the amount of superoxide anion (O_2^-) generated from the 5 different types of Mϕ. Notably, intracellular, but not extracellular, O_2^- generation was indicated to play a major role in UA-induced IL-1β release. Together, our results indicate that the UA-induced IL-1β release was strain-dependent, and the expression status of CD36 and gp91^{phox} is strongly associated with inducibility.

Introduction

Ursolic acid (UA; 3β-hydroxy-12-urs-12-en-28-oic acid), a pentacyclic triterpene carboxylic acid, is found in various medicinal plants and present in the human diet (Liu, 1995; Tokuda et al., 1986). UA has a number of important biological and biochemical functions (Hollosy et al., 2001; Manez et al., 1997; Najid et al., 1992; Tokuda et al., 1986), including attenuation of the expression of inducible nitric oxide synthase and cyclooxygenase-2 through nuclear factor-kappaB (NF-κB) repression in activated mouse macrophages (Mϕ) (Suh et al., 1998). In addition, You et al. recently reported an interesting finding that UA activates NF-κB for inducing nitric oxide and tumor necrosis factor-α production in resting RAW264.7 mouse Mϕ (You et al., 2001). Those findings imply that UA has contrasting activities and its effects on NF-κB activities are dependent on the biological status of Mϕ. Further, we previously demonstrated that UA promotes the release of macrophage migration inhibitory factor (MIF) via extracellular signal-regulated kinase (ERK) 2 activation in the same cell line (Ikeda et al., 2005), and also found that UA bound to CD36, a scavenger receptor (SR), and then generated intracellular reactive oxygen species (ROS) via NADPH oxidase (NOX) activation, thereby releasing interleukin (IL)-1β protein from murine peritoneal macrophages (pMϕ) of ICR mice (Ikeda et al., 2007).

IL-1β, an anti-apoptotic and proinflammatory cytokine, is one of the most pronounced mediators of inflammatory reactions, and is primarily produced in activated monocytes and Mϕ (Dinarello, 1998). Enhanced production of IL-1β has been detected at both mRNA and protein levels in human inflammatory bowel disease (IBD) and dextran sulfate sodium (DSS)-induced colitis murine models, which have features similar to symptomatic and histological findings in humans (Cappello et al., 1992; Savendahl et al., 1997). In addition, our previous results suggested that IL-1β is involved in the development of DSS-induced colitis in mice, and targets IL-1β and IL-6, leading to the development and progression of IBD (Kwon et al., 2005a,b). Further, Mϕ-derived IL-1β has several functions, such as increasing production of eicosanoids, collagenase, and prostaglandin E2, in the chronic disease process of rheumatoid arthritis (RA) (Ridderstad et al., 1991). Thus, this cytokine is considered to be a primary component in the pathogenesis of colitis and RA (Cappello et al., 1992; Kwon et al., 2005a,b; Ridderstad et al., 1991; Savendahl et al., 1997).

It is well known that different strains of mice have different sensitivities to DSS. For example, Balb/c, C3H/HeJ, and C57BL/6J mice are relatively susceptible to DSS, while DBA/2J mice are virtually resistant (Mahler et al., 1998; Stevceva et al., 1999). Since IL-1β may play a critical role in the pathogenesis of colitis, these different sensitivities to DSS-induced colitis may be due to different levels of IL-

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1β released from Mϕ in each strain. On the other hand, we recently found that both UA and DSS induce IL-1β production in peritoneal Mϕ (pMϕ) of ICR mice through an SR-mediated mechanism (Ikeda et al., 2007; Kwon et al., 2007). Moreover, i.p. administration of UA induced IL-1β production in pMϕ, and IL-1β production and myeloperoxidase activity in colonic mucosa of ICR mice were increased (Ikeda et al., 2007), as well as DSS (Vowinkel et al., 2004). Therefore, it may be possible that the genetic background differences of the mice have varying effects on UA-induced IL-1β release.

In the present study, we attempted to determine if the levels of UA-induced IL-1β production in pMϕ differed in 4 mouse strains (C57BL/6J, C3H/He, DDY, and ICR), as well as an established line of mouse Mϕ (RAW264.7 cells), by profiling the mRNA expressions of 5 SRs, including CD36 and 6 NOX subunits. In addition, we investigated superoxide (O_2^-) generation in 5 lines of Mϕ treated with UA to evaluate whether O_2^- generation is related to the sensitivity of IL-1β release. Our previous (Ikeda et al., 2007) and present findings showed that strain differences in regards to susceptibility to UA-induced IL-1β release may be influenced by intracellular, but not extracellular, O_2^- generation via NOX activity, and are dependent on both the expression statuses of CD36 and gp91^{phox}.

Materials and methods

Mice

Specific pathogen-free 5-week-old female C57BL/6J, C3H/He, DDY, and ICR mice were purchased from Japan SLC (Shizuoka, Japan). On arrival, the animals were randomized and transferred to plastic cages containing sawdust bedding (5 mice per cage), which was changed every third day. They were given MF rodent chow (Oriental Yeast, Kyoto, Japan) and fresh tap water ad libitum, which was freshly changed twice a week, and handled according to the Guidelines for the Regulation of Animals, provided by the Experimentation Committee of Kyoto University. The mice were maintained in a controlled environment of 24±2 °C with a relative humidity of 60±5% and a 12-h light/dark cycle (lights on from 06:00 to 18:00). All mice were quarantined for 1 week before starting the experiments.

Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and TRIZOL® were obtained from Invitrogen (Carlsbad, CA). Lipopolysaccharide (LPS, Escherichia coli serotype 0127, B8) was purchased from Difco Labs (Detroit, MI) and UA came from Funakoshi (Tokyo, Japan). Oligonucleotide primers were synthesized by Proligo (Kyoto, Japan). Diphenyleneiodonium (DPI) came from Calbiochem (La Jolla, CA). A mouse IL-1β enzyme-linked immunosorbent assay kit was purchased from R&D Systems Inc. (Minneapolis, MN). All other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan), unless specified otherwise.

Cell culture

pMϕ monolayers were prepared as described previously (Kwon et al., 2002), with some modifications. Briefly, non-treated 6-week-old female mice were killed by cervical dislocation and 10 ml of ice-cold DMEM containing 10% FBS, L-glutamine (330 μg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml) was injected intraperitoneally. Medium containing peritoneal exudates cells (PEC) was then re-collected and kept on ice. The cell suspension was centrifuged at 800 ×g for 5 min and resuspended with DMEM. PEC (5×10^6 cells/ml/well) were seeded into culture plates and allowed to adhere for 24 h at 37 °C under a humidified atmosphere with 5% CO_2. After washing twice with phosphate-buffered saline (PBS), non-adherent cells were removed and the remaining monolayers were designated as pMϕ. Cell viability was 90% or more in all experiments, unless specified otherwise. Murine Mϕ, RAW264.7 cells, were obtained from American Type Culture Collection (Rockville, MD) and grown in DMEM supplemented with 10% FBS, L-glutamine (330 μg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37 °C under a humidified atmosphere with 5% CO_2.

Protein determination

Total protein concentrations in pMϕ and RAW264.7 cells were determined using a DC protein assay (Bio-Rad Laboratories, Hercules, CA) according to the protocol of the manufacturer (dilution factor=50), with γ-globulin used as the standard.

ELISA

ELISA was done as described previously (Ikeda et al., 2007, 2005). Briefly, pMϕ (1×10^6 cells/200 μl) and RAW264.7 cells (1×10^5 cells/200 μl) were treated with UA (4 or 20 μM) or LPS (100 ng/ml), which had been dissolved in dimethylsulfoxide (DMSO) (0.1% v/v, as a final concentration) or phosphate-buffered saline (PBS), respectively. Control cells were treated only with 0.1% (v/v) DMSO and PBS, which showed no significant effects on the assay systems in our preliminary examinations (data not shown). After incubation for 12 h, IL-1β in the supernatants (50 μl) was quantified using ELISA according to the protocols of the kit.

Reverse transcription-polymerase chain reaction (RT-PCR)

pMϕ (5×10^6 cells/ml) and RAW264.7 cells (1×10^6 cells/ml) were cultured for 24 h in the presence of media alone. Total cellular RNA was extracted from the cells using TRIZOL® reagent. RNA was precipitated

Table 1
List of primer sequences and product size for RT-PCR

Gene	Primer	Sequence (5′–3′)	Product size (bp)
CD36[a]	Forward	GTT TTA TCC TTA CAA TGA CA	420
	Reverse	GGA AAT GTG GAA GCG AAA TA	
SR-A[b]	Forward	GGG AGA CAG AGG GCT TAC TGG A	389
	Reverse	TTG TCC AAA GTG AGC TCT CTT G	
SR-BI[c]	Forward	TTT CAG CAG GAT CCA TCT GGT	469
	Reverse	AGT TCA TGG GGA TCC CAG TGA	
CD68[d]	Forward	TTG GGA ACT ACA CAC GTG GGC	67
	Reverse	CGG ATT TGA ATT TGG GCT TG	
CXCL16[e]	Forward	AGG CAA ATG TTT TTG GTG G	263
	Reverse	AAC CAG GGC AGT GTC GC	
TLR4[f]	Forward	GCA ATG TCT CTG GCA GGT GTA	406
	Reverse	CAA GGG ATA AGA ACG CTG AGA	
p22^{phox}[g]	Forward	TTC CTG TTG TCG GTG CCT GC	195
	Reverse	TTC TTT CGG ACC TCT GCG GG	
gp91^{phox}[h]	Forward	TCC AGT CTC CAA CAA TAC GGA TAT G	712
	Reverse	AGT CGG GAT TTC TGA CCG GTA T	
p40^{phox}[i]	Forward	TGT CTT CAT AGA AGT AGC ATC GTA GCC	469
	Reverse	CAA AGT CTA CAT GGG CGC AAA	
p47^{phox}[j]	Forward	GTG GAG AAG AGC GAG AGC GG	365
	Reverse	GGT GGA TGC TCT GTG CGT TG	
p67^{phox}[k]	Forward	CAC AAA GCC AAA CAA TAC GCG	165
	Reverse	CTA TCT GGG CAA GGC TAC GGT T	
Rac1[l]	Forward	CTG CCT GCT CAT CAG TTA CAC G	485
	Reverse	GGA CAG AGA ACC GCT CGG ATA	
GAPDH[m]	Forward	AGC CTT CTC CAT GGT GGT GAA GAC	496
	Reverse	CGG AGT CAA CGG ATT TGG TCG TAT	

[a]30 s of denaturation at 95 °C, 30 s of annealing at 50 °C, and 60 s of primer extension at 72 °C.
[b]30 s at 95 °C, 30 s at 56 °C, and 60 s at 72 °C.
[c]30 s at 94 °C, 30 s at 58 °C, and 60 s at 68 °C.
[d]15 s at 95 °C, and 60 s at 60 °C.
[e]30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C.
[f]45 s at 95 °C, 45 s at 61 °C, and 45 s at 72 °C.
[g, j]30 s at 94 °C, 45 s at 58 °C, and 60 s at 72 °C.
[h, i, k, l]30 s at 95 °C, 30 s at 58 °C, and 60 s at 72 °C.
[m]60 s at 94 °C, and 60 s at 60 °C.
[a, k]25, [b]23, [c, e, f]28, [d]32, [g, h, i, l]22, [j]24, and [m]19 cycles.