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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

# IL33 Is a Stomach Alarmin That Initiates a Skewed Th2 Response to Injury and Infection

Jon N. Buzzelli,[1,2] Heather V. Chalinor,[1] Daniel I. Pavlic,[1] Philip Sutton,[1,3] Trevelyan R. Menheniott,[1] Andrew S. Giraud,[1,2] and Louise M. Judd[1,2]

[1]Murdoch Children's Research Institute, [2]Department of Paediatrics, Royal Children's Hospital, [3]Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, Parkville, Victoria, Australia

**SUMMARY**

Interleukin 33 is a stomach alarmin that is increased immediately after gastric insult and infection but is suppressed during long-term *Helicobacter pylori* infection. Interleukin 33 potently activates gastric T helper 2 immunity, which suggests that its loss during *H pylori* infection may be important in establishing T helper 1 immunity.

**BACKGROUND & AIMS:** Interleukin (IL)33 is a recently described alarmin that is highly expressed in the gastric mucosa and potently activates Th2 immunity. It may play a pivotal role during *Helicobacter pylori* infection. Here, we delineate the role of IL33 in the normal gastric mucosa and in response to gastropathy.

**METHODS:** IL33 expression was evaluated in mice and human biopsy specimens infected with *H pylori* and in mice after dosing with aspirin. IL33 expression was localized in the gastric mucosa using immunofluorescence. Mice were given 1 or 7 daily doses of recombinant IL33 (1 $\mu$g/dose), and the stomach and the spleen responses were quantified morphologically, by flow cytometry and using quantitative reverse-transcription polymerase chain reaction and immunoblotting.

**RESULTS:** In mice, the IL33 protein was localized to the nucleus of a subpopulation of surface mucus cells, and co-localized with the surface mucus cell markers Ulex Europaeus 1 (UEA1), and Mucin 5AC (Muc5AC). A small proportion of IL33-positive epithelial cells also were Ki-67 positive. IL33 and its receptor Interleukin 1 receptor-like 1 (ST2) were increased 4-fold after acute (1-day) *H pylori* infection, however, this increase was not apparent after 7 days and IL33 expression was reduced 2-fold after 2 months. Similarly, human biopsy specimens positive for *H pylori* had a reduced IL33 expression. Chronic IL33 treatment in mice caused systemic activation of innate lymphoid cell 2 and polarization of macrophages to the M2 phenotype. In the stomach, IL33-treated mice developed transmural inflammation and mucous metaplasia that was mediated by Th2/signal transducer and activator of transcription 3 signaling. Rag-1$^{-/-}$ mice, lacking mature lymphocytes, were protected from IL33-induced gastric pathology.

**CONCLUSIONS:** IL33 is highly expressed in the gastric mucosa and promotes the activation of T helper 2–cytokine–expressing cells. The loss of IL33 expression after prolonged *H pylori* infection may be permissive for the T helper 1–biased immune response observed during *H pylori* infection and subsequent precancerous progression. *(Cell Mol Gastroenterol Hepatol 2015;1:203–221; http://dx.doi.org/10.1016/j.jcmgh.2014.12.003)*

*Keywords:* IL33; *Helicobacter pylori*; Inflammatory Response; Gastric Cancer.

Recently, it was suggested that a class of specialized immune regulators, called *alarmins*, are involved in activating an acute immune response after infection or injury.[1] Alarmins describe a class of multifunctional cytokines released by necrotic cells in response to infection or injury to promote an innate and adaptive immune response.[1] One such cytokine, interleukin 33 (IL33),[2] enhances expression of T helper (Th)2 cytokines[3] and activates multiple immune regulatory cells including group 2 innate lymphoid cells (ILC2),[4] basophils,[5–7] mast cells,[8] eosinophils,[7] natural killer T cells,[6] and Th2 lymphocytes.[7]

Current research on IL33 has focused mainly on its role in lung pathology. However, IL33 also has been shown to provide protection during gastrointestinal infection and dextran sodium sulfate–induced colitis by vigorously enhancing Th2 immunity.[9–12] Collectively, these findings suggest that IL33 may be a crucial mediator of the immune response after damage or infection in epithelial tissues. IL33 is highly expressed in the stomach,[3] however, little is known of its gastric function. In this study, we address which cells of the gastric mucosa express IL33, how IL33 expression changes with damage and infection, and characterize the function of IL33 in the stomach.

Similar to the lung and colon, the gastric mucosa is vulnerable to chronic infection and inflammation, which may

***Abbreviations used in this paper:*** **AB, Alcian blue; DC, dendritic cell; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal–regulated kinase; FBS, fetal bovine serum; HBSS, Hank's balanced salt solution; IL, interleukin; ILC, innate lymphoid cell; mRNA, messenger RNA; NF-$\kappa$B, nuclear factor-$\kappa$B; PAS, periodic acid–Schiff; PCR, polymerase chain reaction; QRT-PCR, quantitative reverse-transcription polymerase chain reaction; SMC, surface mucus cells; SPF, specific pathogen free; SS1, Sydney strain 1; STAT, signal transducer and activator of transcription; TFF, trefoil factor; Th, T-helper; WT, wild type.**



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promote serious pathologic outcomes such as peptic ulceration, intestinal metaplasia, and adenocarcinoma. The primary causative agent in this regard is *Helicobacter pylori*. The capacity of *H pylori* to colonize the gastric mucosa and promote a favorable environment for gastric disease is highly dependent on the response of the host immunity to insult and invasion. IL33 is highly expressed in the gastric epithelium[3] and therefore may be an important factor in limiting *H pylori* colonization and consequent inflammatory pathology. Despite its disposition, a functional relationship between IL33 expression, *H pylori* infection, and resultant pathology has yet to be described. Here, we address the role of IL33 during the gastric immune response to the ulcerogen aspirin and in acute and chronic *H pylori* infection. We show IL33 protein to be localized predominately to the nuclei of a subset of surface mucus cells (SMCs), and that systemic administration of IL33 causes a Th2-biased immune response in the stomach and atypical gastric pathology. Furthermore, IL33 messenger RNA (mRNA) is lost in human gastric samples positive for *H pylori* and mice with prolonged *H pylori* infection; events that may result in the skewed Th1/Th17 immunity observed during *H pylori* infection and subsequent pathology.

## Materials and Methods

### Cells

MKN28 cells were grown in RPMI media containing 10% fetal bovine serum (FBS), 100 mmol/L nonessential amino acids (Sigma, St. Louis, MO), and 100 mmol/L penicillin-streptomycin (Sigma). One hour before experiments cell cultures were given fresh RPMI media containing 0.5% FBS.

*Time course.* Cells were given fresh media containing 100 ng/mL of recombinant human IL33 (Shenandoah, St. Louis, MO). Media was left on cells for 0, 1, 5, 15, 30, or 60 minutes before cells were collected (n = 3/time point). The 0-minute time point did not receive fresh media.

*Dose response.* Cells were given fresh media containing 0, 0.01, 0.1, 1, 10, or 100 ng/mL of recombinant human IL33 (Shenandoah) (n = 3/concentration). Media was left on cells for 5 minutes before cell harvest.

*Cell harvesting.* All cells were harvested using RIPA buffer containing 2 nmol/L of sodium fluoride, 2 nmol/L sodium orthovanadate, and 1 protease inhibitor cocktail per 10 mL of RIPA buffer solution (Roche Diagnostic, Indianapolis, IN).

### Mice

Wild-type (WT) mice were from a C57Bl/6 background, 10–12 weeks old. Most mice were housed in individually ventilated, high-efficiency particulate absorption–filtered cages (specific pathogen free [SPF] conditions), and a small subset of mice were kept in a different facility with covered, but not individually ventilated, shoe box cages (conventional conditions). All mice had autoclaved water and irradiated food. Genetically modified mice were genotyped by multiplex polymerase chain reaction (PCR) as previously described.[13] Approval was obtained from Murdoch Children's Research Institute.

### Aspirin Treatment

WT mice ($n \geq 5$) and trefoil factor (TFF)$2^{-/-}$ mice ($n \geq 4$) were treated with 300 mg (200 $\mu$L) of aspirin (Sigma) via an oral gavage. Aspirin was suspended in 1% methylcellulose aqueous solution (Sigma) and control mice were gavaged with 200 $\mu$L of 1% methylcellulose. Mice were starved overnight before aspirin administration. One hour after treatment, mice were given food and killed either 4 or 24 hours after treatment.

### Cytokine Treatment

WT mice ($n \geq 5$) were injected intraperitoneally once daily with 1 $\mu$g of recombinant human IL33 (Shenandoah) or saline for 7 days. One hour before culling, mice were given an additional dose of IL33.

### Tissue Preparation

Mouse stomachs were prepared and analyzed as previously described.[14] Half of the fundus and antrum were collected in liquid nitrogen for RNA and protein. For histologic examination, bisected tissue was fixed in 4% paraformaldehyde in phosphate-buffered saline. The spleen also was collected for analysis. One half was frozen in liquid nitrogen for RNA and protein. The second half was fixed in 4% paraformaldehyde in phosphate-buffered saline.

### Cell Isolation and Flow Cytometry (Fluorescence-Activated Cell Sorting) Analysis

*Spleen cell isolation.* One third of the spleen was made into a single-cell suspension. Red blood cells were lysed with ammonium-Tris chloride buffer for 5 minutes at room temperature. Cell suspensions were stained with fluorescently labeled antibodies (Supplementary Table 1) in Hank's balanced salt solution (HBSS) (2 mmol/L EDTA, 2% FBS), washed, and resuspended in HBSS for fluorescence-activated cell sorter analysis (LSRII and FACSDiva v6.1.1; Becton Dickinson, Franklin Lakes, NJ).

*Stomach cell isolation.* Stomachs were collected in HBSS (2 mmol/L EDTA, 2% FBS), perfused with digestion media (1 × HBSS [without calcium and magnesium], 5 mmol/L EDTA, 5% FBS, and 1 mmol/L dithiothreitol), and incubated at 37°C for 15 minutes. Stomachs then were cut and incubated in digestion media for a further 15 minutes, and then passed through a 70-$\mu$m cell strainer. Cell suspensions were centrifuged and resuspended in 1 × HBSS. Cells were analyzed as described for spleen samples.

*Fluorescence-activated cell sorter analysis.* Dead, autofluorescent, and aggregated cells were removed from the anlysis on the basis of forward scatter (FSC), side scatter (SSC), and propidium iodide staining. The total number of events for each cell type (Supplementary Table 1) was quantified using CountBright beads (#36950; Invitrogen, Carlsbad, CA).

### Macrophage Isolation and Analysis

To harvest peritoneal macrophages, 5 mL of HBSS (Sigma) containing 10 U/mL of heparin (Sigma) was