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Interleukin-1 β increases the risk of gastric cancer through induction of aberrant DNA methylation in a mouse model

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Abstract. Interleukin-1 β (IL-1 β) has a significant role in chronic gastric inflammation and manifestations of gastric diseases. The present study aimed to elucidate the specific role of IL-1 β in induction of DNA methylation using IL-1 receptor type 1 knockout (IL-1R1^{-/-}) mice. In the present study, wild-type (WT) and IL-1R1^{-/-} mice were injected with IL-1 β (5 μ g/kg/day). Serum levels of IL-1 β , interleukin-6 (IL-6) and nitric oxide (NO) were measured by enzyme-linked immunosorbent or NO assays. E-cadherin (E-cad) methylation status and messenger (m)RNA expression of IL-1 β , IL-6, E-cad and inducible nitric oxide synthase (iNOS) were analyzed. Results from the present study indicated significantly higher IL-1 β mRNA expression (P<0.001) in WT mice compared with IL-1R1^{-/-} mice. IL-1 β and IL-6 release was significantly increased in treated WT mice compared with IL-1R1^{-/-} mice at 1 h, 4 h and 8 h (all P<0.005). IL-1 β release was only detected in WT mice following a second dose measured at day 3, week 1 and week 2 when compared with IL-1R1^{-/-} mice. Promoter methylation of E-cad and a decrease in gene expression was observed in treated WT mice. mRNA expression of iNOS in WT mice was significantly increased at week 1 compared with IL-1R1^{-/-} mice (P=0.0411). Furthermore, a significantly increased level of NO production was observed in treated WT mice (P<0.005 at 8 h and week 1; P<0.001 at 4 h and day 3) when compared with IL-1R1^{-/-} mice. The present results indicated that IL-1 β was able to directly induce DNA methylation, which may link inflammation-induced epigenetic changes and the development of gastric diseases.

Introduction

The inflammatory response is the body's natural response to invasion by an infectious agents or tissue damage. However, prolonged inflammation may activate cell proliferation and induce deregulation of cell death in affected tissues (1). Thus, chronic inflammation is now recognized as a predisposing cause of many types of cancer, including liver and gastric (2). Proinflammatory cytokines, including interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and tumor necrosis factor (TNF- α), are produced by stimulated inflammatory cells and epithelial cells during mucosal inflammation (3). IL-1 β is a potent inflammatory mediator that has a significant role in initiating and amplifying the inflammatory response against *Helicobacter pylori* infection (4). The present authors and others have previously demonstrated that IL-1 β induced by *H. pylori* infection promotes gastric carcinogenesis (5,6). Furthermore, the significant role of IL-1 β in increasing the risk of gastric inflammation and cancer has been demonstrated by using a transgenic model overexpressing human IL-1 β protein (7). IL-1 β is known to activate the nuclear factor κ B (NF- κ B) signaling pathway and elicit IL-6 production (8). IL-1 β induced by *H. pylori* infection led to upregulation of inducible nitric oxide synthase (iNOS) and overproduction of nitric oxide (NO) (9). Accumulating evidence demonstrates that overproduction of NO may induce irreversible mucosal DNA damage, which has been proposed to be involved in the initiation and promotion of gastric tumor growth (10).

Promoter CpG island methylation is frequently present in non-neoplastic gastric mucosa with *H. pylori*-induced gastritis (11,12). E-cadherin (E-cad) is a tumor-suppressor gene and its promoter hypermethylation has been observed to have a significant role in gastric carcinogenesis (11,13,14). Therefore, inflammation has been suggested to be an inducer of aberrant DNA methylation and a crucial driving force in the development of gastric cancer. This is evidenced by the frequent detection of E-cad methylation in non-neoplastic gastric mucosa with *H. pylori* infection (15).

It has been demonstrated that IL-1 β may induce DNA methylation *in vitro* (16-18). This methylation-dependent gene silencing mechanism is via IL-1 β activation of NO

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production (16,18). Thus, the present study used mice in which the interleukin 1 receptor type 1 (IL-1R1) was deleted in order to characterize the specific role of IL-1 β in *H. pylori*-induced DNA methylation. Previously the present authors demonstrated that *H. pylori* infection induced severe gastritis with enhanced IL-1 β production is likely a prerequisite mechanism for DNA methylation (5). However, whether *H. pylori*-induced DNA methylation is IL-1 β -dependent, or whether this phenomenon is the result of a complex interaction due to the host immune response to *H. pylori* infection, irrespective of IL-1 β , remains to be elucidated.

The present study aimed to elucidate the biological activity of IL-1 β in the initiation of gastric inflammation, and additionally investigated the specific role of IL-1 β in linking inflammation with DNA methylation by using IL-1R1-homologous-knockout (IL-1R1^{-/-}) mice.

Materials and methods

Animals. IL-1R1^{-/-} mice from a C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The IL-1R1^{-/-} mice and their C57BL/6 wild-type cohorts (WT) were bred and housed in the Animal Unit at the University of Hong Kong (Hong Kong, China). The mice were kept in individually ventilated cages (Tecniplast, West Chester, PA, USA) in an air-conditioned room at 23±1°C with a 12/12 h light/dark cycle with access *ad libitum* to water and food (Ralston Purina Co., Chicago, IL, USA). Male WT and IL-1R1^{-/-} mice (4-6 weeks old), weighing 20-25 g were randomly split into groups (n=4-8 for each time point). All experimental protocols were approved by the Hong Kong Department of Health and the Committee on the Use of Live Animals for Teaching and Research of The University of Hong Kong (Hong Kong, China; approval no., 1327-06).

Intraperitoneal challenges. To elucidate the role of IL-1 β in induction of DNA methylation *in vivo*, WT and IL-1R1^{-/-} mice (n=8 in each group) were treated with intraperitoneal injection of recombinant mouse IL-1 β (5 μ g/kg/day; R&D Systems, Inc., Minneapolis, MN, USA) on day 0 and day 2. Controls for the two strains (n=4) were treated with an identical volume of phosphate-buffered saline (PBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) solution. The dose of IL-1 β was selected based on dose response studies to induce an inflammatory response (19) and preliminary experiments where it was observed that 5 μ g/kg/day of IL-1 β caused measurable effects.

Preparation of specimens. Mice were sacrificed using anesthesia at 1 day, 3 days, 1 week and 2 weeks subsequent to injection. Blood was taken from the tail vein 0, 1, 4 and 8 h following IL-1 β injection and subsequent to sacrifice to investigate the serum levels of IL-1 β , IL-6 and NO. The stomach was resected and snap-frozen for additional analysis.

Measurement of cytokines. The blood levels of proinflammatory cytokines IL-1 β and IL-6 were determined using the mouse IL-1 β and IL-6 Quantikine[®] Colorimetric Sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc.) according to the manufacturer's protocol. Absorbance

was measured at 450 nm by a microplate reader (μ Quant[™] Microplate Spectrophotometer; Bio-Tek Instruments, Inc., Winooski, VT, USA). Each measurement was repeated in triplicate, and the mean value was recorded (in pg/ml).

Methylation analysis. Genomic DNA was extracted from frozen mucosa specimens using the DNeasy Blood & Tissue kit (Qiagen GmbH, Hilden, Germany). The promoter methylation status of E-cad was assessed by restriction enzyme digestion followed by polymerase chain reaction (PCR) as previously described (20). Briefly, 500 ng of genomic DNA was digested with restriction enzymes *Hpa*II (40 units/ml) and *Msp*I (50 units/ml; both Invitrogen; Thermo Fisher Scientific) at 37°C overnight in a total volume of 20 μ l. *Hpa*II and *Msp*I cut DNA at the CCGG sequence; however, *Hpa*II does not cut when the internal cytosine is methylated. A parallel assay using heat inactivated *Msp*I was performed for each DNA sample as the negative control (20). DNA samples extracted from the mouse tail was methylated *in vitro* with CpG methylase (M.SssI; Zymo Research, Irvine, CA, USA) was used as a positive control. Following digestion, 1 μ l of each digest underwent PCR amplification. The PCR reaction was performed using AmpliTaq Gold[®] DNA Polymerase (Invitrogen; Thermo Fisher Scientific, Inc.) at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 sec and elongation at 72°C for 45 sec, followed by a final extension step of 5 min at 72°C. The PCR amplification was performed using the GeneAmp[®] 9700 PCR System (Applied Biosystems[®]; Thermo Fisher Scientific, Inc.). PCR products and a 50 bp DNA ladder (Invitrogen; Thermo Fisher Scientific, Inc.) were subjected to electrophoresis on a 2% agarose gel, stained with ethidium bromide (Invitrogen; Thermo Fisher Scientific, Inc.) and visualized using the Gel Doc XR System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Methylation-positive PCR fragments were purified and confirmed by sequencing using the TA-cloning method (Invitrogen; Thermo Fisher Scientific, Inc.) as previously described (21). This experiment was repeated in triplicate. Gene-specific PCR primers are listed in Table I (synthesised by Invitrogen; Thermo Fisher Scientific, Inc.).

Reverse transcription (RT)-quantitative (q)PCR analysis of gene expression. Total RNA was extracted from snap-frozen mucosa specimens using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA (2 μ g) was reverse transcribed using SuperScript[®] First-Strand Synthesis system (Invitrogen; Thermo Fisher Scientific, Inc.) at 65°C for 10 min, followed by 50°C for 30 min and 85°C for 5 min using the Opticon 2 Real-Time PCR system (Bio-Rad Laboratories, Inc.). A control reaction without reverse transcriptase was included to identify any DNA contamination in the samples. RT-qPCR amplification of IL-1 β , IL-6, E-cad and iNOS was performed in the Opticon 2 Real-Time PCR system (Bio-Rad Laboratories, Inc.) using SYBR Green (Bio-Rad Laboratories, Inc.). Gene expression levels were normalized using glyceraldehyde-3-phosphate dehydrogenase as an internal control gene, and compared with the data of untreated mice using the $\Delta\Delta C_q$ method (22). The experiment was repeated in three times. Gene-specific PCR primers are listed in Table I (synthesised by Invitrogen; Thermo Fisher Scientific, Inc.).

Table I. Primer sequences (5' to 3') used for methylation-specific PCR and RT-PCR analysis.

Gene name	Forward	Reverse
E-cadherin ^a	TCCAGGAACCTCCGTGATGA	CCGGTGTCCCTATTGACAG
E-cadherin ^b	GCGTCCCCAGCCAATCAG	GCAGACGCCGAGCAAACAC
Interleukin-1 β ^a	CAGGATGAGGACATGAGCACC	CTCTGCAGACTCAAACCTCCAC
Interleukin-6 ^a	GATGATGCACTTGCAGAA	GTACTCCAGAAGACCAGAGG
Inducible nitric oxide synthase ^a	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGTTTCG
Glyceraldehyde-3-Phosphate Dehydrogenase ^a	GACATCAAGAAGGTGGTGAAGC	GTCCACCACCCTGTTGCTGTAG

^aprimers for RT-PCR analysis. ^bprimers for methylation analysis. PCR, polymerase chain reaction; RT, reverse transcription.

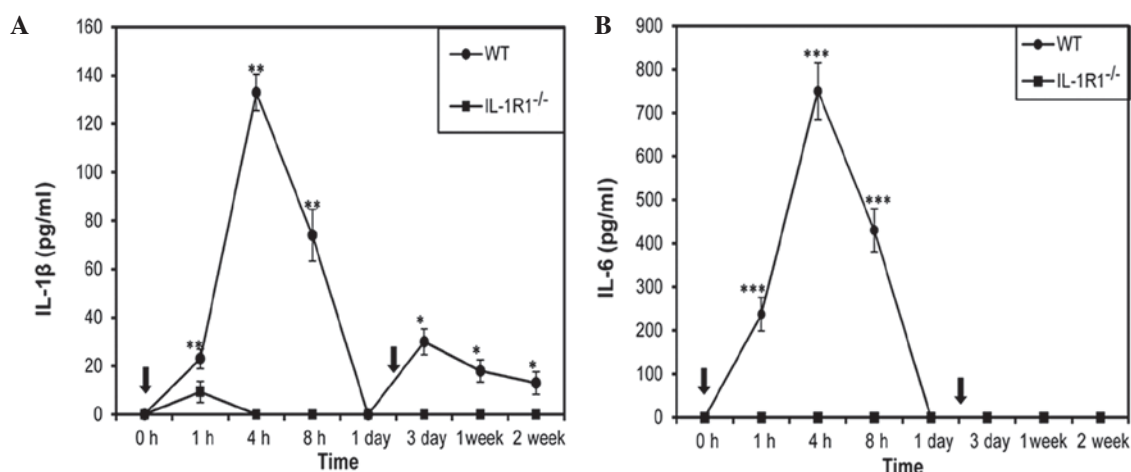


Figure 1. Effect of peripheral IL-1 β challenge on serum levels of (A) IL-1 β and (B) IL-6 in WT and IL-1R1^{-/-} mice. Mice were injected with IL-1 β (5 μ g/kg/day) on day 0 and day 2 (indicated by arrows). Blood was collected and measured by enzyme-linked immunosorbent assay at 0, 1, 4, 8 h subsequent to IL-1 β injection, and after sacrifice at 1 day, 3 days, 1 week and 2 weeks. Data are presented as the mean \pm standard error. Significant differences are shown by *P<0.05, **P<0.005, ***P<0.0005 vs. control mice without injection. IL, interleukin; WT, wild-type; IL-1R1, interleukin 1 receptor type 1.

Nitric oxide assay. Production of NO in mouse serum was measured as nitrate, a more stable end product of NO, based on the Griess reaction method (23) using a Total Nitric Oxide Assay kit (Enzo Life Sciences, Inc., Farmingdale, NY, USA). The optical density was measured at 550 nm using a microplate reader (μ Quant Microplate Spectrophotometer). All standards and samples were measured in triplicate.

Measurement of total DNA methyltransferase (DNMT) activity. Nuclear protein extract was obtained from the frozen gastric mucosa samples according to the manufacturer's protocol using the EpiQuikTM Nuclear Extraction kit (Epigentek, Farmingdale, NY, USA). Protein concentrations were determined using the Bradford method (Bio-Rad Laboratories, Inc.) (24). The total DNMT activity was measured using the EpiQuikTM DNA methyltransferase activity assay kit (Epigentek). Absorbance was determined using a microplate spectrophotometer (μ Quant Microplate Spectrophotometer) at 450 nm, and DNMT activity (OD/h/mg) was calculated according to the manufacturer's protocol.

Statistical analysis. All data are expressed as the mean \pm standard error. The statistical significance between the two experimental groups was analyzed using the student's t-test. All statistical analysis was performed using GraphPad Prism version 5.0

(GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of IL-1 β on induction of inflammatory cytokines. To investigate the impact of peripheral IL-1 β challenge on serum levels of IL-1 β and IL-6, ELISAs were performed on blood collected at 0, 1, 4 and 8 h subsequent to IL-1 β injection, and 1 day, 3 days, 1 week and 2 weeks following sacrifice. Control mice injected with PBS solution demonstrated an undetectable serum level of IL-1 β and IL-6 at all the time points. As shown in Fig. 1A, the IL-1 β level increased significantly between 1 h and 8 h post-injection in wild-type (WT) mice (23 \pm 2.6 pg/ml at 1 h, 133 \pm 4.8 pg/ml at 4 h, 74 \pm 5.2 pg/ml at 8 h; all P<0.005) when compared with IL-1R1^{-/-} mice. Following a peak at 4 h, the levels of IL-1 β progressively decreased to an undetectable level at 1-day post-injection in WT mice. In IL-1R1^{-/-} mice, a low level of IL-1 β was observed only at 1 h post-injection (9.3 \pm 1.4 pg/ml), and the IL-1 β level became undetectable even following a second dose of IL-1 β on day 2. By contrast, in WT mice, following a second dose of IL-1 β , serum levels of IL-1 β remained detectable at 3 days, 1 week and 2 weeks post-injection (30 \pm 2.3 pg/ml, 18 \pm 1.2 pg/ml and 13 \pm 2.3 pg/ml, respectively; all P<0.05) when compared with IL-1R1^{-/-} mice.

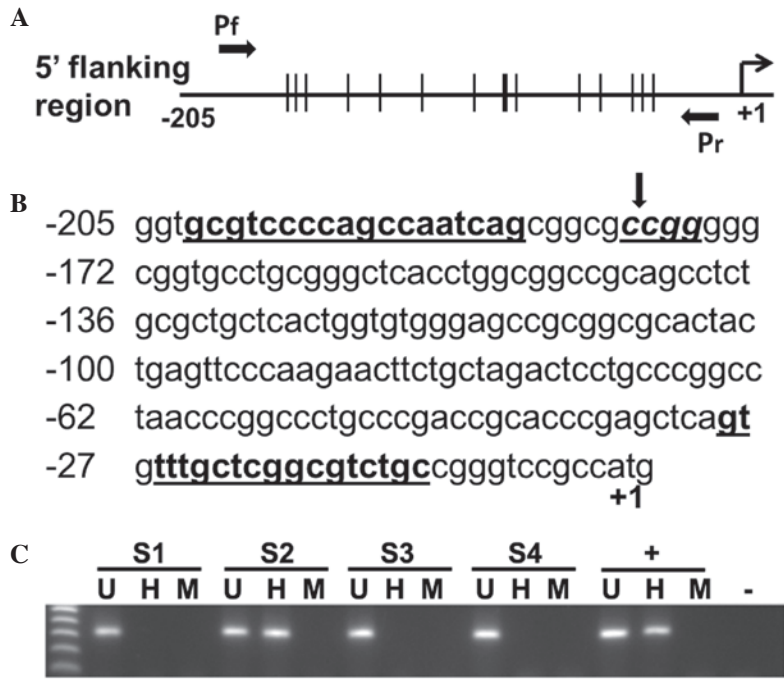


Figure 2. Promoter methylation analysis of the E-cad gene in gastric mucosal specimens from WT mice following IL-1 β treatment. (A) Diagrammatic representation of the 5' flanking region of the E-cad gene. Black arrow indicates the location of the primers used for amplification of the E-cad CpG island. Relative positions of the CpG dinucleotides are indicated as vertical lines (not drawn to scale). (B) Sequence of the region of the E-cad gene analyzed for methylation. Primer sequences and restriction site (CCGG) for *MspI* and *HpaII* are underlined. (C) Representative gel picture revealing the methylation status of E-cad in WT mouse gastric mucosa. E-cad methylation was detected in S2 but not in S1 or S3. Positive control demonstrated methylated normal DNA and negative control without DNA template. E-cad, E-cadherin; WT, wild-type; IL, interleukin; S, sample; U, inactivated *MspI*; H, *HpaII*; M, *MspI*; Pf, primer forward; Pr, primer reverse.

The serum level of IL-6 was assessed to confirm that the induced IL-1 β was due to peripheral inflammatory cytokine IL-1 β challenge and not injection. IL-6 levels increased significantly at 1 h post-injection and reached a peak at 4 h, and subsequently became undetectable at 1-day post-injection (237 \pm 11.5 pg/ml at 1 h, 750 \pm 24.7 pg/ml at 4 h, 430 \pm 5.6 pg/ml at 8 h; all $P < 0.0005$) in WT mice when compared with IL-1R1 $^{-/-}$ mice (Fig. 1B). Unlike IL-1 β , the IL-6 level was not detectable at day 3, 1 week or 2 weeks, even following a second dose of IL-1 β on day 2. In injected IL-1R1 $^{-/-}$ mice and control mice treated with PBS solution, no IL-6 induction was observed, and the IL-6 level was undetectable at all the time points analyzed (Fig. 1B).

Induction of promoter methylation of E-cad by IL-1 β injection. The promoter methylation status of E-cad was assessed in mice gastric mucosae collected at 1 day, 3 days, 1 week and 2 weeks post-injection of IL-1 β . Methylation of E-cad was absent in the injected WT and IL-1R1 $^{-/-}$ mice (n=8 in each group) at 1-day post-injection. Following a second dose of IL-1 β on day 2, promoter methylation of E-cad was observed in 3/8 (37.5%) WT mice at the 3 day and 1 week time points, and 1/8 (12.5%) WT mice at 2-weeks post-injection (Fig. 2). However, methylation of E-cad was not detected in IL-1R1 $^{-/-}$ mice (n=8) and PBS-treated control mice (n=4) at all time points.

Effect of IL-1 β treatment on mRNA expression of proinflammatory genes, E-cad and iNOS. A significant change in IL-1 β messenger (m)RNA expression in the gastric mucosa

was observed on day 3 in treated WT mice when compared with IL-1R1 $^{-/-}$ mice or controls treated with PBS (all $P < 0.001$). Otherwise, the mRNA expression was comparable between treated WT and IL-1R1 $^{-/-}$ mice or controls at all other time points measured (data not shown). IL-1 β treatment did not induce a statistically significant difference in mRNA expression of IL-6 in WT and IL-1R1 $^{-/-}$ mouse gastric mucosae, and expression of IL-6 was almost undetectable in the control group (data not shown). A slight decrease in the mRNA expression of E-cad was observed between the IL-1 β -treated WT and IL-1R1 $^{-/-}$ mice, as well as in the control mice treated with PBS solution (Fig. 3A). However, IL-1 β treatment induced a higher expression of iNOS in WT mice when compared with IL-1R1 $^{-/-}$ mice. The expression of iNOS was comparable between treated WT mice and IL-1R1 $^{-/-}$ mice on day 3 ($P = 0.058$). A statistically significant difference in iNOS expression was observed at 1-week post-infection in WT mice ($P = 0.0411$; Fig. 3B).

Effect of IL-1 β treatment on NO production. The serum NO levels obtained in the various groups of mice at different time points are illustrated in Fig. 4. The serum level of NO was significantly increased in IL-1 β -injected WT mice when compared with IL-1R1 $^{-/-}$ mice at 4 h (195.33 \pm 22.33 μ M; $P < 0.001$), 8 h (138.34 \pm 16.24 μ M; $P < 0.005$), 3 days (182.60 \pm 32.2 μ M; $P < 0.001$) and 1 week (127.34 \pm 12.14 μ M; $P < 0.005$), respectively. IL-1 β injection did not affect the NO level in IL-1R1 $^{-/-}$ mice; the nitrate concentrations measured at all three time points were comparable to the basal physiological levels measured in the control mice (Fig. 4).

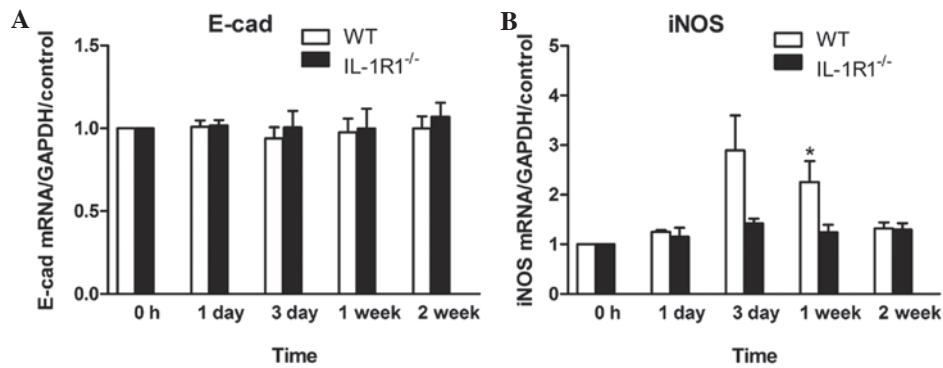


Figure 3. Expression of (A) E-cad and (B) iNOS in gastric mucosa of mice challenged with IL-1 β was analyzed by reverse-transcription quantitative polymerase chain reaction 1 day, 3 days, 1 week and 2 weeks post-injection. The fold increase was calculated at the time point specified relative to internal control genes (GAPDH) and compared with control mice without injection at time 0 h. Data are presented as the mean \pm standard error. Significant differences are shown by * $P < 0.05$. E-cad, E-cadherin; iNOS, inducible nitric oxide synthase; IL, interleukin; WT, wild-type; IL-1R1, interleukin 1 receptor type 1; mRNA, messenger RNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Discussion

Proinflammatory cytokine IL-1 β has a significant role in gastric carcinogenesis (16,17,25). In the present study, IL-1R1^{-/-} mice, which are unresponsive to IL-1 β , were used to fully elucidate the specific role of IL-1 β in linking gastric inflammation to DNA methylation induction. The results indicated that peripheral IL-1 β challenge may induce proinflammatory cytokine release. This is evidenced by the inducible serum IL-1 β and IL-6 in injected WT mice when compared with IL-1R1^{-/-} mice lacking functional receptors to IL-1 β , or control mice without IL-1 β injection (these mice did not have inducible production of serum IL-1 β and IL-6). These findings were in agreement with previous studies that reported that IL-1 β is a significant mediator in initiation of the immune response (26,27). The present study additionally assayed the serum levels of IL-6 to confirm that the increase in IL-1 β was due to induction instead of exogenous injection. A previous study additionally demonstrated that peripheral challenge of IL-1 β was able to induce IL-6 release (28). However, the serum levels of IL-6 were very low and dissipated quickly, suggesting that the immune response initiated by IL-1 β treatment may not be a potent stimulator for IL-6 production (28).

The authors of the present study previously demonstrated that *H. pylori* infection induced IL-1 β production and E-cad methylation in *in vitro* and *in vivo* experiments. This methylation induction effect could be reversed following the administration of IL-1 β antagonist, interleukin-1 receptor antagonist (5,17,18). These findings suggest the potential mechanistic basis of *H. pylori* in induction of epigenetic changes for gastric diseases. In the present study, having demonstrated that IL-1 β injection was able to initiate an immune response evidenced by release of proinflammatory cytokines, the methylation status of E-cad was assessed. Methylation of E-cad was detected in IL-1 β injected WT mice on day 3, following a second dose of IL-1 β . This finding was supported by a significant upregulation of NO detected on day 3. The need for a second dose of IL-1 β may be associated with the short half-life of the IL-1 β protein, which inadequately elicited a strong immune response following a single dose (29). Disappearance of E-cad methylation was detected 2 weeks post-infection, which was consistent with the plateau of IL-1 β .

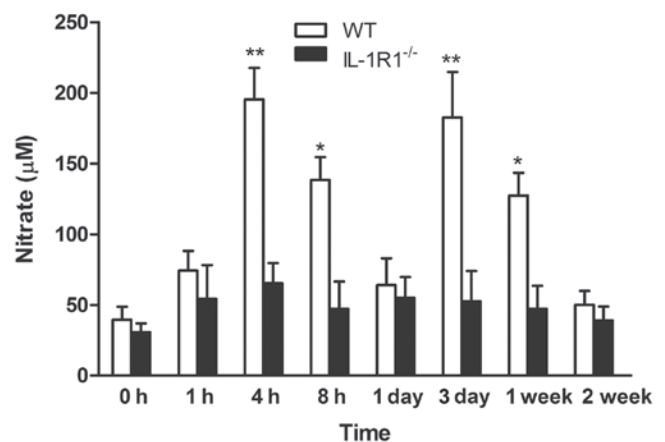


Figure 4. Effect of peripheral IL-1 β challenge on serum levels of NO production. WT and IL-1R1^{-/-} mice were treated with IL-1 β (5 μ g/kg/day) on day 0 and day 2. NO was measured as nitrate in mouse serum 0, 1, 4 and 8 h subsequent to IL-1 β injection, and following sacrifice at 1 day, 3 days, 1 week and 2 weeks. Data are presented as the mean \pm standard error. Significant differences are shown by * $P < 0.005$, ** $P < 0.001$ vs. control mice without injection. IL, interleukin; NO, nitric oxide; WT, wild-type; IL-1R1, interleukin 1 receptor type 1.

This is additionally in agreement with previous reports that severe inflammation is required for initiation and maintenance of methylation induction (5,30). Mechanistically, the present study observed the absence of E-cad methylation in IL-1R1^{-/-} mice and controls, suggesting that E-cad methylation may be mediated through an IL-1 β -dependent signaling pathway. To the best of our knowledge, no other study has demonstrated that IL-1 β injection may induce promoter methylation of E-cad *in vivo*. Furthermore, the results of the present study suggest that inflammatory cytokines may directly trigger epigenetic changes.

The results of the present study additionally support the mechanism of *H. pylori*-induced epigenetic changes and gastric carcinogenesis via IL-1 β activation of iNOS and NO production, as proposed in our previous studies (5,17,18). Excessive NO production causes tissue damage with subsequent cellular genome injuries (10). Previous studies have demonstrated that NO may activate expression of DNMTs, which are the final effector of DNA methylation (16,18,31).

However, no significant changes in total DNMT activity were detected in the present study (data not shown). These findings are consistent with previous studies, suggesting that mRNA expression of DNMTs was not increased in non-cancerous gastric mucosa in humans and mice (5,32). It is likely that aberrant DNA methylation results from an imbalance between various DNMTs (32-34). However, the present study provides an improved insight into the role of NO in the IL-1 β activated methylation-dependent silencing pathway that may link inflammation with gastric carcinogenesis.

Previous studies have reported that IL-1R1 $^{-/-}$ mice demonstrate attenuated infiltration of inflammatory cells in the stomach with *H. pylori* infection compared with WT mice (5,6). Among the infiltrating inflammatory cells in *H. pylori*-infected gastric mucosa, the IL-1R1 $^{-/-}$ mice demonstrated a significantly reduced number of macrophages and neutrophils compared with the WT mice. Based on the absence of E-cad methylation in IL-1R1 $^{-/-}$ mice, it may be speculated that IL-1 β directly induces epigenetic deregulation and thus promotes gastric carcinogenesis. This is supported by the observation that IL-1R1 $^{-/-}$ mice demonstrate reduced susceptibility to gastric carcinogenesis (5,6). Although significant progress has been made with regard to our understanding of the mechanistic link between inflammation and gastric cancer development, more comprehensive characterization of the role of inflammation in the initiation and maintenance of epigenetic changes is required.

In conclusion, the current study demonstrated that IL-1 β may directly trigger epigenetic alterations that lead to suppression of gene expression. The results provide a significant mechanistic link between inflammation and gastric cancer development. These findings also highlight the importance of developing treatment strategies that target persistent inflammatory responses to achieve the prevention and eradication of disease.

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