ORIGINAL ARTICLE

A pro-inflammatory role for Th22 cells in *Helicobacter pylori*-associated gastritis

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ABSTRACT

Objective Helper T (Th) cell responses are critical for the pathogenesis of *Helicobacter pylori*-induced gastritis. Th22 cells represent a newly discovered Th cell subset, but their relevance to *H. pylori*-induced gastritis is unknown.

Design Flow cytometry, real-time PCR and ELISA analyses were performed to examine cell, protein and transcript levels in gastric samples from patients and mice infected with *H. pylori*. Gastric tissues from interleukin (IL)-22-deficient and wild-type (control) mice were also examined. Tissue inflammation was determined for pro-inflammatory cell infiltration and pro-inflammatory protein production. Gastric epithelial cells and myeloid-derived suppressor cells (MDSC) were isolated, stimulated and/or cultured for Th22 cell function assays.

Results Th22 cells accumulated in gastric mucosa of both patients and mice infected with *H. pylori*. Th22 cell polarisation was promoted via the production of IL-23 by dendritic cells (DC) during *H. pylori* infection, and resulted in increased inflammation within the gastric mucosa. This inflammation was characterised by the CXCR2-dependent influx of MDSCs, whose migration was induced via the IL-22-dependent production of CXCL2 by gastric epithelial cells. Under the influence of IL-22, MDSCs, in turn, produced pro-inflammatory proteins, such as S100A8 and S100A9, and suppressed Th1 cell responses, thereby contributing to the development of *H. pylori*-associated gastritis.

Conclusions This study, therefore, identifies a novel regulatory network involving *H. pylori*, DCs, Th22 cells, gastric epithelial cells and MDSCs, which collectively exert a pro-inflammatory effect within the gastric microenvironment. Efforts to inhibit this Th22-dependent pathway may therefore prove a valuable strategy in the therapy of *H. pylori*-associated gastritis.

Significance of this study

What is already known on this subject?

- Th22 cells represent a newly discovered Th cell subset, exerting either pathogenic or protective properties depending on the context of inflammation.
- Th22 cells and IL-22 have been confirmed to play critical roles in chronic inflammatory conditions.
- IL-22 is expressed in the mucosa of various species, including humans.
- Inflammatory cell infiltration is the most likely result in pathophysiology for gastritis and is considered a clinical hallmark of the disease.

What are the new findings?

- Th22 cells accumulated in gastric mucosa of both patients and mice infected with *Helicobacter pylori.*
- Gastric IL-22 expression correlated with *H. pylori* colonisation and the severity of gastritis and contributed to a proinflammatory role.
- *H. pylori* infection-induced inflammation was characterised by the CXCR2-dependent influx of myeloid-derived suppressor cells (MDSCs), whose migration was induced via the IL-22-dependent production of CXCL2 by gastric epithelial cells

How might it impact on clinical practice in the foreseeable future?

- Our in vitro and in vivo data together provide a multistep model of inflammation during *H. pylori* infection involving interactions between *H. pylori*, Th22 cells, dendritic cells, gastric epithelial cells and MDSCs within the gastric mucosa. In this regard, our findings suggest several possible therapeutic targets, including IL-22, S100A8 and S100A9. Given the apparent relationship between IL-22 levels and the severity of gastric inflammation observed in *H. pylori*-infected patients, thought should be given to the use of IL-22 and/or Th22 cells as novel diagnostic biomarkers for *H. pylori* infection.

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INTRODUCTION

Helicobacter pylori is a human pathogen that infects nearly half the world's population. Infection with *H. pylori* is frequently associated with chronic inflammation of the gastric mucosa (gastritis) and can lead to peptic ulceration and gastric cancer.[1] Although the development of *H. pylori*-associated

gastritis remains poorly understood, it is believed that the nature of the $CD4^+$ helper T (Th) cell response is a key contributing factor. For example, a mouse model of *H. pylori*-induced gastritis, Th cells were found to be both 'necessary and sufficient' for the development of *H. pylori*-associated gastritis.[2]

Cells of the Th22 lineage secrete IL-22 (Th22 cells) represent a newly discovered Th cell subset and can contribute to both protective and pathological immune responses. In mice, it has been reported that IL-22 has protective effects in IBD[3] and hepatitis.[4] By contrast, others have found that IL-22 has a pathological role in mouse models of *Toxoplasma gondii* infection[5] and psoriasis,[6] while in human IBD, IL-22 appeared to be pro-inflammatory.[7] To date, virtually nothing is known about Th22 cells during *H. pylori* infection in either humans or mice and we were therefore interested to explore a possible relationship.

In the current study, we have for the first time demonstrated that *H. pylori*-infected patients have an over-abundance of Th22 cells and that this result is paralleled in mice infected with *H. pylori*. The differentiation of these Th22 cells is induced by IL-23 derived from *H. pylori*-activated dendritic cells (DC) and its overall effect is to promote inflammation. In this regard, Th22 polarisation stimulates gastric epithelial cells to secrete CXCL2, which, in turn, recruits myeloid-derived suppressor cells (MDSC) that produce the pro-inflammatory proteins S100A8 and S100A9 and inhibit Th1 cell responses. Collectively, these data highlight a pathological role for Th22 cells in *H. pylori*-induced gastritis.

MATERIALS AND METHODS

Patients and specimens

The gastric biopsy specimens and blood were collected from 78 *H. pylori*-infected and 59 uninfected patients who underwent upper oesophagogastroduodenoscopy for dyspeptic symptoms at XinQiao Hospital (see online supplementary table S1). *H. pylori* infection was determined by [^{14}C] urea breath test and rapid urease test of biopsy specimens taken from the antrum and subsequently conformed by real-time PCR for 16S rDNA and serology test for specific anti-*H. pylori* antibodies (Abs). For isolation of human primary gastric epithelial cells, fresh non-tumour gastric tissues (at least 5 cm distant from the tumour site) were obtained from patients with gastric cancer who underwent surgical resection and were determined as *H. pylori*-negative individuals as above at the Southwest Hospital. None of these patients had received chemotherapy or radiotherapy before sampling. Individuals with atrophic gastritis, hypochlorhydria, antibiotics treatment, autoimmune disease, infectious diseases and multiprimary cancer were excluded. The study was approved by the ethics committee of XinQiao Hospital and Southwest Hospital of Third Military Medical University. Written informed consent was obtained from each patient.

Antibodies and other reagents

See online supplementary methods.

Mice

All breeding and experiments were undertaken with review and approval from the Animal Ethical and Experimental Committee of Third Military Medical University. Specific pathogen free (SPF) female BALB/c and C57BL/6 wild-type (WT) mice were purchased from the Experimental Animal Centre of the Third Military Medicine University. Through material transfer agreements, C57BL/6 IL-23p19 knockout (KO) (IL-23 KO) and BALB/c IL-22 KO mice were obtained from Dr Wenjun Ouyang (Genentech). All mice were viral Ab free for pathogenic murine viruses and negative for pathogenic bacteria including *Helicobacter* spp and parasites (see online supplementary table S2), and were maintained under SPF conditions in a barrier-sustained facility and provided with sterile food and water.

Bacteria culture and infection of mice with bacteria

H. pylori NCTC 11637 (*cagA* positive) (WT *H. pylori*) and *cagA*-KO mutant *H. pylori* NCTC 11637 (*ΔcagA*) were grown in brain-heart infusion plates containing 10% rabbit blood at 37°C under microaerophilic conditions. For infecting mouse, bacteria were amplified in Brucella broth with 5% fetal bovine serum (FBS) with gentle shaking at 37°C under microaerobic conditions. After culture for 1 day, live bacteria were collected and adjusted to 10^9 colony forming units (CFU)/mL. The mice were fasted overnight and orogastrically inoculated twice at a 1-day interval with 3×10^8 CFU bacteria. *H. pylori* infection status and *H. pylori*-induced gastritis in murine experiments were confirmed (data not shown).

Generation of bone marrow chimaera mice

See online supplementary methods.

Cytokine/antibodies/CXCR2 antagonist administration

One day after infection, mice were injected intraperitoneally with 25 μg of recombinant murine IL-22 or IL-23, or anti-IL-22, anti-IL-17A, anti-IL-17F, anti-interferon (IFN)-γ or isotype control Abs (100 μg), or anti-CXCR2 and/or anti-CXCL2 Abs or rat immunoglobin (Ig)G2a and/or IgG2b (100 μg), or 4 mg/kg SB225002 or dimethylsulfoxide (DMSO) control, and repeated every week until the mice were sacrificed.

Evaluation of inflammation

The mice were sacrificed at the indicated times. The greater curvature of the stomach was cut to perform H&E staining and immunofluorescence. The intensity of inflammation was evaluated independently by two pathologists according to previously established criteria.[8]

Isolation of single cells from tissues and DCs preparation

See online supplementary methods.

Cell/tissue culture and stimulation

Human primary gastric epithelial cells were purified from gastric tissue single-cell suspensions in a Magnetic-activated cell sorting (MACS) column purification system using anti-CD326 magnetic beads (Miltenyi Biotec). Human gastric epithelial cell line AGS cells, primary gastric epithelial cells or gastric tissues were stimulated with WT *H. pylori* and/or *ΔcagA* at different multiplicity of infection (MOI). AGS cells and primary gastric epithelial cells were also stimulated with IL-22 (100 ng/mL) for 1, 3, 6, 12 and/or 24 h. For signal pathway inhibition experiments, AGS cells were pretreated with FLLL32 (10 μM) for 2 h, or STAT3 siRNA or control siRNA (100 nM) for 24 h. DCs were stimulated with WT *H. pylori* and/or *ΔcagA* at different MOI for 6 h. Then the gentamycin was added to kill the bacteria for 2 h and then cells were washed three times. MDSCs were sorted with FACSAria II (BD Biosciences) from blood of *H. pylori*-infected patients and stimulated with IL-22 (100 ng/mL) for 1, 3, 6, 12 and/or 24 h. After coculture, cells were collected for microarray, real-time PCR and western blot, and the culture supernatants were harvested for ELISA.

In vitro T cell culture system

In a 5-day incubation, purified human peripheral or mouse spleen $CD4^+$ T cells (StemCell Technologies) were cocultured (2×10^5 cells/well) with WT *H. pylori* or *ΔcagA* stimulated-DCs from autologous blood; or WT *H. pylori* or *ΔcagA* stimulated-bone marrow–derived dendritic cells (BMDCs) from WT or IL-23 KO mice at 2:1 ratio. Alternatively, $CD4^+$ T cells were cocultured with autologous *ΔcagA*-stimulated DCs at 2:1 ratio supplemented with IL-23 (10 ng/mL) or media alone, or with autologous WT *H. pylori*-stimulated DCs at 2:1 ratio supplemented with IL-23 Ab (10 μg/mL) or control IgG (10 μg/mL). $CD4^+$ T cells were also first labelled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and cocultured (1×10^5 cells/well) with MDSCs at different ratios. After such 5-d incubation, cells were collected and analysed by intracellular cytokine staining.

Chemotaxis assay

Th cell-polarising culture supernatants derived from WT *H. pylori* or *ΔcagA*-stimulated DCs were collected to stimulate primary gastric epithelial cells for 48 h. Then, the secondary primary gastric epithelial cell culture supernatants were again collected as chemoattractant liquids. Sorted MDSCs (1×10^5) were transferred into the upper chambers of transwells (Corning). CXCL2 (10 ng/mL) and chemoattractant liquids under various conditions were placed in the lower chambers. After 24 h culture, migration was quantified by counting cells in the lower chamber and cells adhering to the bottom of the membrane. In some cases, blocking Ab for CXCR2 (10 μg/mL) were added into MDSC suspensions and incubated for 2 h before chemotaxis assay.

Immunofluorescence, real-time PCR, flow cytometry, ELISA, western blot analysis and microarray experiments are described in online supplementary methods.

Statistical analysis

Results are expressed as mean±SEM. Student t test was generally used to analyse the differences between two groups, but when the variances differed, the Mann–Whitney U test was used. Inflammation score data were analysed by the Mann–Whitney U test. For multigroup data analysis, an analysis of variance was used. Correlations between parameters were assessed using Pearson correlation analysis and linear regression analysis, as appropriate. SPSS statistical software (V.13.0) was used for all statistical analysis. All data were analysed using two-tailed tests, and $p<0.05$ was considered statistically significant. Microarray data analysis was performed with the assistance of Genminix Informatics. Clustering was performed using Cluster V.3.0 and patterns were created and viewed using Java TreeView V.1.0.13 software. Raw data from each array were analysed using TwoClassDif.

RESULTS

Th22 cells are enriched in gastric mucosa of *H. pylori*-infected patients and mice

To evaluate the potential role of Th22 cells in *H. pylori*-associated pathology, we compared the Th22 cell levels in gastric tissues. Notably, the gastric mucosa of *H. pylori*-infected patients showed a higher frequency of Th22 cells (figure 1A). Also, the overall levels of IL-22 mRNA (figure 1B) and protein (figure 1C) were higher, respectively, in the gastric mucosa of *H. pylori*-infected patients. Next, IL-22 expression was positively correlated with *H. pylori* colonisation (figure 1D), suggesting induction and/or maintenance of Th22 cells by *H. pylori*.

The presence of *cagA* is strongly associated with the development of gastritis.[9] Notably, we found that IL-22 expression in *cagA*-positive patients was significantly higher than that in *cagA*-negative individuals (figure 1E). Consistent with our findings in humans, Th22 cells were only detected in WT *H. pylori*-infected mice, reaching a peak 35 days postinfection (p.i.) (figure 1F). Similar observations were made in C57BL/6 mice (see online supplementary figure S1A, B), indicating a role for *cagA* across multiple host genetic backgrounds.

It has previously been reported that—apart from Th cells—IL-22 can also be produced by natural killer cells, lymphoid tissue inducer-like cells and innate lymphoid cells.[10] Using our mouse model of *H. pylori* infection, we found no evidence for IL-22 expression in these cells (see online supplementary figure S1E), suggesting that Th cells are the only immune cells that produce IL-22 in gastric mucosa during *H. pylori* infection. Finally, we also assessed whether we could detect Th22 cells outside the gastric mucosa during *H. pylori* infection in mice, but found minimal numbers of Th22 cells in bone marrow (BM), blood, spleen, mesenteric lymph node and Peyer's patches (see online supplementary figure S2).

DCs stimulated by *H. pylori* induce Th22 cells via IL-23

DCs are known to be critically important in both priming and maintaining Th22 cells.[11] We, therefore, sought to determine whether DCs were responsible for the development of Th22 cells during *H. pylori* infection. Interestingly, *H. pylori*-stimulated DCs were able to potently induce $CD4^+$ T cells to differentiate into Th22 cells (figure 2A), and this was most noticeable when using a WT *H. pylori* strain. Similarly in mice, BMDCs can effectively induce Th22 cell differentiation following WT *H. pylori* exposure (figure 2B).

It has previously been shown that Th22 cells are induced by IL-23[12] and that DCs are potent producers of IL-23 at sites of bacterial infection.[13] To see whether similar mechanisms might operate in *H. pylori* infection, we first found that IL-23 protein were significantly upregulated in WT *H. pylori*-stimulated DCs compared with those stimulated with *ΔcagA* or no bacteria (figure 2C). Next, we found that blocking IL-23 with neutralising Ab effectively inhibited the generation of Th22 cells (figure 2D). Consistent with this, BMDCs from IL-23 KO mice failed to induce Th22 cell polarisation (figure 2B). Conversely, provision of exogenous IL-23 significantly increased Th22 cell polarisation (figure 2D). Collectively, these findings indicate that *H. pylori*-stimulated DCs express IL-23 which, in turn, promotes Th22 cell differentiation in vitro.

To assess if a similar phenomenon might occur in vivo, we infected WT and IL-23 KO mice with *H. pylori* and found that, compared with WT mice, IL-23 KO mice developed significantly fewer Th22 cells in gastric mucosa (figure 2E), indicating that IL-23 does indeed have a permissive role in inducing Th22 cell development in vivo. By generation of BM chimaera mice, we found that IL-23-producing BM-derived cells are largely responsible for Th22 cell development during *H. pylori* infection in this model (figure 2F). Taken together, our data demonstrate that IL-23 plays an essential role in Th22 cell induction by DCs in vitro and are consistent with the operation of similar mechanisms in vivo.

IL-22 has proinflammatory effects during *H. pylori* infection

To understand the possible biological effects of Th22 cell induction during *H. pylori* infection, we compared IL-22 expression

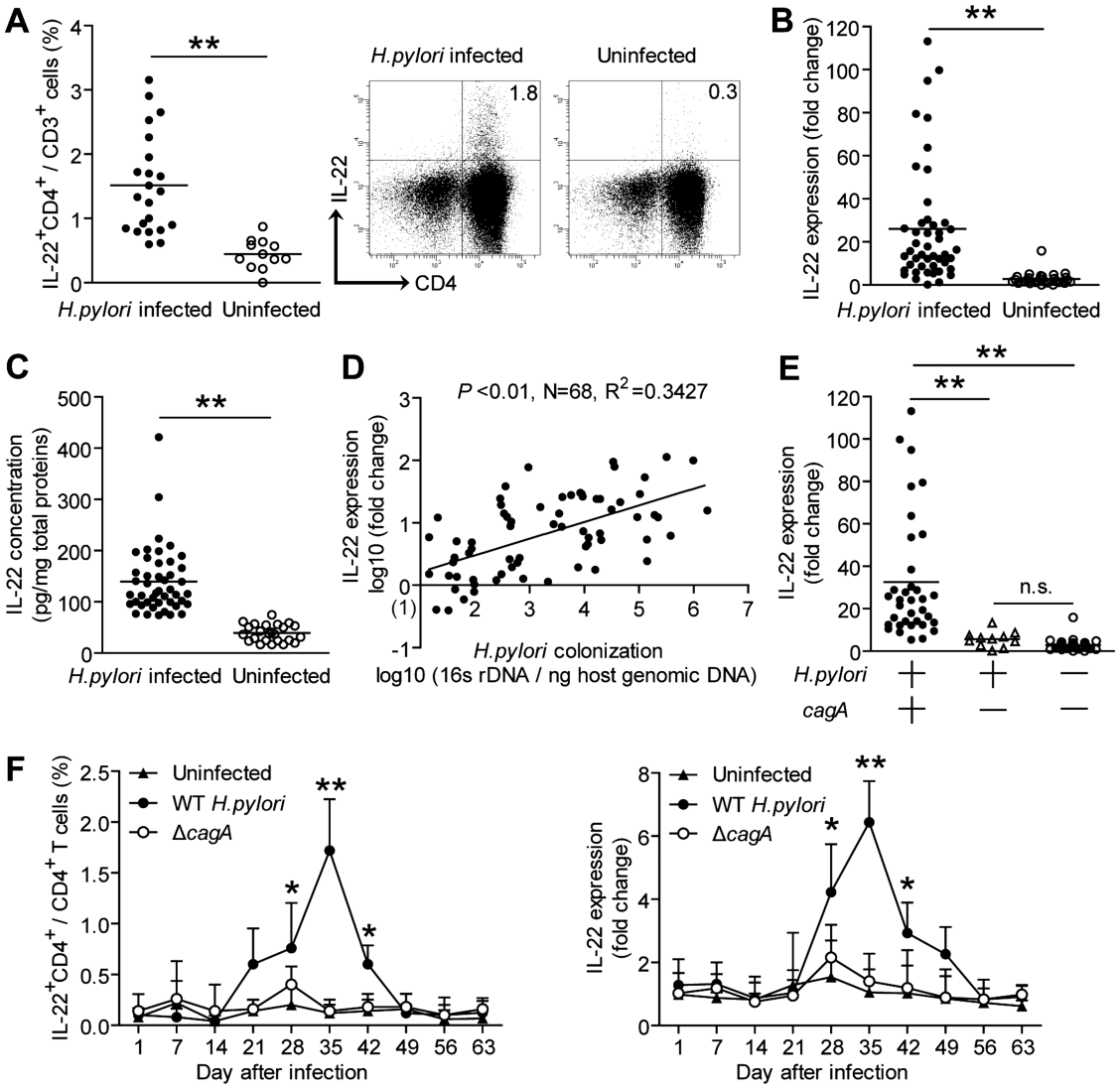

Figure 1 Th22 cells accumulated in gastric mucosa of *Helicobacter pylori*-infected patients and mice. (A) The percentage of T helper type 22 (Th22) cells in $CD3^+$ cells in gastric mucosa of *H. pylori*-infected (n=22) and uninfected donors (n=12) was compared. Results are expressed as percentage of Th22 cells in $CD3^+$ T cells. (B and C) Interleukin (IL)-22 mRNA expression (B) and IL-22 protein concentrations (C) in gastric mucosa of *H. pylori*-infected (n=46) and uninfected donors (n=27) were compared. (D) The correlation between IL-22 expression and *H. pylori* colonisation was analysed. (E) IL-22 mRNA expression in gastric mucosa of $cagA^+$ *H. pylori*-infected (n=35), $cagA^-$ *H. pylori*-infected (n=11) and uninfected donors (n=29) were compared. (F) Dynamic changes of Th22 cell response and IL-22 mRNA expression in wild-type (WT) *H. pylori*-infected, *ΔcagA*-infected and uninfected BALB/c mice. n=5 per group per time point in *F*. *p<0.05, **p<0.01 n.s. p>0.05 for groups connected by horizontal lines compared, or compared with uninfected mice. n.s. not significant.

within the gastric mucosa with the severity of gastritis observed in patients infected with *H. pylori*. Notably, higher IL-22 expression was strongly associated with more severe gastritis (figure 3A). This led us to hypothesise that IL-22 might exert proinflammatory effects during *H. pylori* infection and, thus, contribute to gastritis.

To test this hypothesis in vivo, we conducted a series of loss-of-function and gain-of-function experiments involving IL-22 and evaluated the inflammatory response in gastric mucosa on day 49 p.i. Compared with WT mice, IL-22 KO mice showed significantly less inflammation in gastric mucosa (figure 3B). Neutralisation of IL-22 significantly reduced gastric inflammation (figure 3B). Conversely, injection of IL-22 significantly increased gastric inflammation (figure 3B). Finally, consistent IL-22 being derived from Th cells, the effect of IL-22 appears to be mediated by BM-derived cells (see online supplementary figure S3A). Collectively, these results suggest that IL-22 has proinflammatory effects during *H. pylori* infection in vivo.

Gastric epithelial cells are induced by *H. pylori* to upregulate IL-22R1

Given the critical importance for IL-22R1 in IL-22 signalling, we sought to evaluate IL-22R1 expression during *H. pylori* infection. First, IL-22R1 expression was increased in gastric mucosa of *H. pylori*-infected patients (figure 3C), and was also higher in individuals carrying *cagA*-positive strains (figure 3D). Similarly, in mice, IL-22R1 expression was significantly increased in gastric mucosa of mice infected with WT *H. pylori* compared with those either uninfected or infected with *ΔcagA* (figure 3C, D). Since IL-22 typically acts on epithelial cells, we next examined expression of IL-22R1 on human primary gastric epithelial cells. Infection with WT *H. pylori* infection, upregulated IL-22R1 gene expression compared with either no

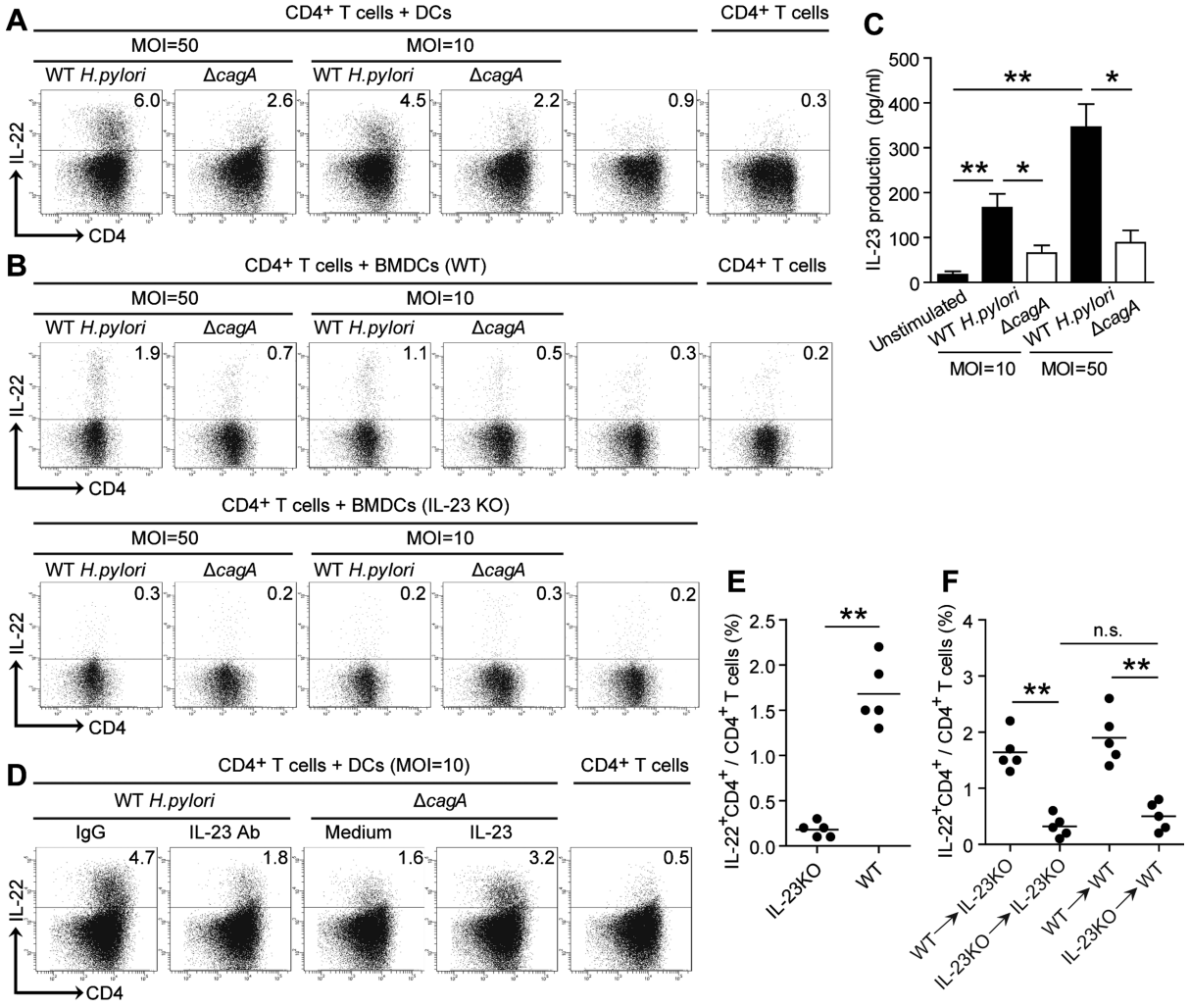

Figure 2 *Helicobacter pylori*-stimulated DCs induce Th22 cell polarisation via IL-23. (A and B) Th22 cell polarisation was assessed by flow cytometry, as described in the Methods section, and statistically analysed. Results are expressed as percentage of Th22 cells in $CD4^+$ T cells. (C) Concentrations of IL-22 protein in unstimulated, WT *H. pylori*-stimulated or *ΔcagA*-stimulated DCs derived from blood monocytes and in the DC supernatants were compared (n=3). (D) Th22 cell polarisation was assessed by flow cytometry, as described in the Methods section, and statistically analysed. Results are expressed as percentage of Th22 cells in $CD4^+$ T cells. Results are representative of three independent experiments. (E and F) Th22 cell response in gastric mucosa of WT *H. pylori*-infected WT C57BL/6 and IL-23 KO (E) or WT *H. pylori*-infected BM chimaera mice (F) on day 35 postinfection were compared. *p<0.05, **p<0.01, n.s. p>0.05 for groups connected by horizontal lines compared. DC, dendritic cells; Th; helper T cells; WT; wild-type; KO, knockout; BM, bone marrow; MOI, multiplicity of infection; BMDC, bone marrow–derived dendritic cells; IL, interleukin; n.s., not significant.

infection or infection with *ΔcagA* (figure 3E). Similar results were obtained with AGS cells, an immortalised human gastric epithelial cell line (see online supplementary figure S3D). Collectively, these results indicate that *H. pylori* infection induces IL-22R1 expression on gastric epithelial cells, implying that these cells are a major target of IL-22 action within the inflamed gastric mucosa.

IL-22 promotes CXCL2 production and attracts MDSCs into the gastric mucosa during *H. pylori* infection via CXCR2

IL-22 is known to induce the production of various chemokines within the brain.[14] We were, therefore, interested to know if IL-22 similarly induces chemokine production in gastric mucosa. To begin, we found that IL-22 induced AGS cells to produce CXCL2 in a dose-dependent and STAT3-dependent manner (figure 4A). Similarly, IL-22 (but not IL-23) induced CXCL2 production by primary gastric epithelial cells (figure 4B). IL-22 KO mice or neutralisation of IL-22 significantly reduced CXCL2 production in gastric mucosa (figure 4C). Conversely, injection of IL-22 significantly increased CXCL2 production (figure 4C).

CXCL2 promotes cell migration by binding to the chemokine receptor CXCR2. We first found that mice infected with WT *H. pylori* showed a higher frequency of MDSCs with abundant expression of CXCR2 in gastric mucosa than those infected with *ΔcagA* or uninfected (figure 4D,E and see online supplementary figure S4C). This accumulation of MDSCs peaked on day 49 p.i. Notably, MDSCs expressed high levels of Ly6C and minimal Ly6G, and should thus be regarded as monocytic MDSC (M-MDSCs)[15] (figure 4E). Consistent with this, we found a higher frequency of MDSCs with a $CD14^+HLA\text{-}DR^{low/-}$ M-MDSC phenotype in peripheral blood of *H. pylori*-infected patients compared with uninfected donors. Notably, these MDSCs showed abundant expression of CXCR2 (figure 4E, F).

Next, neutralisation of IL-22 significantly reduced MDSC accumulation, whereas, neutralisation of IL-17A, IL-17F and IFN-γ all had no effect (see online supplementary figure S5A). Similarly, both IL-22 KO and IL-23 KO mice showed

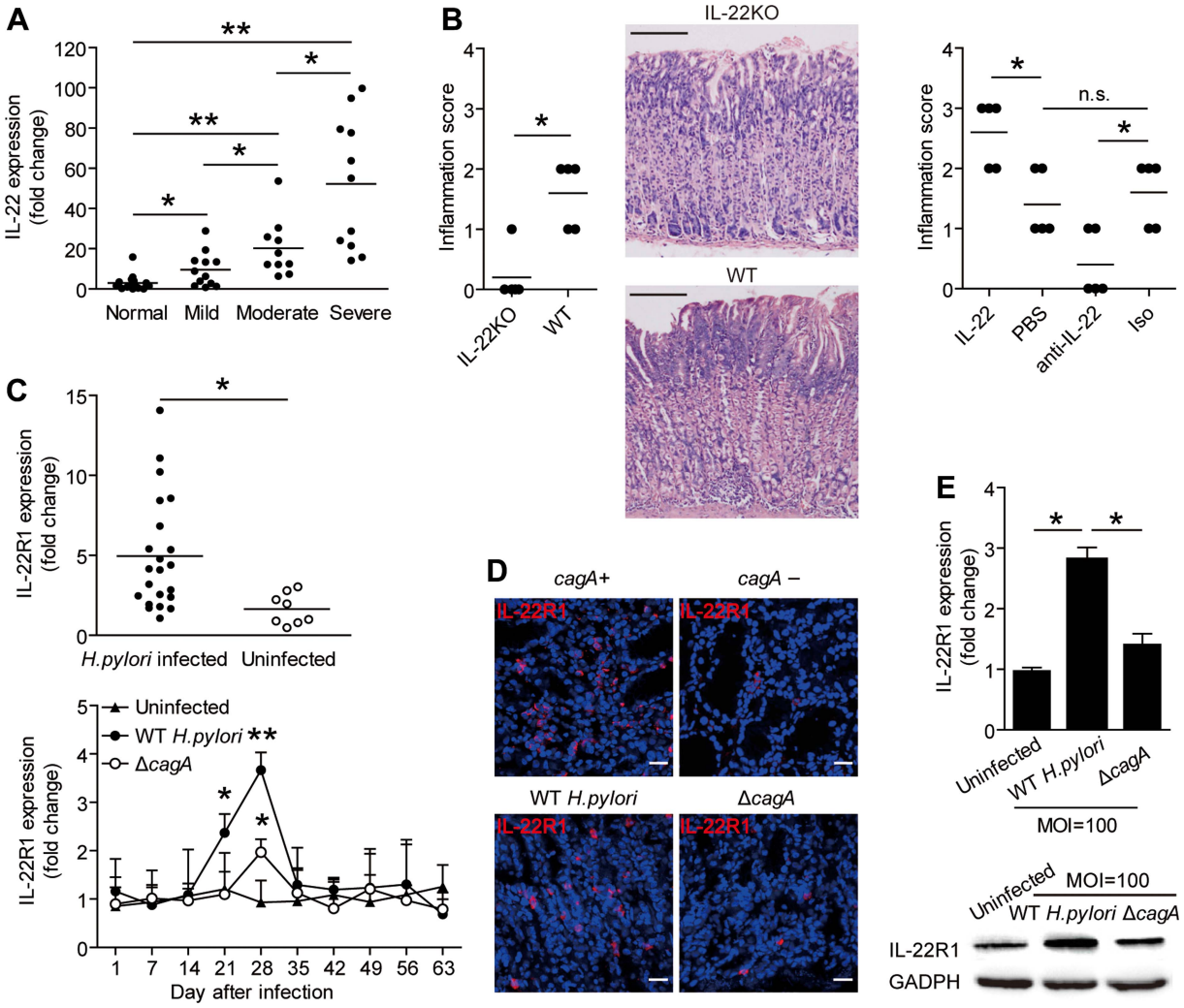

Figure 3 IL-22 has pro-inflammatory effects during *Helicobacter pylori* infection and *H. pylori* induce gastric epithelial cells to upregulate IL-22R1. (A) IL-22 mRNA expression in gastric mucosa of *H. pylori*-infected patients with mild (n=12), moderate (n=10), severe inflammation (n=11) and uninfected donors with normal gastric histopathology (n=15) was compared. (B) Histological scores of inflammation in gastric antra of the WT *H. pylori*-infected WT BALB/c or IL-22 KO mice or WT *H. pylori*-infected WT BALB/c mice injected with IL-22 or Abs against IL-22 on day 49 postinfection were compared. H&E staining, scale bars: 100 μ. (C) Expression of IL-22 mRNA in gastric mucosa of *H. pylori*-infected patients (n=22) and uninfected donors (n=8) was compared. Dynamic change of IL-22R1 mRNA expression in WT *H. pylori*-infected, *ΔcagA*-infected and uninfected BALB/c mice. (D) Representative immunofluorescence staining images showed IL-22R1 expression in gastric mucosa of WT *H. pylori*-infected and *ΔcagA*-infected mice or *cagA*$^+$ *H. pylori*-infected and *cagA*$^-$ *H. pylori*-infected patients. Scale bars: 20 μ. (E) IL-22R1 mRNA expression or IL-22R1 protein in WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected primary gastric epithelial cells from uninfected donors were compared (n=3) or analysed by western blot. *p<0.05, **p<0.01, n.s. p>0.05 for groups connected by horizontal lines compared. WT; wild-type; KO, knockout; IL, interleukin; MOI, multiplicity of infection; PBS, phosphate-buffered saline; GADPH, glyceraldehyde 3-phosphate dehydrogenase; Abs, antibodies; n.s., not significant.

significantly fewer MDSCs (figure 5B). However, injection of IL-22 significantly increased MDSC accumulation in IL-23 KO mice, while injection of IL-23 had no significant effect on MDSC numbers in IL-22 KO mice (figure 5B), suggesting that IL-22 and not IL-23 is a more proximal influence on MDSC accumulation.

To evaluate the contribution of an IL-22-CXCL2-CXCR2 axis to the accumulation of MDSCs, MDSC chemotaxis assay was performed and demonstrated that culture supernatants from primary gastric epithelial cells treated with WT *H. pylori*-stimulated DC-derived Th22 cell-polarising culture supernatants induced significantly more MDSC migration than those supernatants from gastric epithelial cells treated with *ΔcagA*-stimulated DC-derived culture supernatants and this effect was lost upon pretreatment with neutralising Abs against IL-22 and CXCR2 (figure 5C). Neutralisation of IL-22 or CXCL2 with Ab, inhibition of CXCR2 with SB225002 or simultaneous blocking of both CXCL2 and CXCR2, all significantly reduced *H. pylori*-induced MDSC accumulation (figure 5A and see online supplementary figure S5B). Conversely, injection of IL-22 significantly increased *H. pylori*-induced MDSC accumulation (figure 5A and see online supplementary figure S5B). Finally, the effect of IL-22 again appears to be mediated by BM-derived cells (see online supplementary figure S5C). Collectively, these results, therefore, suggest that an IL-22-CXCL2-CXCR2 axis contributes to MDSC accumulation within the gastric mucosa of *H. pylori*-infected mice.

IL-22 stimulates production of proinflammatory proteins S100A8 and S100A9 by MDSCs

It has previously been shown that proinflammatory S100A8 and S100A9 proteins are highly expressed in the inflamed gastric

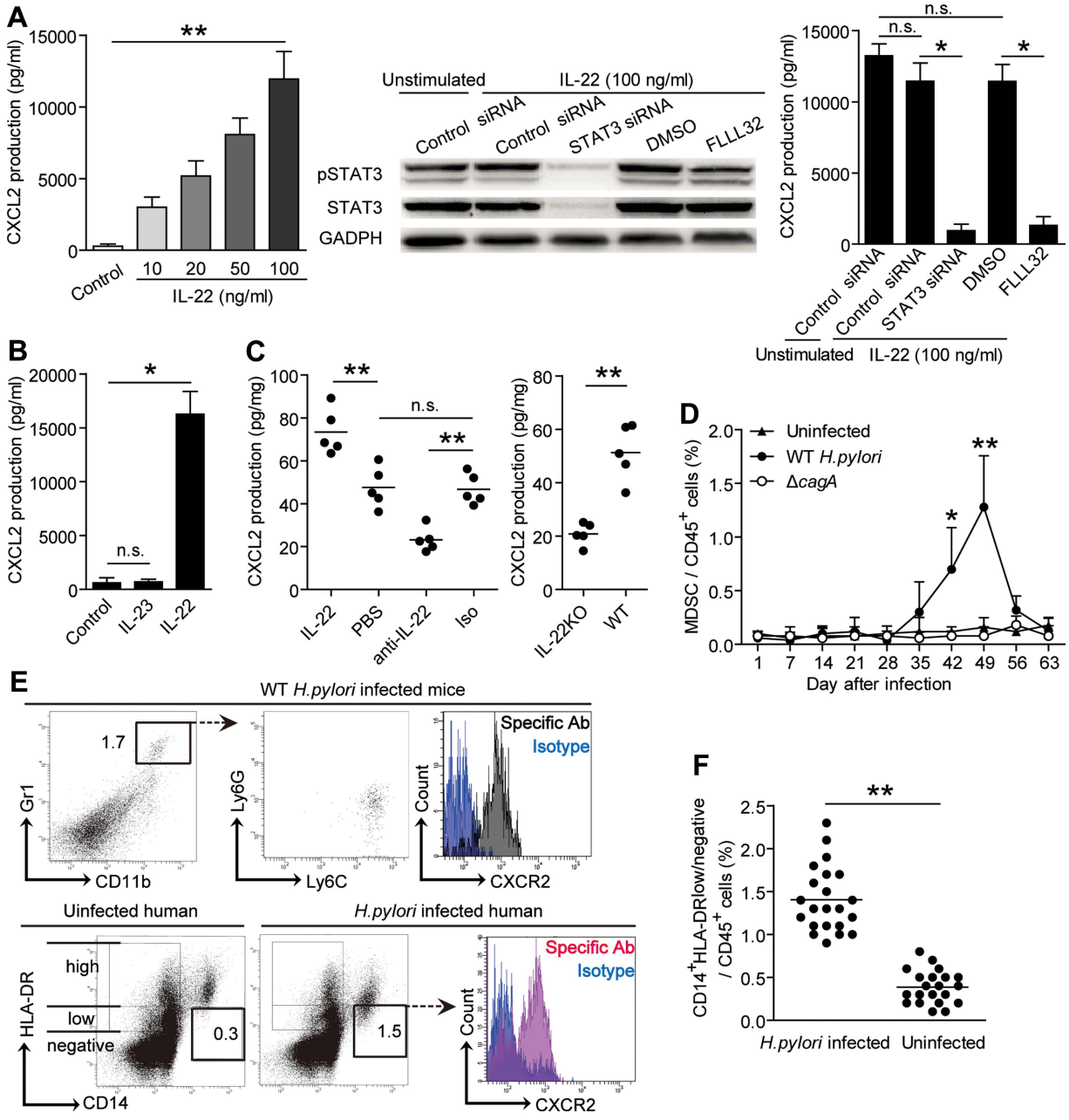

Figure 4 IL-22 promotes CXCL2 production in vivo and in vivo and CXCR2-expressing MDSCs accumulated in gastric mucosa during *Helicobacter pylori* infection. (A and B) AGS cells (A) and primary gastric epithelial cells (B) were pretreated and stimulated as described in Methods. CXC12 production was detected in cell supernatants by ELISA (n=3). STAT3 and p-STAT3 proteins were analysed by western blot. (C) Concentrations of CXCL2 protein in gastric mucosa of WT *H. pylori*-infected WT BALB/c mice injected with IL-22 or PBS control, or Abs against IL-22 or corresponding isotype control Ab, or of WT *H. pylori*-infected WT BALB/c and IL-22 KO mice on day 42 postinfection (p.i) were compared. (D) Dynamic change of MDSCs in WT *H. pylori*-infected, *ΔcagA*-infected and uninfected BALB/c mice. (E) Representative dot plots of MDSCs by gating on $CD45^+$ cells and expression of Ly6C, Ly6G and CXCR2 on MDSCs in gastric mucosa of WT *H. pylori*-infected mice on day 49 p.i., and representative dot plots of MDSCs by gating on $CD45^+$ cells and expression of CXCR2 on MDSCs in peripheral blood of *H. pylori*-infected and uninfected donors. Numbers indicate relative percentages in $CD45^+$ cells. (F) MDSC level in peripheral blood of *H. pylori*-infected patients (n=22) and uninfected donors (n=21) was compared; n=5 per group per time point in D. *p<0.05, **p<0.01, n.s. p>0.05 for groups connected by horizontal lines compared, or compared with uninfected mice. WT; wild-type; KO, knockout; IL, interleukin; PBS, phosphate-buffered saline; GADPH, glyceraldehyde 3-phosphate dehydrogenase; Abs, antibodies; MDSC, myeloid-derived suppressor cell; DMSO, dimethylsulfoxide; n.s., not significant.

mucosa of *H. pylori*-infected individuals,[16] and our own recent data provide a similar result (data not shown). The underlying basis for this induction of S100 proteins has remained unclear. We were, therefore, interested to observe a positive correlation between IL-22 and S100A8/S100A9 (see online supplementary figure S6A), prompting us to speculate whether IL-22 might regulate S100A8/S100A9. Since S100A8/S100A9 are known to be secreted by MDSCs,[17] we isolated MDSCs and stimulated them with IL-22. This potently induced MDSCs to synthesise and express S100A8/S100A9 in vitro (figure 6A). Further,IL-22 KO mice or neutralisation of IL-22 produced significantly less S100A8/S100A9 in gastric mucosa (figure 6B, C). Conversely, injection of IL-22 significantly increased S100A8/S100A9 production (figure 6C). Finally, the effect of IL-22 once again

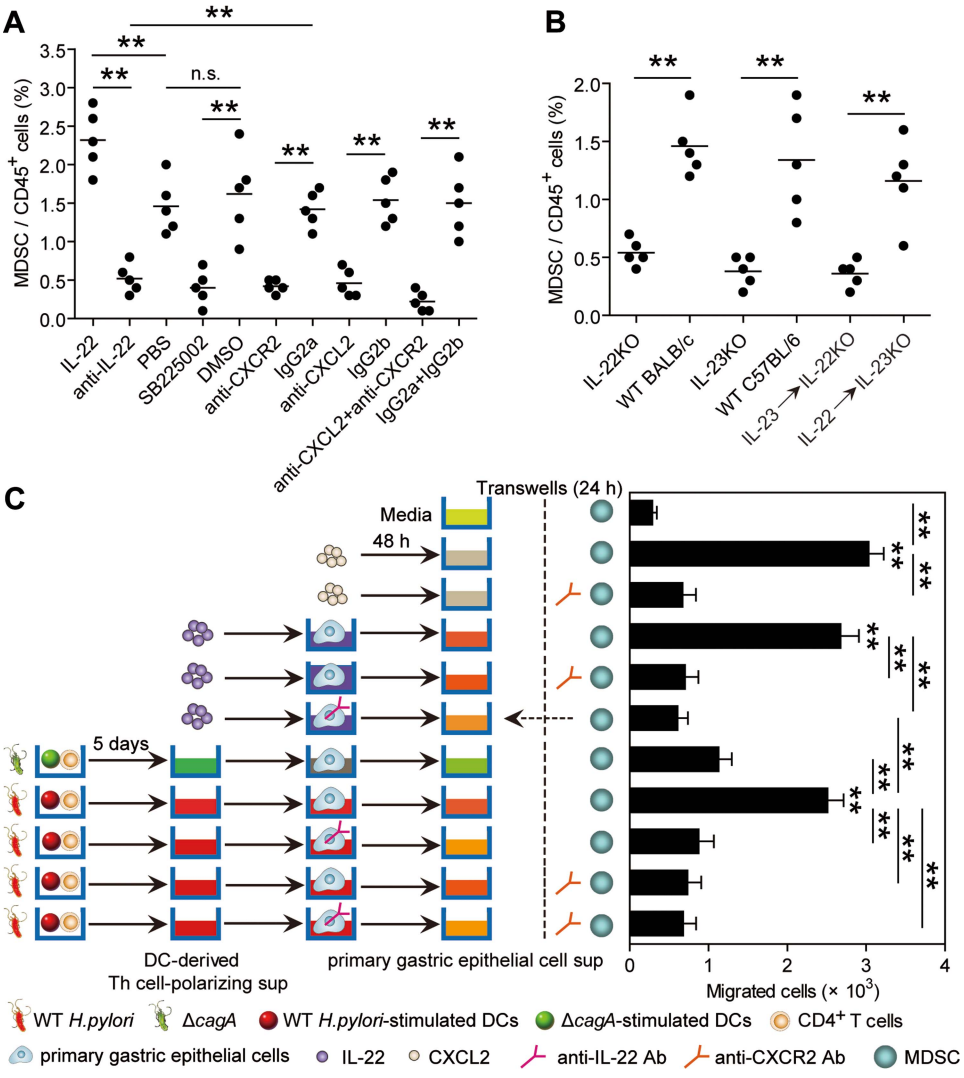

Figure 5 IL-22 promotes MDSC accumulation in gastric mucosa in vivo and migration in vitro during *Helicobacter pylori* infection by CXCL2-CXCR2 axis. (A) MDSC responses in gastric mucosa of WT *H. pylori*-infected WT BALB/c mice injected with IL-22 or PBS control, Abs against IL-22 (IgG2a), CXCR2 (IgG2a) and/or CXCL2 (IgG2b) or corresponding isotype control Abs, or SB225002 or DMSO control on day 49 p.i. were compared. (B) MDSC responses in gastric mucosa of WT *H. pylori*-infected WT BALB/c and IL-22 KO mice, or WT C57BL/6 and IL-23 KO mice, or WT *H. pylori*-infected IL-22 KO mice injected with IL-23 and IL-23 KO mice injected with IL-22 (B) on day 49 p.i. were compared. (C) MDSC migration was assessed by transwell assay, as described in Methods, and statistically analysed (n=3). *p<0.05, **p<0.01, n.s. p>0.05 for groups connected by horizontal lines compared. WT; wild-type; KO, knockout; IL, interleukin; PBS, phosphate-buffered saline; Abs, antibodies; MDSC, myeloid-derived suppressor cell; DMSO, dimethylsulfoxide; DC, dendritic cells; p.i., postinfection; n.s., not significant.

appears to be mediated by BM-derived cells (see online supplementary figure S6F). Collectively, our data demonstrate that IL-22 plays an essential role in inducing S100A8/S100A9 expression in gastric mucosa during *H. pylori* infection.

IL-22-induced MDSCs suppress Th1 cell responses in *H. pylori* infection

Th1 cells have previously been implicated in *H. pylori* gastritis.[2 18] Since Th1 cell responses can be inhibited by MDSCs in other contexts,[19] we were interested to learn whether IL-22-induced MDSCs would affect Th1 cell responses during *H. pylori* infection. Neutralisation of IL-22 or CXCL2 with Ab, inhibition of CXCR2 with SB225002 or simultaneous blocking of both CXCL2 and CXCR2 all significantly increased *H. pylori*-induced Th1 cell responses (figure 6D and see online supplementary figure S7A). Similarly, IL-22 KO mice showed significantly greater Th1 cell responses (figure 6E). Conversely, injection of IL-22 significantly reduced *H. pylori*-induced Th1 cell response (figure 6D and see online supplementary figure S7A). Finally, the effect of IL-22 again appears to be mediated by BM-derived cells (see online supplementary figure S7B). Collectively, these data demonstrated that IL-22 plays an essential role in the inhibition of Th1 cells in gastric mucosa during *H. pylori* infection in vivo.

Next, we wanted to test whether MDSCs might directly suppress Th1 cell development. We, therefore, cocultured CFSE-labelled peripheral $CD4^+$ T cells of healthy donors with peripheral $CD14^+HLA\text{-}DR^{low/-}$ MDSCs from *H. pylori*-infected patients for 5 days, and found that—in comparison with $CD14^+HLA\text{-}DR^{high}$ monocytes—$CD14^+HLA\text{-}DR^{low/-}$ MDSCs suppressed Th1 cell development (figure 6F and see online supplementary figure S7C).

DISCUSSION

Th22 cells and IL-22 appear to have different roles depending upon the nature of the infection. For example, in infections with *Trichuris trichiura* roundworm[20] and *Influenza A* virus,[21] Th22 cells and IL-22 provide the host with protection. By contrast, in *Toxoplasma gondii*,[5] *West Nile* virus,[14] *Hepatitis B* virus[22] and persistent fungal[23] infections, Th22 cells and IL-22 contribute to pathology by promoting inflammation. Our data are in keeping with the latter studies, since they clearly indicate that, during *H pylori* infection, Th22 cells and IL-22 are proinflammatory and contribute to the pathogenesis of gastritis. What remains unclear is why Th22 cell responses are protective in some infections, but pathogenic in others. To this end, our findings that the *H. pylori*-associated virulence factor *cagA* was necessary to induce maximal IL-22R1 expression, CXCL2 production, MDSC accumulation and S100A8/9 expression suggest that

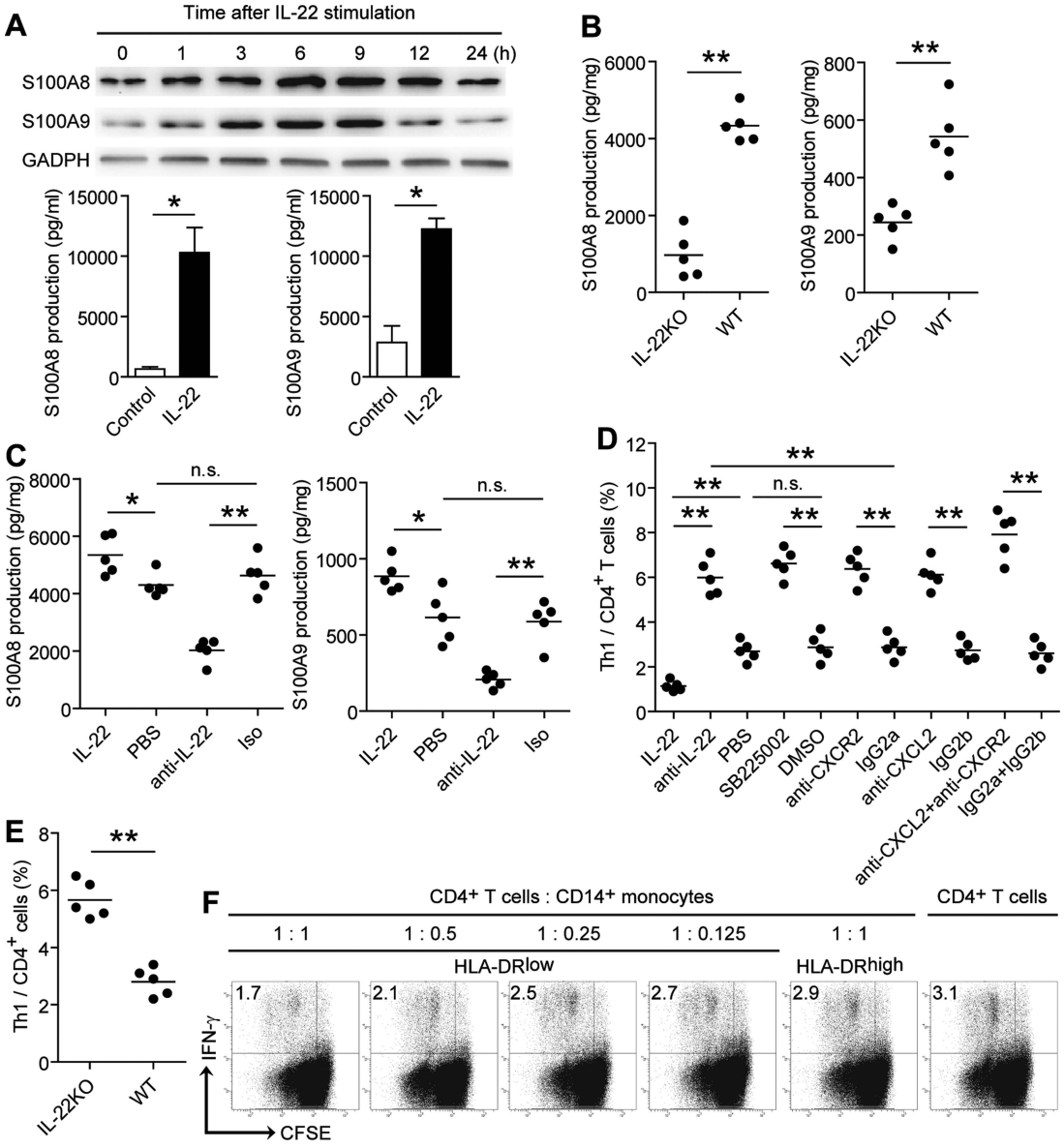

Figure 6 IL-22 induces proinflammatory proteins S100A8 and S100A9 production from MDSCs and regulates S100A8 and S100A9 in vivo, and IL-22-induced MDSCs suppress Th1 cell response in *Helicobacter pylori* infection. (A) S100A8 and S100A9 proteins in IL-22-stimulated human $CD45^+CD14^+HLA\text{-}DR^{low/-}$ MDSCs for different time points or 24 h were analysed. (B and C) S100A8 and S100A9 protein in gastric mucosa of WT *H pylori*-infected WT BALB/c and IL-22 KO mice (B), or WT *H. pylori*-infected WT BALB/c mice injected with IL-22 or Abs against IL-22 (C) on day 49 p.i. were compared. (D and E) Th1 cell responses in gastric mucosa of *H. pylori*-infected WT BALB/c mice injected with IL-22 or PBS control, Abs against IL-22 (IgG2a), CXCR2 (IgG2a), and/or CXCL2 (IgG2b) or corresponding isotype control Abs, or SB225002 or DMSO control (D), or *H pylori*-infected WT BALB/c and IL-22 KO mice (E) on day 49 p.i. were compared. (F) T cell-MDSC coculture was assessed by flow cytometry as described in Methods and statistically analysed (n=3). Results are expressed as percentage of proliferated Th1 cells in $CD4^+$ T cells. Results are representative of three independent experiments. *p<0.05, **p<0.01, n.s p>0.05 for groups connected by horizontal lines compared, or compared with uninfected mice. WT; wild-type; KO, knockout; IL, interleukin; PBS, phosphate-buffered saline; Abs, antibodies; MDSC, myeloid-derived suppressor cell; DMSO, dimethylsulfoxide; DC, dendritic cells; p.i., postinfection; GADPH, glyceraldehyde 3-phosphate dehydrogenase; IFN, interferon; CFSE, carboxyfluorescein diacetate succinimidyl ester; n.s., not significant.

intrinsic factors encoded by the infection itself are likely to be important in influencing the outcome of the Th22 cell response. Previous studies detailed that mice were preferentially infected with *cag*PAI-negative *H. pylori* clinic isolates[24] and *H. pylori* B128 strains[25] that induced less inflammation, which resembles our data on *ΔcagA* compared with WT *H. pylori* (data not shown).

The proinflammatory nature of IL-22 has been suggested to involve various mechanisms in the past. For example, enhanced expression of IL-22 was accompanied by increased CXCL2 levels in a mouse model of psoriasis.[26] Meanwhile, IL-22 was observed to promote myeloid cell infiltration into the central nervous system.[27] And IL-22 was also reported to induce S100 family members in acanthosis.[6] Our findings are consistent with these studies and demonstrate for the first time how each of these individual findings fit together in the context of a single experimental model: in this case, *H. pylori* gastritis. Specifically, our in vitro and in vivo data together provide a multistep model of inflammation during *H. pylori* infection involving interactions between *H. pylori*, Th22 cells, DCs, gastric epithelial cells and MDSCs within the gastric mucosa (figure 7).

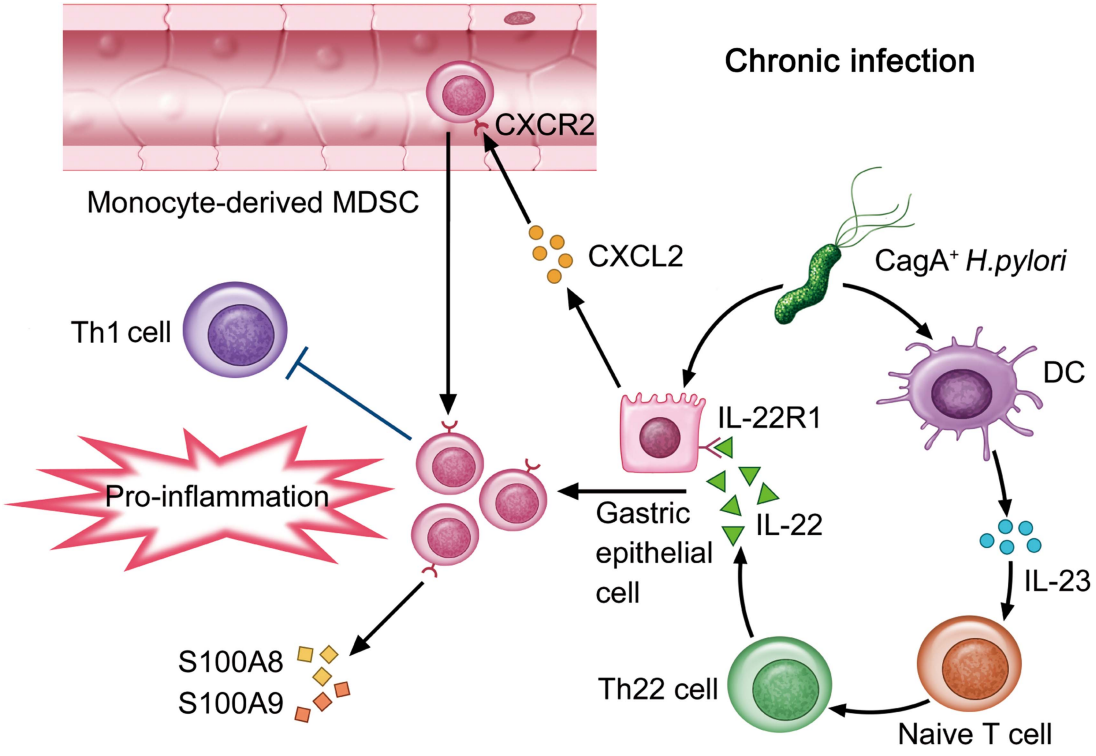

Figure 7 A proposed model of cross-talk among *Helicobacter pylori*, dendritic cells (DC), gastric epithelial cells and myeloid-derived suppressor cells (MDSC) leading to Th22 cell differentiation and MDSC-mediated proinflammation in gastric mucosa during *H. pylori* infection. *H. pylori* stimulate DCs to secrete interleukin (IL)-23 and *H. pylori* induce gastric epithelial cells to express IL-22R1. Release of IL-23 induces the polarisation of Th22 cells. Polarised Th22 cells expand in gastric mucosa where they release cytokine IL-22 that stimulates gastric epithelial cells to secrete CXCL2. Responding to the CXCL2 chemokine gradient, myeloid progenitor cell-derived, CXCR2-expressing MDSCs migrate into gastric mucosa where they exert a proinflammatory effect by producing inflammatory proteins, S100A8 and S100A9, and suppressing T helper type 1 (Th1) cell response.

MDSCs are a heterogeneous population of immature myeloid cells with the capacity to potently suppress T cell immunity.[15] MDSCs have been most intensively studied in the context of cancer, but their role in the pathogenesis of viral,[28] parasitic[20] and bacterial[29] diseases is now starting to be appreciated. Our data suggest that MDSCs may be a key cellular player in *H. pylori*-induced gastritis via the inhibition of Th1 responses that differed in C57BL/6 and BALB/c mice[30] and were associated with *cag*PAI$^+$ *H. pylori*-induced and IFN-γ-inducible chemokine responses in gastric epithelial cells[31]—which is consistent with their canonical role in T cell suppression—and also via the production of key inflammatory mediators, such as S100A8 and S100A9.

The clinical outcome for patients with *H. pylori*-associated gastritis remains diverse, with sequelae ranging from an asymptomatic illness at one of the spectrum to life-threatening peptic ulceration and gastric carcinoma at the other end. Given the apparent relationship between IL-22 levels and the severity of gastric inflammation observed in *H. pylori*-infected patients (figure 3A), thought should be given to the use of IL-22 and/or Th22 cells as novel diagnostic biomarkers for *H. pylori* infection.

Although *H. pylori* remains reasonably straightforward to eradicate in most patients using oral antibiotics, it is noteworthy that chronic gastritis commonly persists even after successful eradication therapy.[32] Treatments that can address the underlying inflammatory process may therefore be of clinical value in such cases. In this regard, our findings suggest several possible therapeutic targets, including IL-22, S100A8 and S100A9. At the same time, it will be interesting to test whether the same proinflammatory cellular networks and molecular pathways described here for *H. pylori* gastritis operate in other chronic infections where eradication is more difficult (eg, hepatitis B). If this is true, then targeting these same molecular pathways may also prove to be of clinical benefit.

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Correction Notice This article has been corrected since it was published online first. The funding number 81201265 has now been added.

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Competing interests None.

Patient consent Obtained.

Ethics approval The biopsy specimens were obtained under protocols approved by the ethics committees of XinQiao Hospital and Southwest Hospital of Third Military

Medical University. All animal experiments were undertaken with approval from the Animal Ethical and Experimental Committee of Third Military Medical University.

Provenance and peer review Not commissioned; externally peer reviewed.

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Supplementary Methods

Antibodies and other reagents

The Abs for immunofluorescence: mouse anti-human IL-22R1, and rat anti-mouse IL-22R1 from R&D Systems; rat anti-mouse CD11b, rat anti-mouse Ly6C from Biolegend; goat anti-mouse-TRITC, goat anti-rat-TRITC, goat anti-rat-FITC from Zhongshan Biotechnology. The Abs for flow cytometry: for human, anti-CD3-APC-H7 from BD Pharmingen; anti-CD4-PE, anti-IL-22-Alexa Fluor 647, anti-IFN-γ-APC, anti-CD14-PerCP-Cy5.5, and anti-CXCR2-PerCP-eFluor 710 from eBioscience; anti-CD45-PE-Cy7, anti-HLA-DR-Alexa Fluor 647, anti-HLA-DR-FITC, and anti-CD14-PE from Biolegend; anti-IL-22R1-APC from R&D Systems; for mouse, anti-CD3-FITC, anti-CD4-APC, anti-IL-22-PE, anti-IFN-γ-PE from eBioscience, anti-CD45-PE-Cy7, anti-CD11b-PerCP-Cy5.5, anti-Gr1-FITC, anti-CXCR2-Alexa Fluor 647, anti-Ly6G-APC-Cy7, anti-Ly6C-PE, anti-lineage panel-biotin, streptavidin-APC-Cy7, anti-NKp46-PE, and anti-IL-7Rα-FITC from Biolegend. The Abs for neutralizing and blocking were as follows: anti-human/mouse IL-22, anti-mouse IL-17A, anti-mouse IL-17F, and anti-mouse IFN-γ from eBioscience; anti-mouse CXCR2 and anti-mouse CXCL2 from R&D Systems. The Abs for Western blot: anti-human IL-22R1 and anti-mouse IL-22R1 from R&D Systems; anti-human STAT3 from Santa Cruz; anti-human p-STAT3 (Y705) from Cell signaling technology; anti-human S100A8 and anti-human S100A9 from Abcom. Purified anti-CD3 and anti-CD28 Abs were from Biolegend. ELISA kits for human IL-22, mouse IL-22, and mouse CXCL2 were from R&D Systems; ELISA kits for human S100A8, mouse S100A8, human S100A9, mouse S100A9, and human CXCL2 were from Uscn Life Science; ELISA kit for human IFN-γ was from eBioscience. Collagenase IV, DNase I, DMSO, PMA, ionomycin, and gentamycin were from Sigma-Aldrich. The potent and selective nonpeptide CXCR2 antagonist SB225002 (*N*-(2-hydroxy-4-nitrophenyl)-*N*'-(2-bromophenyl)urea) was from R&D Systems. The potent STAT3 inhibitor FLLL32 was from MedKoo Biosciences. CFSE was from eBioscience. Protein Extraction Reagent was from Pierce. All recombinant cytokines and chemokines were from PeproTech except recombinant murine IL-22 that was from eBioscience.

Generation of BM chimera mice

The following BM chimeric mice were prepared: male WT BM→female IL-23 KO mice, male IL-23 KO BM→female IL-23 KO mice, male WT BM→female WT mice, and male IL-23 KO BM→female WT mice; or male WT BM→female IL-22 KO mice, male IL-22 KO BM→female IL-22 KO mice, male WT BM→female WT mice, and male IL-22 KO BM→female WT mice. BM cells were collected from the femurs and tibia of donor mice by aspiration and flushing, and were suspended in PBS at the concentration of 5×10^7/ml. The BM in recipient mice was ablated with lethal irradiation (8 Gy). Then, the animals received intravenously 5×10^6 BM cells from donor mice in a volume of 300 μl sterile PBS under the anaesthesia. Thereafter, the transplanted BM was allowed to regenerate for 4-6 weeks before subsequent experimental procedures were performed. To verify successful engraftment and reconstitution of the BM in the transplanted mice, genomic DNA was isolated from tail tissues of each chimera mouse 4 weeks after BM transplantation. Quantitative PCR was performed to detect the *Sry* gene present in the Y chromosome (primers seen in Supplementary Table 3) and mouse β2-microglobulin (β2-M) gene as an internal control. The chimeric rates were calculated on the assumption that the ratio of the Sry to β2-M gene was 100% in male mice. We confirmed that the chimeric rates were consistently higher than 80%. After BM reconstitution was confirmed, mice were infected with bacteria as described above.

Isolation of single cells from tissues and dendritic cell (DCs) preparation

Fresh tissues were washed three times with Hank's solution containing 1% fetal calf serum (FCS) (Gibco) before being cut into small pieces. The specimen were then collected in RPMI 1640 containing 1mg/ml collagenase IV and 10 mg/ml DNase I, and mechanically dissociated by using the gentle MACS Dissociator (Miltenyi Biotec). Dissociated cell suspensions were further incubated 0.5-1 h at 37°C under continuous rotation. The cell suspensions were then filtered through a 70-μm cell strainer (BD Labware). Peripheral blood mononuclear cells (PBMCs) from healthy donors and gastric cancer patients were isolated by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare). Fresh human peripheral blood monocytes were selected using anti-CD14 magnetic beads (StemCell Technologies). To generate human DCs, monocytes were cultured (2×10^5 cells/well in 24-well culture plates) for 7 days in RPMI 1640 medium supplemented with

10% FCS (R-10) supplemented with recombinant human IL-4 (500 U/ml) and GM-CFS (100 ng/ml). The medium was changed every second day by removing half the medium and adding freshly made medium supplemented with full concentrations of cytokines. Morphologic analysis and high expression of CD1a and CD11c were parameters for quality and purity of DC preparations. To generate mouse bone marrow-derived DCs (BMDCs), BM cells were cultured in R-10 supplemented with 20 ng/ml recombinant murine GM-CSF (20 ng/ml) and IL-4 (10 ng/ml) for 7 days. DCs were purified using anti-CD11c magnetic beads (Miltenyi Biotec).

Immunofluorescence

Paraformaldehyde-fixed cryostat sections of tissues were washed in PBS and blocked for 30 min with 20% goat serum in PBS and stained for IL-22R1, and CD11b and/or Ly6C. Slides were examined with a confocal fluorescence microscope (LSM 510 META, Zeiss).

Real-time PCR

DNA of the biopsy specimens were extracted with QIAamp DNA Mini Kit (QIAGEN) and RNA of biopsy specimens and cultured cells were extracted with TRIzol reagent (Invitrogen). The RNA samples were reversed transcribed to cDNA with PrimeScript^{TM} RT reagent Kit (TaKaRa). Real-time PCR was performed on the IQ5 (Bio-Rad) with the Real-time PCR Master Mix (Toyobo) according to the manufacturer's specifications. Expression of 16S rDNA, *cagA*, IL-23p19, IL-22, IL-22R1, CXCL2, S100A8, and S100A9 was measured using the TaqMan and/or SYBR green method with primers (Supplementary Table 3). For mice, mouse β2-microglobulin served as the normalizer, and uninfected stomach served as the calibrator. For human, human β-actin served as the normalizer, and unstimulated cells served as the calibrator. The relative gene expression was expressed as fold change calculated by the ΔΔCt method.

Flow cytometry

Cells were stained for Abs of surface markers or control isotype Abs. For intracellular molecules measurements, the cells were stimulated for 5 h with PMA (50 ng/ml) plus ionomycin (1 μg/ml) in the presence of Golgistop (BD Pharmingen). Intracellular cytokine staining was performed after

fixation and permeabilization, using Perm/Wash solution (BD Pharmingen). Then, the cells were analyzed by multicolour flow cytometry with FACSCanto II (BD Biosciences). Data were analyzed with Flowjo software (TreeStar) or FACSDiva software (BD Biosciences).

ELISA

Human and mouse gastric tissues from specimens were collected, homogenized in 1 ml sterile Protein Extraction Reagent, and centrifuged. Tissue supernatants were collected for ELISA. Concentrations of IL-22 in the tissue supernatants; concentrations of IL-23 in the DC culture supernatants; concentrations of CXCL2 in the gastric epithelial cell culture supernatants or tissue supernatants; concentrations of S100A8 and S100A9 in the MDSC culture supernatants or tissue supernatants; and concentrations of IFN-γ in the T cell culture system supernatants were determined using ELISA kits according to the manufacturer's instructions.

Western blot analysis

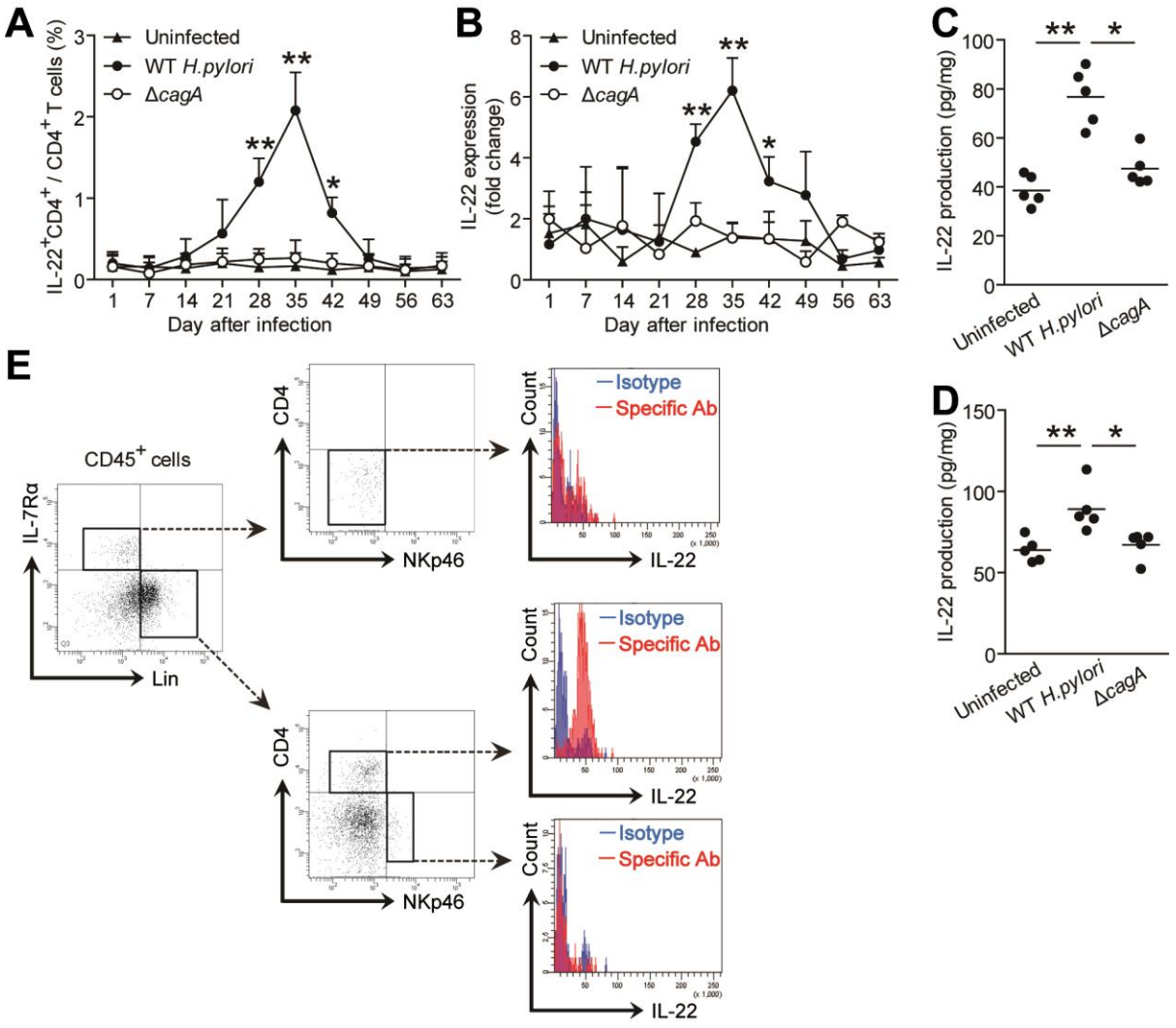
Western blot assays were performed on 10%-15% SDS-PAGE gels using equivalent amounts of cell or tissue lysate proteins of samples. Five percent skimmed milk or three percent BSA was used for blocking the PDF membranes. Mouse IL-22R1 was detected with rat anti-IL22R1 Abs; human IL-22R1, S100A8, S100A9, STAT3, and p-STAT3 were detected with mouse anti-IL-22R1 Abs, rabbit anti-S100A8 Abs, rabbit anti-S100A9 Abs, mouse anti-STAT3 Abs, and rabbit anti-p-STAT3 Abs, respectively. This was followed by incubation with HRP-conjugated secondary Abs (Zhongshan Biotechnology). Bound proteins were visualized by using SuperSignal® West Dura Extended Duration Substrate kit (Thermo).

Microarray experiments

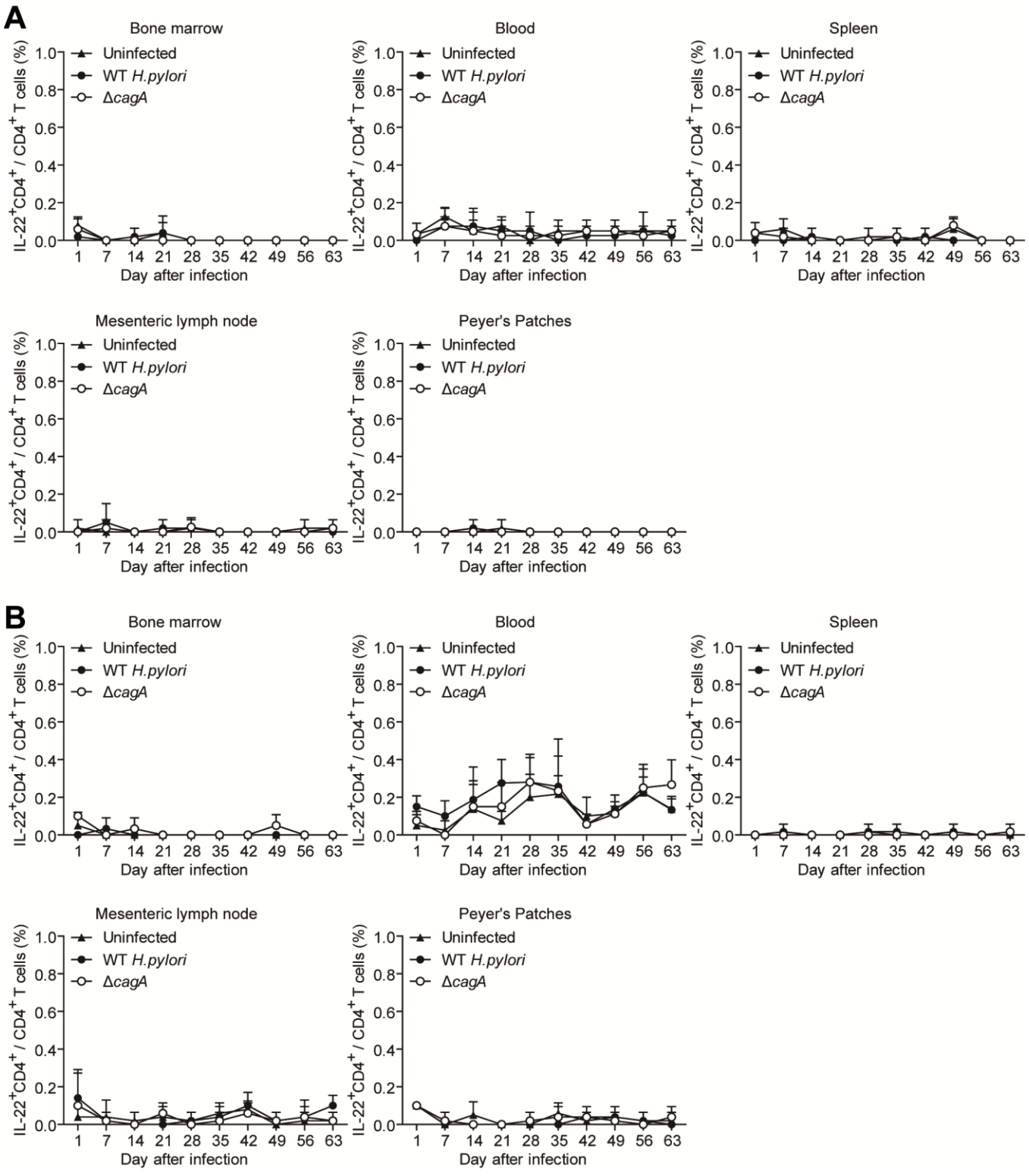
Gene expression profiles of MDSCs were analyzed with the human Exon 1.0 ST GeneChip (Affymetrix), strictly following the manufacturer's protocol. Microarray experiments were performed at the Genminix Informatics (China) with the microarray service certified by Affymetrix.

Supplemental Figures

Supplemental Figure 1. Th22 cells accumulated in gastric mucosa of *H. pylori*-infected patients and mice. (A and B) Dynamic change of Th22 cell response (A) and IL-22 mRNA expression (B) in WT *H. pylori*- infected, *ΔcagA*-infected, and uninfected C57BL/6 mice. (C and D) Concentrations of IL-22 protein in gastric mucosa of WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected BALB/c mice (C) and C57BL/6 mice (D) on day 35 p.i. were compared. (E) Representative dot plots of intracellular cytokine staining for IL- 22 expression on immune cell types (including NK cells, lymphoid tissue inducer-like cells (LTi-like cells), innate lymphoid cells, and Th cells) among total $CD45^+$ haematopoietic cells in gastric mucosa of WT *H. pylori*-infected BALB/c mice on day 35 p.i.. Red histograms represent cells stained for specific markers; blue histograms represent cells stained with isotype control Ab. n=5 per group per time point in *A* and *B*. *p<0.05, **p<0.01 for groups connected by horizontal lines compared, or compared with uninfected mice.

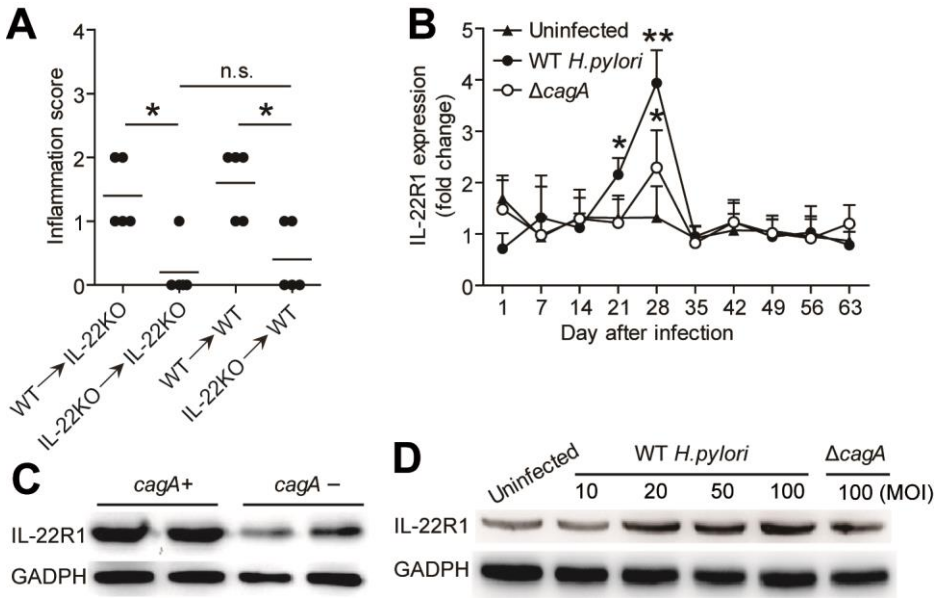


Supplemental Figure 2. Dynamic change of Th22 cell response in BM, blood, spleen mesenteric lymph node, and Peyer's pathes of WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected BALB/c (A) and C57BL/6 (B) mice. Results are expressed as mean ± SEM. n=5 per group per time point.

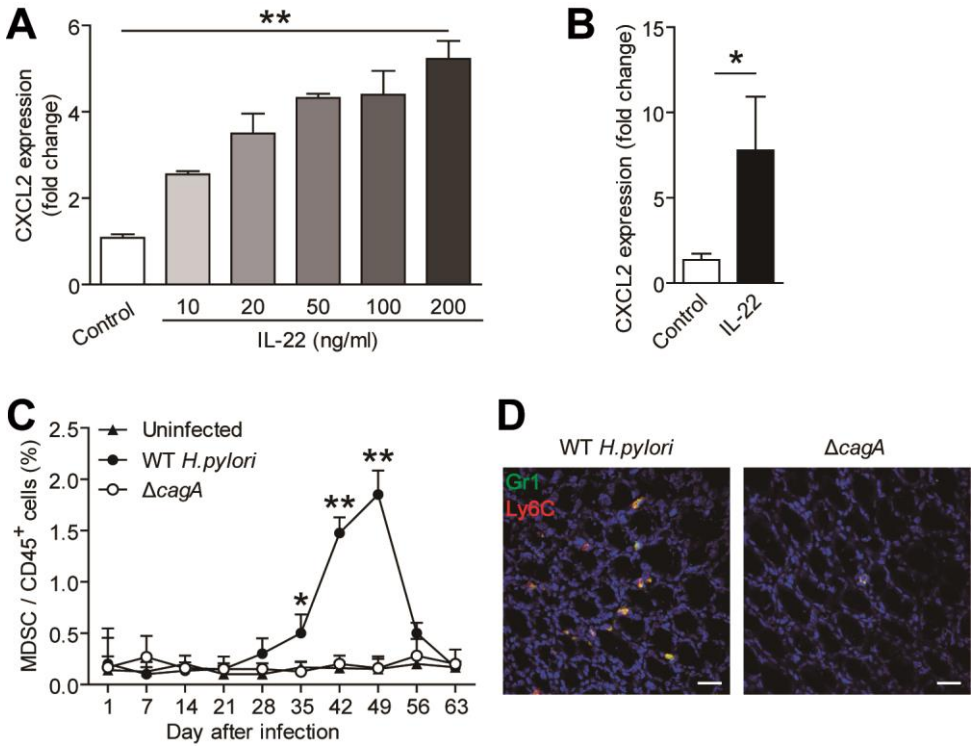


Supplemental Figure 3. IL-22 has pro-inflammatory effects during *H. pylori* infection, and *H. pylori* induces gastric epithelial cells to up-regulate IL-22R1. (A) Histological scores of inflammation in gastric antra of WT *H. pylori*-infected BM chimera mice on day 49 p.i. were compared. (B) Dynamic change of IL-22R1 mRNA expression in WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected C57BL/6 mice. n=5 per group per time point. (C) IL-22R1 protein in gastric mucosa of

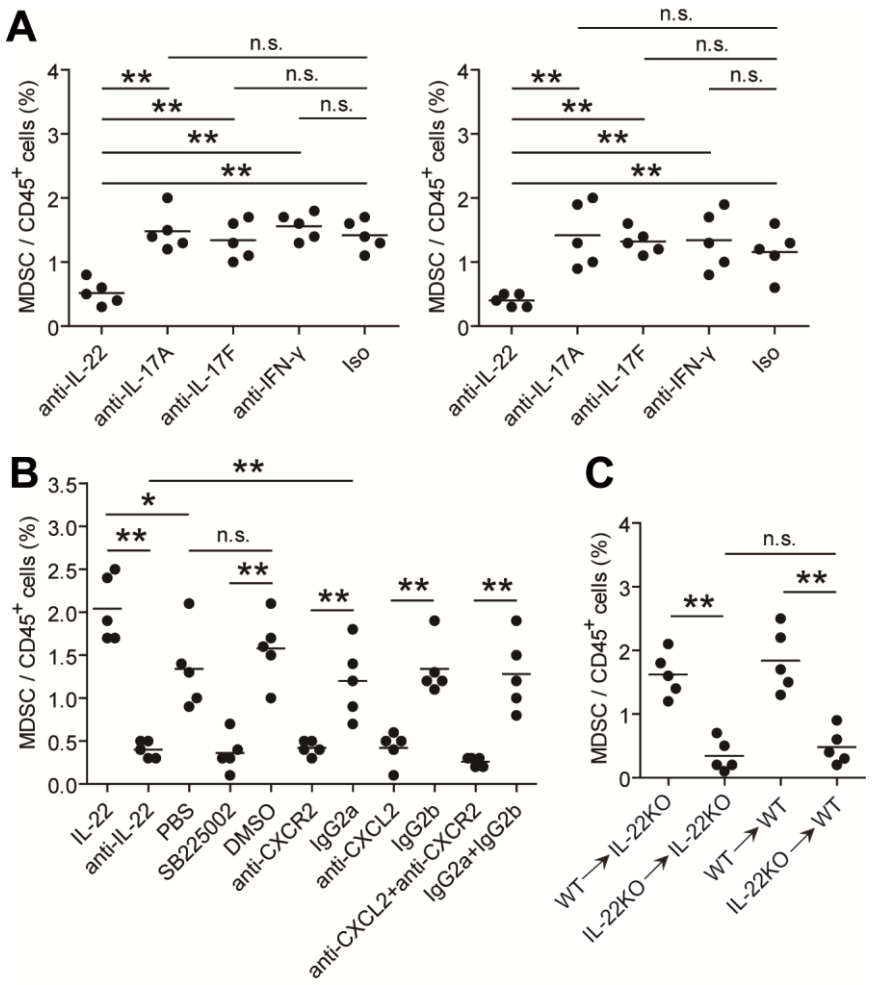
cagA^{+} *H. pylori*-infected and *cagA*$^{-}$ *H. pylori*-infected patients was analyzed by Western blot. (D) IL-22R1 protein in WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected AGS cells was analyzed by Western blot. n=5 per group per time point in *B*. *p<0.05, **p<0.01 for groups compared with uninfected mice.



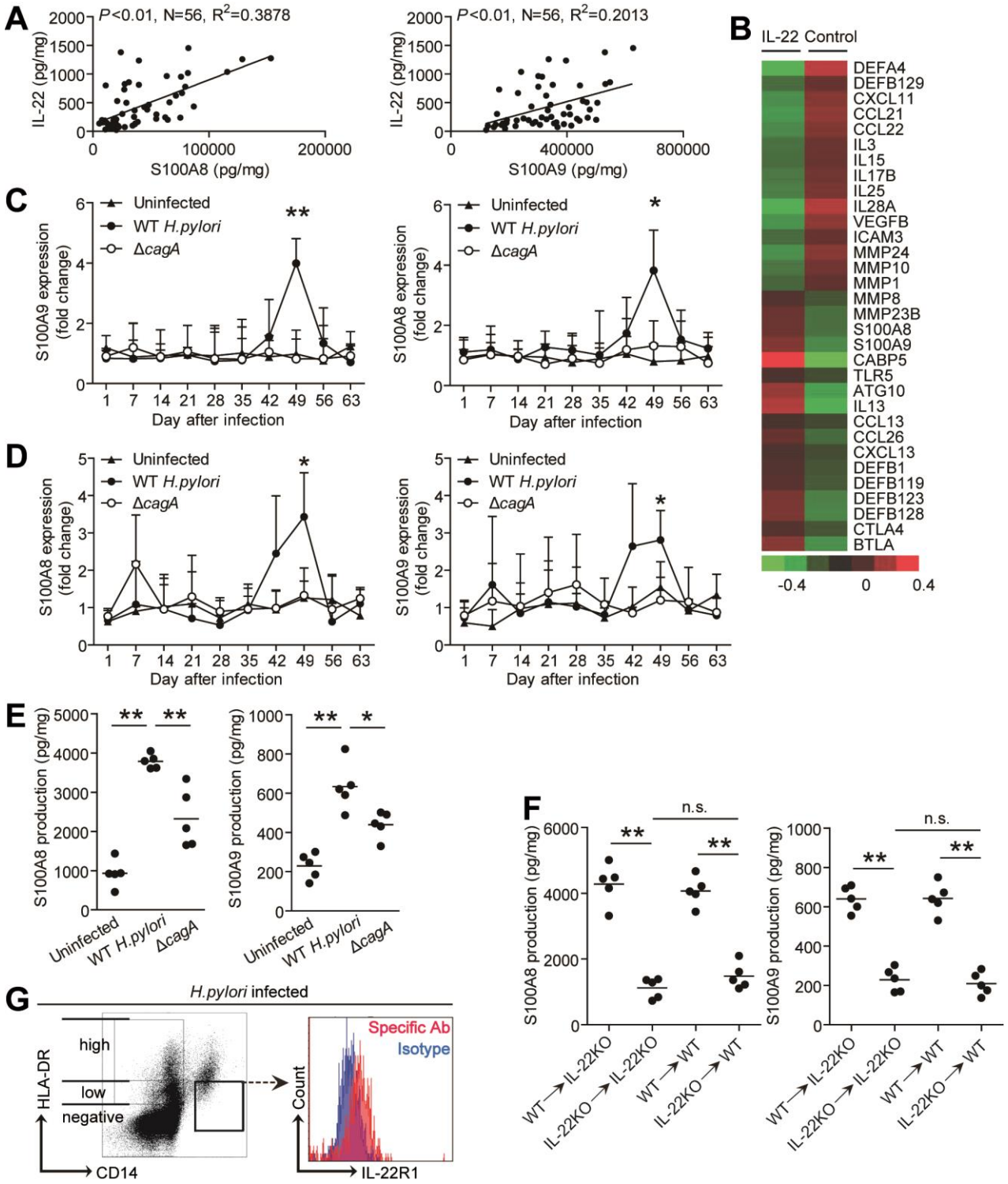
Supplemental Figure 4. IL-22 promotes CXCL2 production, and CXCR2-expressing MDSCs accumulated in gastric mucosa during *H. pylori* infection. (A) Expression of CXCL2 mRNA in unstimulated or IL-22-stimulated AGS cells was compared (n=3). (B) CXCL2 mRNA expression in unstimulated, or IL-22- stimulated primary gastric epithelial cells from uninfected donors were compared (n=3). (C) Dynamic change of MDSCs in WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected C57BL/6 mice. n=5 per group per time point. (D) Representative immunofluorescence staining images showed M-MDSCs infiltration in gastric mucosa of WT *H. pylori*-infected mice on day 49 p.i.. Scale bars: 20 microns. *p<0.05, **p<0.01, n.s p>0.05 for groups connected by horizontal lines compared, or compared with uninfected mice.



Supplemental Figure 5. IL-22 promotes MDSC accumulation in *H. pylori*-infected gastric mucosa by CXCL2-CXCR2 axis *in vivo*. (A) MDSC responses in gastric mucosa of WT *H. pylori*-infected WT BALB/c (left) and WT C57BL/6 (right) mice injected with Abs against IL-22, IL-17A, IL-17F, or IFN-γ or isotype control Abs on day 49 p.i. were compared. (B) MDSC responses in gastric mucosa of in WT *H. pylori*- infected WT C57BL/6 mice injected with IL-22 or PBS control, Abs against IL-22 (IgG2a), CXCR2 (IgG2a), and/or CXCL2 (IgG2b) or corresponding isotype control Abs, or SB225002 or DMSO control on day 49 p.i. were compared. (C) MDSC responses in gastric mucosa of WT *H. pylori*-infected BM chimera mice on day 49 p.i. were compared. *p<0.05, **p<0.01, n.s p>0.05 for groups connected by horizontal lines compared.

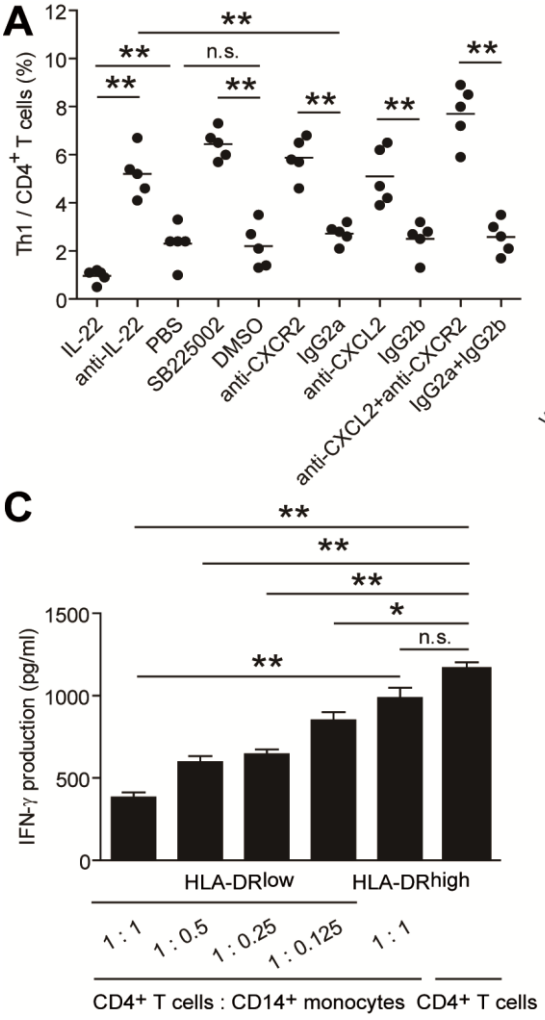


Supplemental Figure 6. IL-22 regulates pro-inflammatory proteins S100A8 and S100A9 expression. (A) Correlations between IL-22 and S100A8 or S100A9 in gastric mucosa of *H. pylori*-infected patients were analyzed. (B) Clustering of microarray data for the genes in human $CD45^{+}CD14^{+}HLA\text{-}DR^{low/-}$ MDSCs. (C and D) Dynamic change of S100A8 and S100A mRNA expression in WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected BALB/c (C) and C57BL/6 (D) mice. (E) S100A8 and S100A9 proteins in gastric mucosa of WT *H. pylori*-infected, *ΔcagA*-infected, and uninfected BALB/c mice on day 49 p.i. were analyzed. (F) S100A8 and S100A9 protein concentrations in gastric mucosa of WT *H. pylori*-infected BM chimera mice on day 49 p.i. were compared. (G) Representative dot plots of MDSCs and expression of IL-22R1 on MDSCs by gating on $CD45^{+}$ cells in peripheral blood of *H. pylori*-infected patients. n=5 per group per time point in *C* and *D*. *p<0.05, **p<0.01, n.s p>0.05 for groups connected by horizontal lines compared, or compared with uninfected mice.



Supplemental Figure 7. IL-22-induced MDSCs suppress Th1 cell response in H. pylori infection. (A and B) Th1 cell responses in gastric mucosa of in H. pylori-infected WT C57BL/6 mice injected with IL-22 or PBS control, Abs against IL-22 (IgG2a), CXCR2 (IgG2a), and/or CXCL2 (IgG2b) or corresponding isotype control Abs, or SB225002 (the potent and selective nonpeptide CXCR2 antagonist) or DMSO control (A), or H. pylori-infected BM chimera mice (B) on day 49 p.i. were compared. (C) CFSE-labelled peripheral CD4+ T cells of uninfected donors were co-cultured for 5 days with human peripheral CD45+CD14+HLA-DRlow/- MDSCs from H. pylori-infected patients at different ratios. Production of IFN-? was detected by ELISA in the co-culture supernatants (n=3). *p<0.05, **p<0.01, n.s p>0.05 for groups connected by horizontal

lines compared.



Supplementary Table 1. Clinical characteristics of patients

Variables	*H. pylori*-infected	Uninfected
Age (median, range)	(43 year, 22–71 years)	(46 year, 25–68 years)
Sex (male/female)	42/36	32/27

Exclusion criteria were: previous treatment for *H. pylori* infection, use of inhibitors of acid secretion and/or antibiotics during the 2 months before the study, use of anticoagulant drugs in the last week, gastrointestinal malignancy, severe concomitant cardiovascular, respiratory or endocrine diseases, clinically significant renal or hepatic disease, haematological disorders, previous gastro-oesophageal surgery, history of allergy to any of the drug used in the study, pregnancy or lactation, alcohol abuse, drug addiction, severe neurological or psychiatric disorders, and long-term use of corticosteroids or anti-inflammatory drugs.

Supplementary Table 2. The detailed health status and health report of all mice

	Date	HMV	Sendai	MPV	TMEV	LCMV	MNV	Tyzzer's organism	*C. kutc.*	*P. pneu.*	*Salmonella* sp	*E coli O-15*	*Helicobacter* spp	*M. pulm.*	*G. muri.*	*Trichomonas* sp	*Entamoeba* sp	*P. cari.*	*Syphacia* sp	*A. tetr.*
BALB/c	03.04.14	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	06.12.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	05.08.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	02.04.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	07.12.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	04.08.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	03.04.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

	Date	HMV	Sendai	MPV	TMEV	LCMV	MNV	Tyzzer's organism	*C. kutc.*	*P. pneu.*	*Salmonella* sp	*E coli O-15*	*Helicobacter* spp	*M. pulm.*	*G. muri.*	*Trichomonas* sp	*Entamoeba* sp	*P. cari.*	*Syphacia* sp	*A. tetr.*
C57BL/6	04.04.14	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	07.12.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	06.08.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	03.04.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	08.12.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	05.08.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	04.04.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

	Date	HMV	Sendai	MPV	TMEV	LCMV	MNV	Tyzzer's org	*C. kutc.*	*P. pneu.*	*Salmonella* sp	*E coli O-15*	*Helicobacter* sp	*M. pulm.*	*G. muri.*	*Trichomonas*	*Entamoeba* sp	*P. cari.*	*Syphacia* sp	*A. tetr.*

								anism					p			sp				
IL-2KO	05.04.14	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	08.12.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	07.08.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	04.04.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	09.12.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	06.08.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	05.04.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

	Date	HMV	Sendai	MPV	TMEV	LCMV	MNV	Tyzzer's organism	*C. kutc.*	*P. pneu.*	*Salmonella* sp	*E coli O-115*	*Helicobacter* sp	*M. pulm.*	*G. muri.*	*Trichomonas* sp	*Entamoeba* sp	*P. cari.*	*Syphacia* sp	*A. tetr.*
IL-3ko	06.04.14	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	09.12.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	08.08.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	05.04.13	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	10.12.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	07.08.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
	06.04.12	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Note. mouse hepatitis virus, HMV; Sendai virus, Sendai ;Mouse pneumonitis virus, MPV ; Theller murine encephalomyelitis virus, TMEV; lymphocytic choriomeningitis virus, LCMV; mouse norovirus, MNV; *Corynebacterium kutcheri*, *C. kutc.*; *Pasteurella pneumotropica*, *P. pneu.*; *Salmonella* sp; *E coli O-115*; *Helicobacter* spp; *Mycoplasma pulmonis*, *M. pulm.*; *Giardia muris*, *G. muri.*; *Trichomonas* sp; *Entamoeba* sp; *Pneumocystis carinii, P. cari.*; *Syphacia* sp; *Aspicularis tetraptera, A. tetr.*

Supplementary Table 3. Primer and probe sequences for real-time PCR analysis

Gene	Primer or probe	Sequence 5′→3′
H. pylori 16S rDNA	forward	TTTGTTAGAGAAGATAATGACGGTATCTAAC
	reverse	CATAGGATTTCACACCTGACTGACTATC
	probe	CGTGCCAGCAGCCGCGGT
H. pylori cagA	forward	GAGTCATAATGGCATAGAACCTGAA
	reverse	TTGTGCAAGAAATTCCATGAAA
Human β-actin	forward	TTCCTTCCTGGGCATGGAGTCC
	reverse	TGGCGTACAGGTCTTTGCGG
Human IL-22	forward	GACAAGTCCAACTTCCAG
	reverse	GCTCACTCATACTGACTC
Human IL-22R1	forward	AGCCCTGCAAGGCAGAAATG
	reverse	GATTGTGAAACTGGCCAGGAATG
Human CXCL2	forward	CCCAAGTTAGTTCAATCCTG
	reverse	TTCCTCAGCCTCTATCACAG
Mouse β2-microglobulin	forward	CCTGCAGAGTTAAGCATGCCAG
	reverse	TGCTTGATCACATGTCTCGATCC
	probe	TGGCCGAGCCCAAGACCGTCTAC
Mouse Sry	forward	TGGGACTGGTGACAATTGTC
	reverse	GAGTACAGGTGTGCAGCTCT
Mouse IL-22	forward	ATACATCGTCAACCGCACCTTT
	reverse	AGCCGGACATCTGTGTTGTTAT
Mouse IL-22R1	forward	CTACGTGTGCCGAGTGAAGA
	reverse	AAGCGTAGGGGTTGAAAGGT
Mouse S100A8	forward	GGAAATCACCATGCCCTCTAC
	reverse	GCCACACCCACTTTTATCACC
Mouse S100A9	forward	AACATCTGTGACTCTTTAGCCTTG
	reverse	ACTGTGCTTCCACCATTTGTCT

For the probes, a FAM fluorescent reporter is coupled to the 5' end, and a TAMRA quencher is coupled to the 3'

end.