Enterohemolysin operon of Shiga toxin-producing *Escherichia coli*: a virulence function of inflammatory cytokine production from human monocytes

Ikue Taneike, Hui-Min Zhang, Noriko Wakisaka-Saito, Tatsuo Yamamoto*

Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University Graduate School of Medical and Dental Sciences, 757 Ichibanchou, Asahimachidori, Niigata, Japan

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Abstract Shiga toxin-producing *Escherichia coli* (STEC) is associated with hemolytic uremic syndrome (HUS). Although most clinical isolates of STEC produce hemolysin (called enterohemolysin), the precise role of enterohemolysin in the pathogenesis of STEC infections is unknown. Here we demonstrated that *E. coli* carrying the cloned enterohemolysin operon (*hlyC*, *A*, *B*, *D* genes) from an STEC human strain induced the production of interleukin-1β (IL-1β) through its mRNA expression but not tumor necrosis factor-α from human monocytes. No IL-1β release was observed with an enterohemolysin (HlyA)-negative, isogenic *E. coli* strain carrying a mutation in the *hlyA* gene. The data suggest that enterohemolysin, a pore-forming toxin, induces the production of IL-1β, which is one of serum risk markers for HUS.

Key words: Enterohemolysin operon; Hemolysis; Interleukin-1β; Human monocyte; mRNA expression; Shiga toxin-producing *Escherichia coli*

1. Introduction

Shiga toxin (Stx)-producing *Escherichia coli* (STEC; alternatively called enterohemorrhagic *E. coli*, EHEC) belonging to serotype O157:H7 was identified as an emerging bacterial pathogen of food-borne infections in the United States in 1982 [1]. In Japan, during 1996, explosive outbreaks occurred with more than 17 877 people infected including 12 fatalities [2]. STEC includes not only serotype O157:H7 (the most prevalent serotype), but other serotypes (e.g.) O26, O111, O128, and O145 have also been implicated in human infections [3,4].

Infection is initially associated with abdominal symptoms such as abdominal pain, water diarrhea, and hemorrhagic colitis and also with serious systemic disorders, such as hemolytic uremic syndrome (HUS), especially in young children [3–5]. STEC, in many cases, also encodes hemolysin (called enterohemolysin [6], or EHEC-hemolysin [7]) in addition to Stx [6]. In the case of the outbreak strains in Japan in 1996, more than 98% of STEC were positive for enterohemolysin [8].

Enterohemolysin and α-hemolysin (which is produced from approximately 50% of *E. coli* isolates from extraintestinal infections [9], or from porcine STEC strains [6]) are members of the RTX family, associated with the ATP-dependent HlyB/HlyD/TolC secretion system through which hemolysin (HlyA) is secreted across the bacterial membranes. Both the enterohemolysin and α-hemolysin operons consist of the *hlyC*, *hlyA*, *hlyB*, and *hlyD* genes [10–12].

Enterohemolysin and α-hemolysin are, however, genetically and immunologically distinct hemolysins. Enterohemolysin is a cell-associated hemolysin, in contrast to α-hemolysin which is released from bacterial cells [7]. In the case of enterohemolysin, hemolysis is observed on blood agar containing washed but not unwashed sheep erythrocytes, in sharp contrast to α-hemolysin, which is detected even on blood agar containing unwashed sheep erythrocytes [6].

In HUS, it has been shown that serum levels of proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are elevated [13–15]. Both IL-1β and TNF-α regulate the expression of the Stx receptor (a glycolipid globotriosylceramide, Gb3) on the endothelial cell membrane [16,17]. Bacterial lipopolysaccharide (LPS) and Stx stimulate the production of IL-1β and TNF-α from human monocytes [17,18]. However, whether enterohemolysin induces the production of proinflammatory cytokines has not yet been investigated.

In the present study, we investigated if *E. coli* carrying the cloned enterohemolysin genes elicited proinflammatory cytokines from human monocytes. For this, we constructed an enterohemolysin-negative (*hlyA*) derivative from the cloned enterohemolysin genes, and evidence was provided that enterohemolysin produces IL-1β, but not TNF-α, from human monocytes.

2. Materials and methods

2.1. Bacterial strains and plasmid

STEC human strain F60 (serotype O128:H12) was isolated on occasions of outbreaks of STEC infections in Japan in 1996. It produces both Stx1 and Stx2, and manifests enterohemolysin activities in a great quantity, compared with other STEC human strains isolated from Japan [8]. *E. coli* HB101 is a hybrid between *E. coli* K-12 and *E. coli* B, lacks restriction ability, and was used as a host of recombinant plasmids.

*Corresponding author. Fax: (81)-25-227 0762.
E-mail address: tatsuoy@med.niigata-u.ac.jp (T. Yamamoto).

Abbreviations: STEC, Shiga toxin-producing *Escherichia coli*; Hly, hemolysin; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; HUS, hemolytic uremic syndrome; RT-PCR, reverse transcription-polymerase chain reaction; Stx, Shiga toxin

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The recombinant plasmid pACH1 (24.5 kbp in size) carried the entire enterohemolysin operon (*hlyC*, *hlyA*, *hlyB*, *hlyD* genes) which originated in the enterohemolysin-positive plasmid pO128-60 (120 kbp in size) of STEC strain F60 [8]. The GenBank accession number for the gene sequence of this enterohemolysin operon is AB032930. The determined sequence covers the 7 869-nucleotide sequence of the enterohemolysin operon region, including the entire enterohemolysin genes (*hlyC*, *hlyA*, *hlyB*, *hlyD*) (7 127 bp; Fig. 1), the 477-nucleotide-upstream region from the *hlyC* gene, and the 265-nucleotide-downstream region to the *hlyD* gene.

2.2. Construction of a mutant hlyA gene

For mutagenesis of the *hlyA* gene, the pACH1 plasmid DNA carrying the entire enterohemolysin operon was cut with *Spl*I, whose site was located 81 bp downstream to the initiation codon ATG within *hlyA*. The generated single stranded ends were polymerized and the two resultant double-stranded ends of the linear pACH1 DNA were ligated using a DNA blunting kit (Takara Shuzo Co., Shiga, Japan). The plasmid (pACHT1) thus obtained was subjected to sequence analysis for the whole mutant enterohemolysin operon region, as described above. pACHT1 was used in experiments on hemolysis and cytokine production.

2.3. Media and bacterial growth

For bacterial growth, tryptic soy agar (Difco Laboratories, Detroit, MI, USA) was used as solid media. We used antibiotics (20 μg of chloramphenicol per ml) to maintain the recombinant plasmids during cultivation.

Luria–Bertani (LB) broth (Difco) was used as liquid media which was inoculated and incubated at 37°C for 12–18 h with agitation. In some experiments, RPMI 1640 medium (ICN Biomedicals, Aurora, OH, USA) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY, USA) was also employed. When bacteria were grown in the liquid media, antibiotics were not added. In the case of *E. coli* HB101 carrying pACH1 or pACHT1, after incubation, a portion of the liquid culture was streaked on tryptic soy agar and the colonies developed were examined for the presence of the recombinant plasmid (chloramphenicol-resistant) with the replica plating method. All of the colonies examined were resistant to chloramphenicol (20 μg/ml).

2.4. Hemolysis assay

For hemolysis assay on agar plates, bacteria were streaked on tryptic soy agar supplemented with 5% fresh, washed, defibrinated sheep erythrocytes and 10 mM $CaCl_2$ [6]. The inoculated plates were then incubated for 3 to 24 h at 37°C. Hemolysis occurring around the streaked bacterial colonies was examined with the naked eye.

Hemolysis assay using liquid cultures was performed essentially by the method previously described [19]. Bacterial cells were added at 1×10^7 CFU/ml to tryptic soy broth (Difco) supplemented with 5% sheep blood cells and 10 mM $CaCl_2$, and incubated at 37°C for 6 h. The mixture was then centrifuged, and the quantity of hemoglobin released from lysed blood cells was measured in the supernatant by spectrophotometry (wavelength, 540 nm). The same quantity of blood cells was added to distilled water, and the quantity of hemoglobin released was regarded as the total hemolysis (A540 of total), and the percent lysis with each sample was calculated by the equation, percent lysis = 100×(A540 of sample−A540 of background)/(A540 of total−A540 of background).

2.5. Cytokine assay

For cytokine induction experiments, human mononuclear cells were prepared from peripheral blood using Ficol-Hypaque (Gibco BRL). Adherent monocytes were obtained after incubation in 24-well culture plates (A/S Nunc, Roskilde, Denmark) for 60 min at 37°C in a CO_2 incubator with subsequent washing under high pressure [20]. For treatment of monocytes with stimuli, bacteria with or without the hemolytic phenotype were first grown in LB broth, suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum and grown to the log-phase, after which culture supernatants were prepared. The culture supernatants were incubated with the monocytes (approximately 10^5 cells per ml) for 2–6 h at 37°C in a CO_2 incubator. IL-1β and TNF-α in the supernatants were assayed with IL-1β and TNF-α kits (Genzyme, Cambridge, MA, USA) in accordance with the manufacturer's instructions.

2.6. Cytokine mRNA assay

To detect mRNA, after incubation, total cellular RNA of the monocytes was extracted using acid guanidinium thiocyanate/phenol/chloroform. The amount of RNA was assessed with a RiboGreen RNA quantitation kit (Molecular Probes, Inc., The Netherlands). A reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously [21]. Primers for IL-1β (5′-AAACAGATGAAGTGCTCCTTCCAGG and 5′-TGGAGAACACCACTTGTTGCTCCA, generating a 391-bp product), TNF-α (5′-GGACGTGGAGCTGGCCGAGGAG and 5′-CACCAGCTGGTTATCTCTCAGCTC, generating a 352-bp product), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5′-GGGAGCCAAAAGGGTCATCATCTC and 5′-CCATGCCAGTGAGCTTCCCGTTC, generating a 353-bp product) were kindly provided by Dr. Yasuhiro Natori (International Medical Center of Japan, Tokyo, Japan) [22,23]. Negative controls were performed by omitting RNA from cDNA synthesis and specific PCR amplification. Cycling conditions were denaturation for 45 s at 94°C, annealing for 45 s at 55°C, and polymerization for 45 s at 72°C (35 cycles). Amplified PCR products were analyzed by gel electrophoresis with 2% agarose and stained with ethidium bromide. ϕX174 RF DNA/*Hae*III fragments (Life Technologies, Gaithersburg, MD, USA) were used as molecular size standards.

2.7. Data analysis

Data were presented as mean ± S.D. of triplicate experiments, and evaluated by Mann–Whitney's *U*-test.

3. Results

3.1. Construction of pACHT1 carrying the mutant hlyA gene

The enterohemolysin$^+$ recombinant plasmid pACH1 carried the unique *Spl*I site within the hlyA gene (Fig. 1). pACH1 DNA was cut with *Spl*I and filled in with DNA polymerase, and the plasmid pACHT1 carrying the mutant *hlyA* gene was constructed. The mutant *hlyA* gene carried the CGTACGTACG sequence (4-base GTAC insertion) instead of the CGTACG sequence (*Spl*I site), resulting in a frame shift from the 31st codon and a new stop codon at the position of the 42nd codon (Fig. 1). The deduced amino acid sequence of the mutant *hlyA* gene is only 41 amino acids long, in contrast to that of the intact *hlyA* gene (998 amino acids long). There were no other mutations within the enterohemolysin operon.

3.2. Hemolytic phenotype

Bacterial cells of a wild-type STEC strain F60 (from which the enterohemolysin operon was cloned), *E. coli* HB101, *E. coli* HB101 carrying pACH1, or *E. coli* HB101 carrying pACHT1 were streaked on blood agar plates, and the plates were incubated for 3–24 h at 37°C. When blood agar plates with washed sheep erythrocytes were employed, hemolysis by STEC strain 60 was observed 12–24 h after incubation at 37°C, as shown in Fig. 2A(1). Hemolysis by *E. coli* HB101 carrying pACH1 was much greater than that by STEC strain F60 (Fig. 2A(3)), and was visible as early as 3 h after incubation at 37°C. No detectable hemolysis occurred on blood agar plates not supplemented with $CaCl_2$ or made of unwashed sheep erythrocytes, as shown in Fig. 2B(1,3).

In the case of *E. coli* HB101 or *E. coli* HB101 carrying pACHT1, hemolysis was not observed even with washed sheep erythrocytes or even after prolonged incubation for 24 h at 37°C, as shown in Fig. 2A(2,4).

The hemolytic activities were also evaluated in liquid cultures (Fig. 2C). The hemolytic activity of *E. coli* HB101 carrying pACH1 was 2.1-fold greater, as compared with the he-

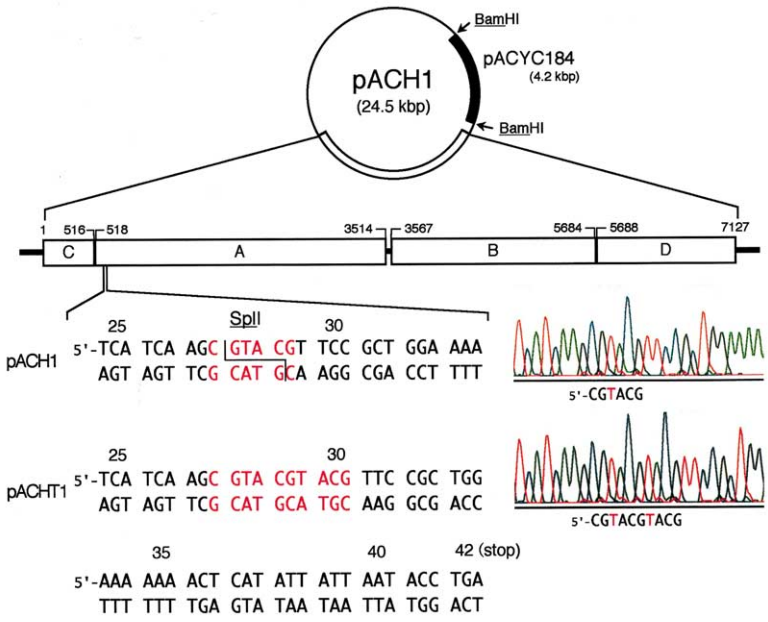

Fig. 1. A restriction endonuclease cleavage map of the recombinant plasmid pACH1 carrying the enterohemolysin operon and of the plasmid pACHT1 carrying the mutant *hlyA* gene. pACH1 consists of pACYC184 (a cloning vector, 4.2 kbp) and a *Bam*HI fragment (20.3 kbp) originating from the Hly^+ plasmid (pO128-60) of a human STEC strain. It exhibits an enterohemolysin phenotype and chloramphenicol resistance of pACYC184. C, A, B, and D in boxes represent the *hlyC*, *hlyA*, *hlyB*, and *hlyD* genes, respectively; the numbers above boxes represent DNA sizes (in bp). In the lower part of this figure, the nucleotide sequences surrounding the *Spl*I site in pACH1 and the *Spl*I-blunt-end-ligated site in the mutant plasmid (pACHT1) are shown. The *Spl*I site in pACH1 represents a unique site, located over the 28th codon position within the *hlyA* gene. pACHT1 was constructed by restriction and a subsequent fill-in reaction. The electropherogram on the right shows the sequencing data around the *Spl*I site in pACH1 and the corresponding site in pACHT1.

molytic activity of STEC strain F60 (Fig. 2C, (3) vs.(1)). The hemolytic activity of *E. coli* HB101 carrying pACHT1 was not significantly observed.

3.3. Enterohemolysin-mediated production of cytokines

Bacterial cells of STEC strain F60, *E. coli* HB101, *E. coli* HB101 carrying pACH1, or *E. coli* HB101 carrying pACHT1 were grown in a liquid medium. Subsequently, the culture filtrates were incubated with monocytes from human peripheral blood for 2 h at 37°C, and cytokines in the supernatants were assayed. The culture filtrates of *E. coli* HB101 carrying pACH1 induced the expression of IL-1β, but not TNF-α from

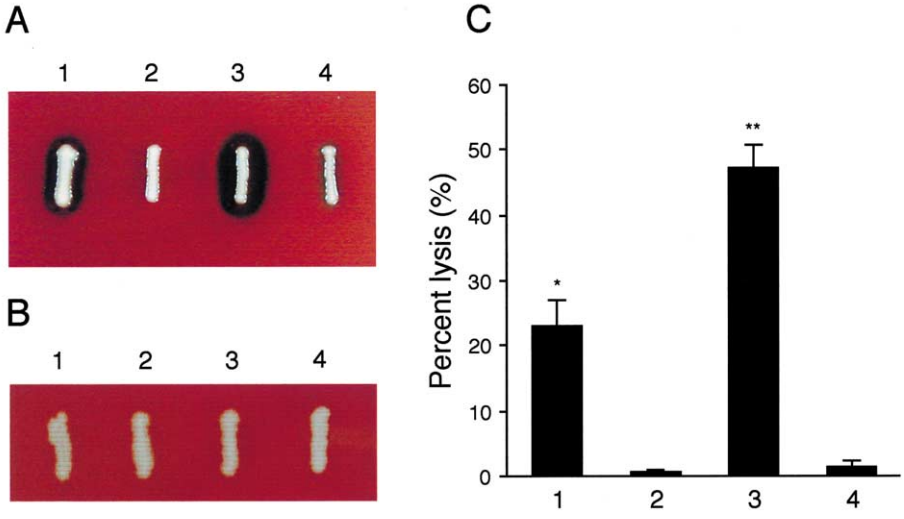

Fig. 2. Hemolytic phenotype of a wild-type STEC strain F60 and *E. coli* HB101 carrying pACH1 (with the intact *hlyA* gene) or pACHT1 (with the mutated *hlyA* gene). Hemolysis was assayed on agar plates in A and B, and in liquid cultures in C. Bacterial cells used were: 1, STEC strain F60; 2, *E. coli* HB101; 3, *E. coli* HB101 carrying pACH1; 4, *E. coli* HB101 carrying pACHT1. In A, streaked blood agar plates containing washed sheep erythrocytes were incubated for 24 h at 37°C. A clear zone of hemolysis (around the streaked bacterial colonies) can be seen in 1, and a larger zone of hemolysis in 3. No detectable hemolysis occurred in 2 or 4. Hemolysis in 3 occurred as early as 3 h after incubation at 37°C. In B, streaked blood agar plates containing unwashed sheep erythrocytes were incubated for 24 h at 37°C. No detectable hemolysis occurred. In C, sheep erythrocytes were incubated with bacteria in a liquid medium for 6 h at 37°C, and the quantity of hemoglobin released from lysed blood cells was measured by spectrophotometry (wavelength, 540 nm) and percent lysis was estimated. The results are presented as mean ± S.D. of triplicate experiments. $^*P < 0.05$, $^{**}P < 0.01$ as compared with the data of *E. coli* HB101.

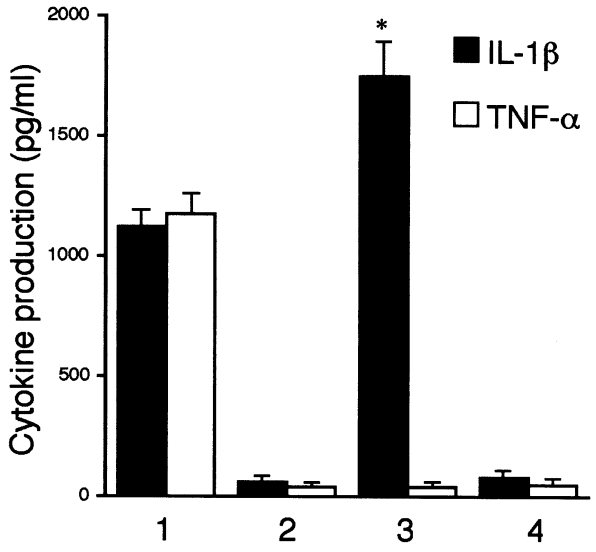

Fig. 3. IL-1β production from human monocytes. Bacteria used were: 1, Hly$^+$ STEC strain F60; 2, *E. coli* HB101; 3, Hly$^+$ *E. coli* HB101 carrying pACH1; 4, Hly$^-$ *E. coli* HB101 carrying pACHT1. The bacterial culture supernatants were incubated with the monocytes (approximately 10^5 cells per ml) for 2 h at 37°C in a CO_2 incubator, and then the IL-1β and TNF-α in the supernatants were assayed. The data are shown with a closed bar and an open bar, respectively, in the figure. The results are presented as mean ± S.D. of triplicate experiments. Marked IL-1β production was observed with *E. coli* HB101 (pACH1), but not with *E. coli* HB101 or *E. coli* HB101 (pACHT1), even after 6 h of incubation. TNF-α production was not stimulated in any case (except for STEC strain F60). *$P < 0.01$, as compared with the level of IL-1β stimulation by *E. coli* HB101 or *E. coli* HB101 (pACHT1); $P < 0.05$, as compared with the level of IL-1β stimulation by STEC strain F60.

human monocytes (Fig. 3(3)). The IL-1β response was diminished when *E. coli* HB101 carrying pACHT1 was used (Fig. 3(4). This was the case even after 6 h of incubation.

In contrast to *E. coli* HB101 carrying pACH1, a wild-type STEC strain F60 induced the expression of both IL-1β and TNF-α from human monocytes (Fig. 3(1)). The IL-1β expression level observed was lower, compared with that for *E. coli* HB101 carrying pACH1 ($P < 0.05$).

3.4. Enterohemolysin-mediated cytokine mRNA expression

The culture filtrates of *E. coli* HB101 carrying pACH1 induced the expression of IL-1β mRNA (Fig. 4, lane 2), while the culture filtrates of *E. coli* HB101 or *E. coli* HB101 carrying pACHT1 did not (Fig. 4, lanes 1 and 3). The house-keeping gene GAPDH (cDNA) was simultaneously amplified in parallel tubes (Fig. 4, lanes 4–6), indicating equivalent loading of the samples. The specificity of the RT-PCR products was confirmed by restriction enzyme digestion; *Hin*dIII digestion generating 82- and 309-bp fragments for IL-1β (the GenBank accession number for the IL-1β sequence, X02532), and *Msp*I digestion generating 119- and 234-bp fragments for GAPDH (the GenBank accession number for the GAPDH sequence, M17851).

4. Discussion

The precise role of enterohemolysin in the pathogenesis of STEC infections is still unknown. However, the following information has been accumulated and indicates an association of enterohemolysin with clinical diseases in humans: (i) most clinical isolates of STEC produce enterohemolysin [6–8], (ii) enterohemolysin lyses erythrocytes resulting in release of heme and hemoglobin, which serve as sources of iron and stimulate the growth of STEC [24], (iii) in the case of STEC belonging to serotype O111:H-, enterohemolysin was observed in 88% of strains from patients with HUS, but only in 22.2% of strains from patients with diarrhea, indicating that the presence of enterohemolysin increases the ability of STEC O111 to cause extraintestinal complications in humans [25], (iv) antibodies to enterohemolysin have been detected in the sera of patients with HUS [7], (v) *E. coli* O157:H- strains that did not produce Stx, but did produce enterohemolysin, have been isolated from HUS patients [26].

STEC belonging to serotype O157:H7, however, does not effectively secrete enterohemolysin in the culture supernatant. And, when the *hlyB* and *hlyD* genes of the α-hemolysin operon are supplied, STEC O157:H7 becomes able to induce hemolytic activity [7].

The enterohemolysin used in this study (which originated from STEC belonging to serotype O128:H12) is better secreted from *E. coli* cells compared with STEC O157:H7. In the case of O128:H12, the HlyB has only one methionine residue at the amino terminus (the same as α-hemolysin), in sharp contrast to O157-HlyB, which has two repeated methionine residues at the amino terminus (as a consequence, the O157-HlyB is longer than O128-HlyB by one amino acid) [8]. It is thought that the transmembrane domain of HlyB constitutes a pocket which binds with HlyA (the signal sequence at the carboxy terminus of HlyA), while the ATP-binding domain (located at the cytoplasm side) of HlyB generates energy for the transport [27]. There is the possibility that the amino terminal sequence of the O157-HlyB possessing two repeated methionine residues interferes with the transportation of HlyA.

Although the present O128-HlyA was also slightly divergent from the reported O157-HlyA with a homology of 98.5% at the amino acid level [8], both HlyAs shared the same enterohemolysin phenotype on blood agar plates, the same predicted sites for acylation (Lys-550 and Lys-675), and almost identical RTX repeat sequences (glycine- and aspartate-rich Ca^{2+}-binding repeats) [7,8,28]. Both the O128-

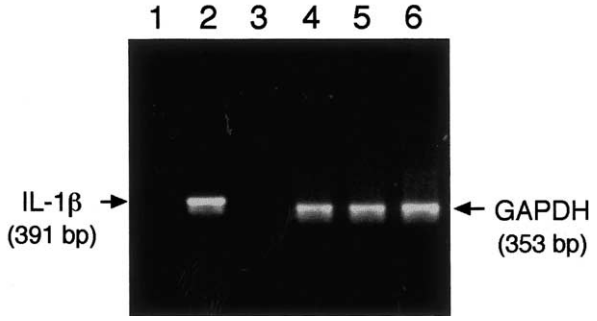

Fig. 4. IL-1β mRNA expression in human monocytes. Human monocytes stimulated for 2 h as in Fig. 3 were procured and total cellular RNA was extracted. The mRNA was reverse-transcribed and amplified with primers for IL-1β (lanes 1 to 3) or for GAPDH (lanes 4 to 6). Stimulation of monocytes was *E. coli* HB101 in lanes 1 and 4, Hly$^+$ *E. coli* HB101 carrying pACH1 in lanes 2 and 5, and Hly$^-$ *E. coli* HB101 carrying pACHT1 in lanes 3 and 6. A representative experiment of three identical experiments is shown. Similar results were obtained even after 6 h of stimulation.

HlyA and O157-HlyA are very divergent from α-HlyA in terms of amino acid length (25 amino acids shorter) and a homology at the amino acid level (61.9% and 61.2–61.6%, respectively).

In this study, we constructed a mutant *hlyA* gene in which only the first 29-amino acid sequence at the N terminus (corresponding to 2.9% of the predicted HlyA amino acid sequence) was conserved. The enterohemolysin operon carrying this mutant *hlyA* gene displayed an enterohemolysin-negative phenotype. By using an *E. coli* strain carrying the intact enterohemolysin operon and an isogenic *E. coli* strain with the mutant *hlyA* gene, we clearly demonstrated that enterohemolysin of a human STEC strain caused the release of IL-1β (but not TNF-α) from human monocytes.

When the enterohemolysin operon of STEC strain F60 was cloned into the pACYC184 (vector with medium copy number), *E. coli* HB101 carrying the recombinant plasmid (pACH1) produced more hemolysin on blood agar plates as well as in liquid cultures. In the latter case, a 2.1-fold greater hemolytic activity was observed as compared with STEC strain F60. In agreement with this, *E. coli* HB101 carrying pACH1 exerted a greater stimulatory effect on human monocytes, and produced more IL-1β. However, since STEC strain F60 produces both Stx and enterohemolysin, further experiments should be performed with STEC strain F60 and its hemolysin-negative (or Stx-negative) mutant to evaluate the stimulatory effects.

In this study, only IL-1β and TNF-α stimulation was examined using human monocytes. Since enterohemolysin is a pore-forming toxin with multiple stress functions on eukaryotic cells, it remains unclear whether the increase in IL-lβ is a specific process or whether it is the cause of the more global cytotoxic effect. Measurement of other cytokines to control the experiments need to be performed.

Around 10% of children infected with STEC develop HUS [29]. HUS is recognized as acute renal failure, with microangiopathic hemolytic anemia and thrombocytopenia [30], and a variety of circulating inflammatory mediators increase during thrombotic microangiopathies, including IL-1β, TNF-α, IL-6 and IL-8 [13–15,31].

Experimental data suggest that the host's inflammatory responses to Stx are involved in the development of HUS. Stx binds to the functional receptor, globotriaosylceramide (Gb3), on human endothelial cells [32,33], monocytes [17], and renal tissue [34]. Stx induces the production of IL-1β, TNF-α, IL-6 and IL-8 from human monocytes [17]. In addition, synthesis of Gb3 is increased by IL-1β and TNF-α, and a combination of Stx and a cytokine exhibits a marked synergistic cytotoxicity on human vascular endothelial cells [33,35,36].

Several hemolysins or cytotoxins have been shown to play a role in pathogenicities via the production of cytokines. Pneumolysin, the membrane-damaging toxin of *Streptococcus pneumoniae*, stimulates the production of IL-1β and TNF-α by human monocytes in vitro [37]. In addition, pneumolysin (and its truncated, non-cytolytic products with C-terminal deletions) has been shown to be capable of inducing IFN-γ production in spleen cells by a mechanism different from pore formation [38]. The α-toxin of *Staphylococcus aureus*, a transmembrane pore-forming toxin, activates NF-κB and induces IL-8 production [39]. Group B streptococcal (GBS) β-hemolysin production has been shown to be associated with systemic and intra-articular IL-6 and IL-1β levels [40]. Furthermore, GBS β-hemolysin induces nitric oxide production via the NF-κB activation [41].

In the case of *E. coli* α-hemolysin, the acylated, hemolytically active form elicits IL-1β release by monocytes, and the acylated α-hemolysin (but not IL-1β) appears to be a critical mediator of the lethality in mice, to which α-hemolysin-producing *E. coli* was given intraperitoneally [42]. However, since STEC colonizes the intestinal mucosa and usually does not invade, STEC can not act as an extraintestinal, bacterial dispatcher of enterohemolysin.

α-Hemolysin production in STEC has been reported. A well-known example is the case of porcine STEC which colonizes the porcine intestinal mucosa, and produces Stx2e (an Stx2 variant) and α-hemolysin (instead of enterohemolysin). The α-hemolysin-producing porcine STEC causes edema disease [43].

In humans, enterohemolysin may contribute to the development of HUS via the production of IL-1β from human monocytes. In addition, enterohemolysin may mediate translocation of other stimulating proteins such as Stx, as Gram-positive bacterial cytolysin (pore-forming toxin) mediates translocation of bacterial effector proteins into host cells [44].

Studies are required with enterohemolysin to clarify its role in STEC pathogenesis.

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