Salmonella enteritidis FliC (Flagella Filament Protein) Induces Human β-Defensin-2 mRNA Production by Caco-2 Cells*

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Ken-ichi Ogushi, a, b Akihiro Wada, a Takuro Niidome, b Naoki Mori, a Kazunori Oishi, d Tsuyoshi Nagatake, a Akira Takahashi, a Hiroshi Asakura, a Sou-ichi Makino, a Hironobu Hojo, g Yoshiaki Nakahara, a Mio Ohsaki, b Tomomitsu Hatakeyama, b Haruhiko Aoyagi, b Hisao Kurazono, a Joel Moss, a and Toshiya Hirayama a, j

From the Departments of aBacteriology, cPreventive Medicine and AIDS Research, and dInternal Medicine, Institute of Tropical Medicine, and the eDepartment of Applied Chemistry, Faculty of Engineering, Nagasaki University, Sakamoto, Nagasaki 852-8523, Japan, the fDepartment of Nutrition, School of Medicine Tokushima University, Kuramoto-cho, Tokushima 770-8503, Japan, the gDepartment of Veterinary Microbiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro Inada, Hokkaido 080-8555, Japan, the hFaculty of Industrial Medicine, Tokai University, Kitakame, Hiratsuka-shi, Kanagawa 259-1292, Japan, the iFaculty of Health Science, Okayama University Medical School, Shikata-cho, Okayama 700-8558, Japan, and the jPulmonary-Critical Care Medicine Branch, NHLBI, National Institutes of Health, Bethesda, Maryland 20892

Antimicrobial peptides are crucial for host defense at mucosal surfaces. Bacterial factors responsible for induction of human β-defensin-2 (hBD-2) mRNA expression in Caco-2 human carcinoma cells were determined. Salmonella enteritidis, Salmonella typhimurium, Salmonella typhi, Salmonella dublin, and culture supernatants of these strains induced hBD-2 mRNA expression in Caco-2 human carcinoma cells. Using luciferase as a reporter gene for a 2.1-kilobase pair hBD-2 promoter, the hBD-2-inducing factor in culture supernatant of S. enteritidis was isolated. The supernatant factor was heat-stable and protease-sensitive. After purification by anion exchange and gel filtration chromatography, the hBD-2-inducing factor was identified as a 53-kDa monomeric protein with the amino-terminal sequence AQVINTNSLSLLTQNNLIMWYR, which is identical to that of the flagella filament structural protein (FliC) of S. enteritidis. Consistent with this finding, the 53-kDa protein reacted with anti-FliC antibody, which prevented its induction of hBD-2 mRNA in Caco-2 cells. In agreement, the hBD-2-inducing activity in culture supernatant was completely neutralized by anti-FliC antibody. In gel retardation analyses, FliC increased binding of NF-κB (p65 homodimer) to hBD-2 gene promoter sequences. We conclude that S. enteritidis FliC induces hBD-2 expression in Caco-2 cells via NF-κB activation and thus plays an important role in up-regulation of the innate immune response.

Antimicrobial peptides play an important role in host defense at mucosal surfaces. The two major groups of vertebrate defensins, α- and β-defensins, differ in the arrangements of their disulfide bonds. Six human α-defensins (HD-1 to HD-6) and two β-defensins (hBD-1 and hBD-2) have been reported to date. HD-1, HD-2, HD-3, and HD-4 are present in neutrophils, where they constitute 30–50% of the total protein in azurophilic granules (1). HD-5 and HD-6 were identified in the Paneth cells of small intestinal crypts (2, 3) and in female reproductive tissue (4). hBD-1 was purified from plasma (5) and detected in a range of epithelial tissues (6). hBD-2 was purified from skin and shown to be expressed in the lung and uterus (7). hBD-2 was induced in mucosal tissues following bacterial infections (7–9).

Salmonella species are Gram-negative organisms that cause gastroenteritis and enteric fever in humans. As pointed out by Bäumler et al. (10), an increase in numbers of human infections with Salmonella enteritidis began in the 1960s but was followed by an almost 50% decrease from 1970 to 1976. Another increase began in 1977, with signs of a decrease beginning in 1992. In contrast, the increasing frequency of S. enteritidis infection in poultry did not begin until 1989, and a decline started in 1996 (11). Reasons for the fluctuations are unknown, but the recent recognition of food-borne infection with S. enteritidis has renewed interest in how these organisms can invade, persist, and spread.

To evaluate the role of defensins in S. enteritidis infection, we investigated the induction of hBD-2 in Caco-2 cells, a human colon carcinoma line. S. enteritidis, Salmonella typhimurium, Salmonella typhi, and Salmonella dublin increased hBD-2 mRNA levels, as did the culture supernatant of these strains. The hBD-2-inducing protein in S. enteritidis supernatant was purified by sequential anion exchange and gel filtration chromatography, and the amino-terminal sequence was determined. It was established that the hBD-2-inducing factor is flagella filament structural protein (FliC) of S. enteritidis, and induction results from NF-κB activation.

MATERIALS AND METHODS

Bacteria Strains and Growth Conditions—S. enteritidis, S. typhimurium, S. typhi, and S. dublin strains were grown on tryptose soy agar and then in liquid culture overnight in 3 ml of tryptic soy broth (TSB). A sample (500 μl) of the overnight culture was added to 20 ml of TSB, and bacteria harvested in log phase were used in this study.

Induction of hBD-2 mRNA in Caco-2 Cells—Caco-2 cells were grown in DMEM supplemented with 10% fetal calf serum (FCS) in 60-mm

PCR, polymerase chain reaction; RT, reverse transcription; TBS, Tris-buffered saline; TSB, tryptic soy broth.
HBD-2 induction by FliC

Preparation of Anti-FliC Peptide Antibody, Anti-FliC Protein Antibody, and Western Blotting—A peptide, NH2-QFTFDDKTKNESAKL (amino acids 339–353 of S. enteritidis Flig with an added carboxyterminal Cys), was synthesized and coupled to KLH. Rabbits were immunized with the KLH-coupled peptide (200 µg) or with purified FliC protein (40 µg) on day 0, with peptide (200 µg) or Flig (40 µg) on day 21 and with peptide (100 µg) or Flig (40 µg) at intervals of a week thereafter until the desired serum antibody titer against the peptide or protein was reached. All antigens were injected subcutaneously, with complete Freund’s and with incomplete Freund’s adjuvants (1:1). Rabbits were bled 7 weeks after the first injection.

For immunoblotting, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Immobilon-P membranes) at 100 V for 1 h with transfer buffer (25 mM Tris, 0.19 mM glycine, 20% methanol) followed by staining with Ponceau-S. The protein band was excised for amino acid sequencing with a gas-phase protein sequencer PPSQ-21 (Shimadzu, Japan).

Preparation of Anti-FliC Peptide Antibody, Anti-FliC Protein Antibody, and Western Blotting—A peptide, NH2-QFTFDDKTKNESAKL (amino acids 339–353 of S. enteritidis Flig with an added carboxyterminal Cys), was synthesized and coupled to KLH. Rabbits were immunized with the KLH-coupled peptide (200 µg) or with purified FliC protein (40 µg) on day 0, with peptide (200 µg) or Flig (40 µg) on day 21 and with peptide (100 µg) or Flig (40 µg) at intervals of a week thereafter until the desired serum antibody titer against the peptide or protein was reached. All antigens were injected subcutaneously in complete Freund’s adjuvant (1:1). Rabbits were bled 7 weeks after the first injection.

For immunoblotting, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Immobilon-P membranes) at 100 V for 1 h at 4 °C with transfer buffer. Membranes were incubated with 5% milk powder in TBS-T (50 mM Tris-HCl, pH 7.5, 15 mM NaCl, 0.1% Tween-20) for 1 h and washed with TBS-T, followed by incubation in a 1: 5000 dilution of anti-FliC peptide IgG in TBS-T for 1 h at room temperature. After washing in TBS-T, membranes were incubated in a

Temperature. After washing in TBS-T, membranes were incubated in 250 mM Tris-HCl, pH 8.3, 50 mM KC, 2 mM MgCl2, 1 mM of each deoxynucleotide triphosphate, and reverse transcriptase for 30 min at 42 °C. cDNA was mixed with 10 µg/ml Tris-HCl, pH 3.5, 15 mM NaCl, 50 pmol each of primer hBD-2 sense (GTTGAAGCTTCCAGCATCA), hBD-2 antisense (TATTTTTGAGACCACTAGGT), GAPDH sense (TGAAGGTCCGAGTCAACGGATTGGT), and GAPDH antisense (TATTTGGGCGAGTCAACACCAC). The mixture was covered with mineral oil before initiating PCR for 40 cycles of 1 min at 94 °C, 2 min at 55 °C, and 2 min at 72 °C. PCR products were identified in ethidium bromide-stained 1.5% agarose gels.

Luciferase Assay—To assess hBD-2 promoter activity, Caco-2 cells were seeded in 24-well culture plates (1 × 105 cells in 1 ml of DMEM per well) and incubated for 24 h at 37 °C. 2.5 µg of the hBD-2 promoter linked to a luciferase reporter gene (5′-deletion constructs of the hBD-2 promoter (pGL3-2110, pGL3-938, pGL3-398, pGL3-229, and pGL3-197) or a mutated hBD-2 promoter construct (pGL3-938/mi) (12) were incubated with 0.5 µg of an internal control Renilla luciferase expression vector (pRL-TK) and 10 µl of 1 µM dextran poly-(l-l-lysine) (K6G) (13) in 250 µl of FCS-free DMEM for 15 min at room temperature before addition of DNA-peptide complexes to Caco-2 cells. After incubation for 3 h at 37 °C, 1 ml of DMEM was added, and 12 h later, the culture medium was replaced.

After DNA transfection, the medium was replaced with 1 ml of fresh FCS-free DMEM, and 250 µl of the sample to be assayed was then added. After incubation for 6 h at 37 °C, Caco-2 cells were washed with 1 ml of PBS and lysed by adding 300 µl of lysis buffer (ToyO Ink Co.). After 15 min at room temperature, the lysate was centrifuged (18,000 × g, 5 min, 4 °C). Luciferase activity of the supernatant, measured using a luminometer (Berthold), was normalized to the activity of an internal control Renilla luciferase.
overnight at room temperature, polymerized FlïC was collected by centrifugation (100,000 × g, 1 h, 4 °C) and dissolved in PBS.

Construction of PET-FliC and Purification of Recombinant FlïC—A complete structural gene of the flïC gene was amplified by PCR using a set of oligonucleotide primers, primer 1 (5’-GGGCAATGCGAAACTCTTAATTG3’) and primer 2 (5’-GGGGATCTTTACGACGCTTTAAGAG3’), followed by digestion with NcoI and BamHI sites of pET15b (Novagen, Darmstadt, Germany) and PET-FliC was yielded. PET-FliC was transformed into Escherichia coli BL21 (pLysS) to express FlïC protein.

Bacterial cells were harvested by centrifugation 5 h after induction of mid-log phase culture with 1 μM isopropyl-β-D-thiogalactopyranoside. Lysis was performed in the PBS by repeated sonication. After centrifugation at 18,000 × g for 15 min at 4 °C, the supernatant was collected and adjusted to pH 2 with 1 M HCl, and it was maintained at that pH with constant stirring for 30 min at room temperature. After centrifugation at 100,000 × g for 1 h at 4 °C, the pH of the supernatant was adjusted to 7.2 with 1 M NaOH and dialyzed against PBS.

Preparation of Nuclear Extract—To prepare nuclear extract, Caco-2 cells were grown to about 90% confluency in 100-mm dishes, the culture medium was replaced with 10 ml of PBS-free medium, and incubation was continued for 1 h at 37 °C. Cells were then incubated with FlïC, 100 ng/ml, for 0, 1, 3, 6, or 12 h, washed with PBS, and scraped in 2 ml of PBS. After addition of 400 μl of Buffer A (10 mM HEPES-KOH, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.2% Nonidet P-40) containing protease inhibitor mixture tablets followed by gentle rotation for 30 min at 4 °C, nuclei were pelleted by centrifugation (100,000 × g, 5 min, 4 °C) and dispersed in 100 μl of Buffer B (20 mM HEPES-KOH, pH 7.9, 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) containing protease inhibitor mixture tablets followed by gentle rotation for 30 min at 4 °C. After centrifugation (18,000 × g, 15 min, 4 °C), the concentration of protein in the supernatant was determined by the method of Bradford (Bio-Rad).

Electrophoretic Mobility Gel Shift Assay—Oligonucleotides were synthesized by Amersham Pharmacia Biotech. The positive strands of the double-stranded oligonucleotides, corresponding to regions of the NF-κB, were prepared as follows, with mutated nucleotide sequence underlined: wild-type NF-κB (−213 to −196) for hBD-2, 5’-TTTCTGGGGTTCTCAGTA-3’; mutated NF-κB for hBD-2, 5’-TTTCTTAAGGTTTAAATGA-3’. The complementary strands in equal concentrations were mixed, heated to 95 °C for 5 min, and annealed by slowly cooling to room temperature. Double-stranded oligonucleotides were stored at −20 °C in 50 mM NaCl. For electrophoretic mobility shift assay, oligonucleotide probes were labeled with [γ-32P]ATP (Amersham Pharmacia Biotech) using T4 polynucleotide kinase (Takara, Shiga, Japan) and purified using ProbeQuant™ G-50 Micro Columns (Amerham Pharmacia Biotech).

Samples (2 μg) of nuclear proteins were incubated with the indicated radiolabeled oligonucleotides for 20 min at 25 °C in binding buffer (20 mM Tris-HCl, pH 7.9, 50 mM NaCl, 10% glycerol, 1 mM dithiothreitol, 2 mM MgCl2, 1 mM EDTA, 0.1% Nonidet P-40, 50 μg/ml bovine serum albumin). Specificities of the binding reactions were tested in竞争 assays in which a 100-fold excess of unlabeled wild-type or mutated oligonucleotide was added. Protein-nucleotide complexes were separated by electrophoresis in a 4% DNA retardation gel with Tris borate/EDTA (36 mM Tris-HCl, 36 mM boric acid, 0.8 mM EDTA) at constant current (20 mA) at 4 °C. Gels were dried, and complexes were analyzed by autoradiography using a Fuji imaging analyzer (Fuji Film).

Supershift experiments were performed by adding antibody (1 μl) to the binding mixture 15 min before addition of the radiolabeled probe; complexes were then separated as described above. Antibodies against synthetic peptides derived from NF-κB/Rel family proteins (p65A) against amino acids 3–19 of p65, p65(C) against amino acids 531–550 of p65, p50 (N-19) against the amino terminus of p50, p50 (K-27) against conserved epitope of p52, c-Rel (N) against the amino terminus of c-Rel, and Rel B (C-19) against the carboxy terminus of Rel B) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

RESULTS

Induction of hBD-2 mRNA in Caco-2 Cells by Salmonella Species—To evaluate the effect of Salmonella species on hBD-2 mRNA levels by RT-PCR, Caco-2 cells were incubated for 6 h with S. typhimurium, S. enteritidis, S. typhi, or S. dublin or their broth culture filtrates, each of which increased hBD-2 mRNA (Fig. 1).

Stability of hBD-2-inducing Factor in S. enteritidis Supernatant—The factor in S. enteritidis culture supernatant responsible for hBD-2 induction was stable to heating at 100 °C for 10 min (Fig. 2). However, incubation for 2 h with trypsin or proteinase K resulted in a loss of hBD-2-inducing activity, indicating that the factor in broth culture of S. enteritidis was heat-stable and proteinase-sensitive.

Purification of hBD-2-inducing Factor from S. enteritidis Supernatant—hBD-2-inducing activity in S. enteritidis supernatant was estimated by induction of luciferase activity of pGL3–2110-transfected Caco-2 cells. pGL3–2110 contains the 5’ flanking region of the hBD-2 gene (−2110 to −1) in a luciferase-reporter plasmid (Fig. 7). pGL3–2110-transfected cells were incubated with S. enteritidis supernatant, before and after heat and proteinase treatment and before assay of luciferase activity (Fig. 3). As shown in the RT-PCR assay (Fig. 2), in the luciferase assay, the hBD-2-inducing factor in S. enteritidis supernatant was stable to heating at 100 °C for 10 min (Fig. 2).

TABLE I

| Volume | Total amount of protein | Total activity | Specific activity | Yield |
|--------|------------------------|---------------|-----------------|-------|
| ml     | mg                     | units*        | units/mg        | %     |
| S. enteritidis supernatant | 100 | 2.35 | 114,320 | 48,647 | 100 |
| Heat | 100 | 2.35 | 114,320 | 48,647 | 100 |
| Saturated ammonium sulfate precipitation | 70% saturated ammonium sulfate precipitation | 40 | 1.55 | 80,000 | 51,780 | 70.0 |
| Resource Q | 16 | 0.29 | 21,760 | 77,034 | 19.0 |
| Superox 12 | 1 | 0.04 | 3,200 | 80,000 | 2.80 |

* 1 Unit = Sample can activate the level of EC50 of luciferase activity for pGL3-2110 transfected Caco-2 cells.

FIG. 3. Stability of hBD-2-inducing factor in S. enteritidis culture supernatant assayed with luciferase as the reporter gene. hBD-2-inducing activity in Caco-2 cells was quantified by luciferase activity of pGL3–2110-transfected Caco-2 cells. The cells were incubated for 6 h with control TSB medium, S. enteritidis supernatant, heat-treated supernatant (10 min at 100 °C), or incubation for 2 h with 40 μg/ml trypsin or 200 μg/ml proteinase K (5), followed by heat treatment. Luciferase activity was then examined. Luciferase activity is reported relative to that of cells transfected with empty vector. Data are the means ± S.D. of values from three separate experiments with assays in duplicate.
culture supernatant was heat-stable and proteinase-sensitive (Fig. 3).

Using the luciferase assay system, the hBD-2-inducing factor in S. enteritidis supernatant was purified by anion exchange and gel filtration chromatography (Fig. 4, A and B, and Table 1). In pooled effluents from Superose 12, a single protein band of 53 kDa was seen by Coomassie Brilliant Blue staining after SDS-PAGE in 10% gel (Fig. 4C). The amino-terminal sequence of the 53-kDa protein, AQQVNTNLSLLTQNNLNK, is identical to that of flagella filament structural protein (FliC) of S. enteritidis. Consistent with this result, the 53-kDa protein reacted with anti-FliC peptide antibody (Fig. 4D).

When pGL3−2110-transfected Caco-2 cells were incubated with FliC for 6 h before assay of the luciferase activity, inducing activity was concentration-dependent up to 0–0.1 μg/ml and was maximal with 0.1–10 μg/ml (data not shown).

Inhibition of hBD-2 induction by the 53-kDa protein or FliC with anti-FliC protein antibody confirmed that FliC is the hBD-2-inducing factor (Fig. 5A). Inhibitory effects of the two proteins on hBD-2-inducing activity in the luciferase assay were similarly relieved by antibody, consistent with the conclusion that the 53-kDa protein in S. enteritidis supernatant is FliC and functions as an hBD-2-inducing factor.

To determine whether FliC is the major hBD-2-inducing factor in S. enteritidis culture supernatant, the effect of anti-FliC protein antibody on this activity was examined. As shown in Fig. 5B, the hBD-2-inducing activity was completely neutralized by anti-53-kDa protein (FliC) antibody, indicating that FliC is responsible for the hBD-2-inducing activity in culture supernatant.

FliC Expressed in E. coli (BL21) Causes hBD-2 Induction—To clarify that FliC is really hBD-2-inducing factor in S. enteritidis culture supernatant, a FliC expression plasmid was constructed, and recombinant FliC was expressed in E. coli, BL21. As shown in Fig. 6A, the lysate with recombinant FliC but not control lysate has hBD-2-inducing activity in a dose-dependent manner.

When pGL3−2110-transfected Caco-2 cells were incubated with recombinant FliC or original FliC for 6 h before assay of luciferase activity, hBD-2-inducing activity exhibited concentration-dependent effects up to 0–0.1 μg/ml and was maximal with 0.1–10 μg/ml (Fig. 6B).

Gram-positive MSSA (ATCC25923) and its culture supernatant and E. coli BL21 strains and their supernatant did not activate the hBD-2 reporter gene in Caco-2 cells (data not shown).

These all results support the hypothesis that FliC is a major hBD-2-inducing factor in S. enteritidis culture supernatant.

Effect of Deletion or Mutation of NF-κB-binding Sequences in the hBD-2 Gene on FliC-Stimulation of Promoter Activity—Recently, we reported that induction of hBD-2 mRNA by H. pylori is regulated by NF-κB (12). To determine FliC effects on hBD-2 mRNA may involve NF-κB activation, Caco-2 cells were transfected with different luciferase-reporter constructs and stimulated with FliC. FliC did not enhance luciferase activity in Caco-2 cells transfected with the control luciferase-reporter plasmid, pGL3 or of those transfected with pGL3−197, or pGL3−938nt (Fig. 7). It did increase activity of cells transfected with pGL3−2110, pGL3−938, pGL3−398, or pGL3−229 (Fig. 7), consistent with involvement of NF-κB in the induction of hBD-2 by FliC.

FliC Activates NF-κB in Caco-2 Cells—Binding of proteins to 18-base pair oligonucleotides, with a sequence corresponding to that of the NF-κB site at position −208 to −199 in the hBD-2 promoter region, was evaluated by electrophoretic mobility shift assay. Caco-2 cells were incubated with FliC, 100 ng/ml, for 0, 1, 3, 6, or 12 h. Specific DNA-protein complexes that bind NF-κB sequence were not observed in control cells (Fig. 8A). After incubation with FliC for 1 h, levels of complexes were increased, but they decreased with longer incubation. Addition of a 100-fold excess of unlabeled oligonucleotide with wild-type sequence, but not that with the mutated sequence, specifically decreased the amount of radiolabeled complex (Fig. 8A).

Identification of NF-κB-binding Proteins in Nuclei of Caco-2 Cells Treated with FliC—To identify proteins that bound to the NF-κB site, mobility shift assays were performed using anti-
bodies raised against p65, p50, p52, c-Rel, and RelB proteins. As shown in Fig. 8B, in the presence of oligonucleotide with sequence of the NF-κB site in the hBD-2 promoter, p65-specific antibodies, but not several others, caused a marked supershift, suggesting that p65-p65 homodimer was a major component of the DNA-binding complex.

**DISCUSSION**

Unlike hBD-1, which is produced constitutively, hBD-2 is synthesized in response to bacterial infection or proinflammatory agonists, suggesting a role for hBD-2 in epithelial innate host defense (7, 16). The bacterial factors that induce hBD-2 in mammalian cells are still unclear, and the regulation of hBD-2
induced expression of IL-8 via Ca\(^{2+}\)-mediated activation of the NF-κB pathway in epithelial cells. *S. typhimurium* invasion is not required for NF-κB activation in intestinal epithelial cells, although bacterial contact is necessary (26). The invasion-defective *S. typhimurium* mutant HiΔ\(\lambda\), which lacks the type III secretion apparatus, was able to induce IL-8 in T84 cells. Thus, IL-8 induction was not dependent on the type III secretion system.

Because IL-8, tumor necrosis factor α, and IL-1 were induced in mammalian cells by bacterial FliC (19–24), we suspected that FliC acting through NF-κB might be responsible for hBD-2 induction. Activation of NF-κB by Gram-negative and Gram-positive cell wall components is well known, although whether FliC activated NF-κB was not shown. As shown here, NF-κB was activated by FliC, and only the p65–p50 NF-κB homodimer was shown to bind the hBD-2 promoter region. Thus, FliC can activate NF-κB in mammalian cells, and it appears to be responsible for a number of the critical effects of *Salmonella* on mammalian cells.\(^2\)

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**REFERENCES**

1. Harwig, S. L., Ganu, T., and Lehrer, R. I. (1994) *Methods Enzymol.* 236, 160–172

2. Jones, D. E., and Bevins, C. L. (1992) *J. Biol. Chem.* 267, 23216–23225

3. Jones, D. E., and Bevins, C. L. (1993) *FEBS Lett.* 315, 182–192

4. Svinarchu, D. M., Wolf, N. A., Gomez, R., Gonik, B., and Romeo, R. (1997) *Annu. J. Obstet. Gynecol.* 176, 470–475

5. Bensch, W. R., Raida, M., Magert, H. J., Schulz-Knappe, P., and Forssmann, W. G. (1995) *FEBS Lett.* 368, 326–330

6. Zhao, C., Wang, J., and Lehrer, R. I. (1996) *FEBS Lett.* 396, 319–322

7. Harder, J., Bartels, J., Christophers, E., and Schroder, J. M. (1997) *Nature* 387, 86

8. Bals, R., Wang, X., Wu, Z., Freeman, T., Bafina, V., Zasloff, M., and Wilson, J. M. (1998) *J. Clin. Invest.* 102, 874–880

9. Wada, A., Mori, N., Oishi, K., Hojo, H., Nakahara, Y., Hamanaka, Y., Nakashima, M., Sekine, I., Ogushi, K., Nidome, T., Nagatake, T., Moss, J., and Hirohata, T. (1999) *Biochem. Biophys. Res. Commun.* 263, 770–774

10. Bäumer, A. J., Hargis, R. M., and Teolis, R. M. (2000) *Science* 287, 50

11. Ward, L. R., Threlfall, J., Smith, H. R., and O’Brien, S. J. (2000) *Science* 287, 1753–1754

12. Wada, A., Ogushi, Kimura, T., Hojo, H., Mori, N., Suzuki, K., Kumatari, A., May Se, Nakahara, Y., Nakamura, M., Joel, M., and Hirohata, T. (2000) *Cell. Microbiol.* 2, 115–123

13. Ohshaki, M., Urakawa, M., Nidome, T., and Aoyagi, H. (1999) *Pept. Sci.* 387, 453–456

14. Ibrahim, G. F., Fleet, G. H., Lyons, M. J., and Walker, R. A. (1985) *J. Clin. Microbiol.* 23, 1040–1044

15. Vonderfechtz, F., Kanto, S., Aizawa, S. I., and Namba, K. (1989) *J. Mol. Biol.* 209, 127–133

16. Singh, P. K., Ji, P. K., Wiles, K., Hesselberth, J., Liu, L., Conway, B. A. D., Bensch, K. W., Raida, M., Magert, H. J., Schulz-Knappe, P., and Forssmann, W. G. (1995) *J. Biol. Chem.* 270, 1346–1349

17. Ciacci-Woolwine, F., McDermott, P. F., and Mizel, S. B. (1999) *J. Clin. Invest.* 103, 5176–5185

18. Wyant, T. L., Tannen, M. K., and Sztein, M. B. (1999) *J. Clin. Invest.* 104, 1338–1346

19. Wyant, T. L., Tannen, M. K., and Sztein, M. B. (1999) *J. Clin. Invest.* 103, 3619–3624

20. Feldman, M., Bryan, R., Rajan, S., Scheffler, L., Brunert, S., and Prince, A. (1998) *J. Infect. Immun.* 66, 43–51

21. Teizer, S. N., Nataro, J. P., Poteet-Smith, C. E., Smith, J. A., and Guerrant, R. L. (2000) *J. Clin. Invest.* 105, 1769–1777

22. Gewirtz, A. T., Rao, A. S., Simon Jr., P. O., Merlin, D., Carnes, D., Madara, J. L., and Neish, A. S. (2000) *J. Clin. Invest.* 105, 79–92

23. Eaves-Pyles, T., Stazo, C., and Salzman, A. L. (1999) *J. Infect. Immun.* 67, 800–804

24. Hayashi, P., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M., and Adem, A. (2001) *Nature* 410, 1099–1103

\(^2\) During the revision of this report, Hayashi et al. (27) identified that the flagellin of *Listeria monocytogenes* and *S. typhimurium* are recognized by Toll-like receptor 5 (TLR5) in host mammalian cells.
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