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# MicroRNA-155 Is Essential for the T Cell-Mediated Control of *Helicobacter pylori* Infection and for the Induction of Chronic Gastritis and Colitis

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MicroRNAs govern immune responses to infectious agents, allergens, and autoantigens and function by posttranscriptional repression of their target genes. In this paper, we have addressed the role of microRNA-155 (miR-155) in the control of *Helicobacter pylori* infection of the gastrointestinal tract and the development of *H. pylori*-induced chronic gastritis and associated gastric preneoplastic pathology. We show that miR-155 is upregulated in the gastric mucosa of experimentally infected mice and that miR-155<sup>-/-</sup> mice fail to control *H. pylori* infection as a result of impaired pathogen-specific Th1 and Th17 responses. miR-155<sup>-/-</sup> mice are also less well protected against challenge infection after *H. pylori*-specific vaccination than their wild-type (wt) counterparts. As a consequence of their impaired T cell responses to *H. pylori*, miR-155<sup>-/-</sup> mice develop less severe infection-induced immunopathology manifesting as chronic atrophic gastritis, epithelial hyperplasia, and intestinal metaplasia. T cells from miR-155<sup>-/-</sup> mice that are activated by CD3/CD28 cross-linking expand less and produce less IFN- $\gamma$  and IL-17 than wt T cells. Finally, we show in this paper using adoptive transfers that the phenotypes of miR-155<sup>-/-</sup> mice are likely due to T cell-intrinsic defects. In contrast to wt T cells, miR-155<sup>-/-</sup> T cells from infected donors do not control *H. pylori* infections in T cell-deficient recipients, do not differentiate into Th1 or Th17 cells, and do not cause immunopathology. In addition, naive miR-155<sup>-/-</sup> T cells fail to induce chronic Th17-driven colitis in an adoptive transfer model. In conclusion, miR-155 expression is required for the Th17/Th1 differentiation that underlies immunity to *H. pylori* infection on the one hand and infection-associated immunopathology on the other. *The Journal of Immunology*, 2011, 187: 3578–3586.

MicroRNAs (miRNAs) are an abundant class of small noncoding RNAs that modulate the expression of their target genes at the posttranscriptional level. They bind to the 3' untranslated regions of specific target mRNAs, thereby suppressing their translation and promoting their degradation. Of the ~700 different miRNAs identified in the human genome to date, at least 100 are expressed in cells of the immune system. The development and function of innate as well as adaptive immune cell populations are now widely believed to be critically dependent on miRNA function (1). One miRNA in particular, miRNA (miR)-155, has received much attention because of its expression and activity in a variety of immune cell types, namely

macrophages, dendritic cells (DCs), and various subsets of lymphocytes. In myeloid cells, miR-155 is transcriptionally induced in response to TLR ligands or TNF- $\alpha$  exposure in a manner depending on the transcription factors AP-1 and NF- $\kappa$ B (2). Both pro- and anti-inflammatory effects of miR-155 expression have been reported in myeloid cells, depending on the context and the availability of target mRNAs; proinflammatory effects in myeloid cells have been linked to the repression of negative immune regulators such as SHIP1 and suppressor of cytokine signaling 1 (SOCS1) (3–5), whereas anti-inflammatory effects have been associated with the repression of the signaling protein TAB2 and inhibition of IL-1 $\beta$  expression (6). B cells upregulate miR-155 following their activation in germinal centers; miR-155<sup>-/-</sup> B cells exhibit defective Ab class switching and fail to differentiate into plasma cells (7, 8). These defects have been attributed to the miR-155-mediated repression of activation-induced deaminase, an enzyme critically involved in class switch recombination and somatic hypermutation (9, 10). T cells in miR-155<sup>-/-</sup> mice are biased toward Th2 polarization, suggesting that miR-155 promotes Th1 cells (7, 8). A recent study has reported a role for miR-155 in Th17 differentiation and, consequently, found miR-155<sup>-/-</sup> mice to be highly resistant to experimental autoimmune encephalomyelitis (11).

In this study, we have examined a possible role for miR-155 in controlling experimental infection with the Gram-negative bacterial pathogen *Helicobacter pylori*. Persistent *H. pylori* colonization of its preferred niche, the human gastric mucosa, results in chronic gastritis (12) and predisposes carriers to a high risk of developing gastric and duodenal ulcers, gastric cancer, and gastric MALT lymphoma (13–15). We and others (16–19) have reported earlier that the control of *H. pylori* infection in both experimental infection and vaccination/challenge models requires the activity of Th1 and

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The online version of this article contains supplemental material.

Abbreviations used in this article: BMDC, bone marrow-derived dendritic cell; DC, dendritic cell; DSS, dextran sulfate sodium; miR, microRNA; miRNA, microRNA; PMSS1, premouse Sydney strain 1; qRT-PCR, quantitative RT-PCR; snoR, small nucleolar RNA; SOCS1, suppressor of cytokine signaling 1; Treg, regulatory T cell; wt, wild type.

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Th17 cells. *Helicobacter*-specific Th1 cells and their signature cytokine IFN- $\gamma$  were further shown to be directly responsible for the induction of infection-associated preneoplastic pathology manifesting as chronic atrophic gastritis, epithelial hyperplasia, and intestinal metaplasia (18, 20). The adoptive transfer of IFN- $\gamma$ -proficient but not -deficient T cells into TCR $\beta^{-/-}$  mice was sufficient to eliminate the infection in the recipients and to induce the full range of preneoplastic pathology (18). Given the major contribution of T cells to the control of—and immunopathology induced by—*H. pylori*, as well as the crucial role of miR-155 in Th1 and Th17 differentiation and the reported upregulation of miR-155 in T cells upon *H. pylori* infection in vitro and in patients (21, 22), we hypothesized that miR-155 should have a clearly discernible role in *H. pylori* pathogenesis. Indeed, we found that miR-155 $^{-/-}$  mice were incapable of spontaneously controlling *H. pylori* and of developing vaccine-induced protective immunity and were consequently protected against infection-associated preneoplastic pathology. The phenotype of miR-155-deficient mice could be attributed to a likely T cell-intrinsic defect and was corroborated by the inability of miR-155 $^{-/-}$  T cells to proliferate and to produce IFN- $\gamma$  and IL-17 upon activation via their TCR in vitro.

## Materials and Methods

### Animal experimentation and *H. pylori* cultures

C57BL/6, Rag1 $^{-/-}$  BL6, TCR- $\beta^{-/-}$  BL6, and miR-155 $^{-/-}$  BL6 (B6.Cg-Mir155tm1.1Rsky/J) mice were purchased from The Jackson Laboratory (distributed by Charles River Laboratories, Sulzfeld, Germany). All strains were bred at a University of Zurich specific pathogen-free facility. Mice were maintained in individually ventilated cages and included in studies at 6 wk of age. All animal experimentation was conducted in accordance with cantonal and federal guidelines for the care and use of laboratory animals and was reviewed and approved by the Zurich cantonal veterinary office. Mice were orally immunized three times at weekly intervals with 1 mg *H. pylori* (strain SS1) sonicate adjuvanted with 10  $\mu$ g cholera toxin (List Biologicals, Campbell, CA) and challenged 1 wk after the last immunization with autologous *H. pylori* by oral gavage of 10<sup>8</sup> bacteria. For adoptive transfer experiments, 300,000 immunomagnetically sorted splenic CD4<sup>+</sup>CD25<sup>-</sup> T cells or naive CD4<sup>+</sup>CD62L<sup>+</sup>CD44<sup>low</sup> T cells (R&D Systems, Minneapolis, MN) were injected into the tail veins of Rag1 $^{-/-}$  or TCR- $\beta^{-/-}$  mice. Dextran sulfate sodium (DSS)-induced colitis was provoked by three cycles of 2% DSS administration in the drinking water (for 5 d) with 7-d compound-free intervals. The *H. pylori* premouse Sydney strain 1 (PMSS1) and its mouse-passaged derivative SS1 as well as agar and liquid culture conditions were described in detail previously (23, 24).

### Preparation of gastric/colonic tissue and single-cell suspensions; assessment of *H. pylori* colonization and histopathology

Stomachs and colons were retrieved and dissected longitudinally into equally sized pieces. For quantitative assessment of *H. pylori* colonization, one section of each stomach was homogenized in *Brucella* broth, and serial dilutions were plated on horse blood plates for colony counting. For the quantitative assessment of gastric histopathology, Giemsa-stained paraffin-embedded longitudinal stomach sections ranging from the forestomach/corpus junction to the duodenum were scored independently and in a blinded fashion by two experimenters as described previously (18). Scores on a scale of 0–6 were assigned for the parameters chronic inflammation (in the antrum and corpus areas) as well as corpus atrophy, epithelial hyperplasia, and metaplasia. For the characterization of gastric immune cell infiltrates, one section of every stomach (antrum and corpus) was digested in 1 mg/ml collagenase (Sigma-Aldrich), disrupted between glass slides, filtered through a cell strainer (40  $\mu$ m; BD Biosciences, San Jose, CA), and processed for flow cytometry (see below). Colonic lamina propria cells were prepared by collagenase digestion, followed by repeated pipetting and processing of single-cell suspensions for flow cytometry. Colon histopathology was scored on Giemsa-stained sections according to the following published guidelines (25): grade 0, no changes observed; grade 1, minimal scattered mucosal inflammatory cell infiltrates, with or without minimal epithelial hyperplasia; grade 2, mild scattered to diffuse inflammatory cell infiltrates, sometimes extending into the submucosa and associated with erosions, with minimal to mild epithelial hyperplasia and minimal to mild

mucin depletion from goblet cells; grade 3, mild to moderate inflammatory cell infiltrates that were sometimes transmural, often associated with ulceration, with moderate epithelial hyperplasia and mucin depletion; grade 4, marked inflammatory cell infiltrates that were often transmural and associated with ulceration, with marked epithelial hyperplasia and mucin depletion; and grade 5, marked transmural inflammation with severe ulceration and loss of intestinal glands. Representative images were recorded at  $\times 100$  and  $\times 200$  final magnification with a Leica Leitz DM RB microscope equipped with a Leica DFC 420C camera. Images were acquired using the Leica Application Suite 3.3.0 software.

### Flow cytometry

The following Abs were used for FACS analysis: IFN- $\gamma$ -PE-Cy7, CD4-FITC (BD Biosciences CA), CD45-Pacific blue, and Ly6-G-allophycocyanin (all BioLegend, San Diego, CA). For intracellular IFN- $\gamma$  staining, cells were restimulated and blocked for 5 h in medium containing 2.5  $\mu$ g/ml brefeldin A (AppliChem, Darmstadt, Germany), 0.2  $\mu$ M ionomycin (Santa Cruz Biotechnology, Santa Cruz, CA), and 50 ng/ml PMA (Sigma-Aldrich). Cells were first stained for CD4 and then fixed in 4% paraformaldehyde and stained for IFN- $\gamma$  in 0.5% saponin permeabilization buffer. Flow cytometric analysis was performed on a CyanADP instrument and analyzed with FlowJo software (Tree Star, Ashland, OR).

### T cell activation assays

CD4<sup>+</sup>CD25<sup>-</sup> T cells were prepared from single-cell suspensions of C57BL/6 wild-type (wt) or miR-155 $^{-/-}$  spleens (R&D Systems). T cells were stimulated with anti-CD3/CD28-coated beads (Invitrogen) in the presence of 10 ng/ml rIL-2 (R&D Systems). Bone marrow-derived DCs (BMDCs) were generated by culturing fresh wt bone marrow cells at a density of 50,000 per 96-well flat bottom in RPMI 1640 medium with 10% FCS and 4 ng/ml GM-CSF for 7 d. DCs were matured by addition of 500 ng/ml *Escherichia coli* LPS (Sigma-Aldrich) for 12 h. DCs and T cells were cocultured at a ratio of 1:2 (50,000 DCs:100,000 T cells) in the presence of 1  $\mu$ g/ml anti-CD3 $\epsilon$  (BD Biosciences) and 10 ng/ml rIL-2. After 72 h of coculture, IFN- $\gamma$  and IL-17 production were quantified by ELISA (R&D Systems) or IFN- $\gamma$ -specific intracellular cytokine staining for FACS analysis (see flow cytometry). Proliferation was assessed by [<sup>3</sup>H]thymidine incorporation assay.

### Cytokine and miR-155 quantitative RT-PCR

For quantitative RT-PCR (qRT-PCR) of IFN- $\gamma$  and IL-17, RNA was isolated from scraped gastric mucosa (antrum and corpus) using a NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany). Total RNA (1.5  $\mu$ g) was used for cDNA synthesis with Superscript Reverse Transcriptase III (Life Technologies). The resulting cDNA served as a template for quantitative PCR performed with a LightCycler 480 using the SYBR Green I Master Kit (Roche, Basel, Switzerland). Absolute values of IFN- $\gamma$  and IL-17 expression were normalized to GAPDH expression. Primers and conditions were as follows: Tm 55°C, 50 cycles—GAPDH, 5'-GAC ATT GTT GCC ATC AAC GAC C-3' (forward) and 5'-CCC GTT GAT GAC CAG CTT CC-3'; IFN- $\gamma$ , 5'-CAT GGC TGT TTC TGG CTG TTA CTG-3' (forward) and 5'-GTT GCT GAT GGC CTG ATT GTC TTT-3' (reverse); and IL-17, 5'-GCT CCA GAA GGC CCT CAG A-3' (forward) and 5'-AGC TTT CCC TCC GCA TTG A-3'. The qRT-PCR for miR-155 quantification was performed according to a two-step manufacturer's protocol (Applied Biosystems) using specific primers for murine miR-155 (Assay-ID 001806) and small nucleolar RNA (snoR)-202 (Assay-ID 001232) and 10 ng total RNA as template.

### Statistics

GraphPad Prism 5.0 software (GraphPad, La Jolla, CA) was used for statistical analyses. All *p* values were calculated by Mann-Whitney *U* test unless otherwise indicated; Bonferroni-Holm corrections were made for multiple comparisons where appropriate. In all scatter plot graphs, the medians are indicated by horizontal bars. In column bar graphs, the SEM is indicated by vertical bars; n.s. stands for "not significant."

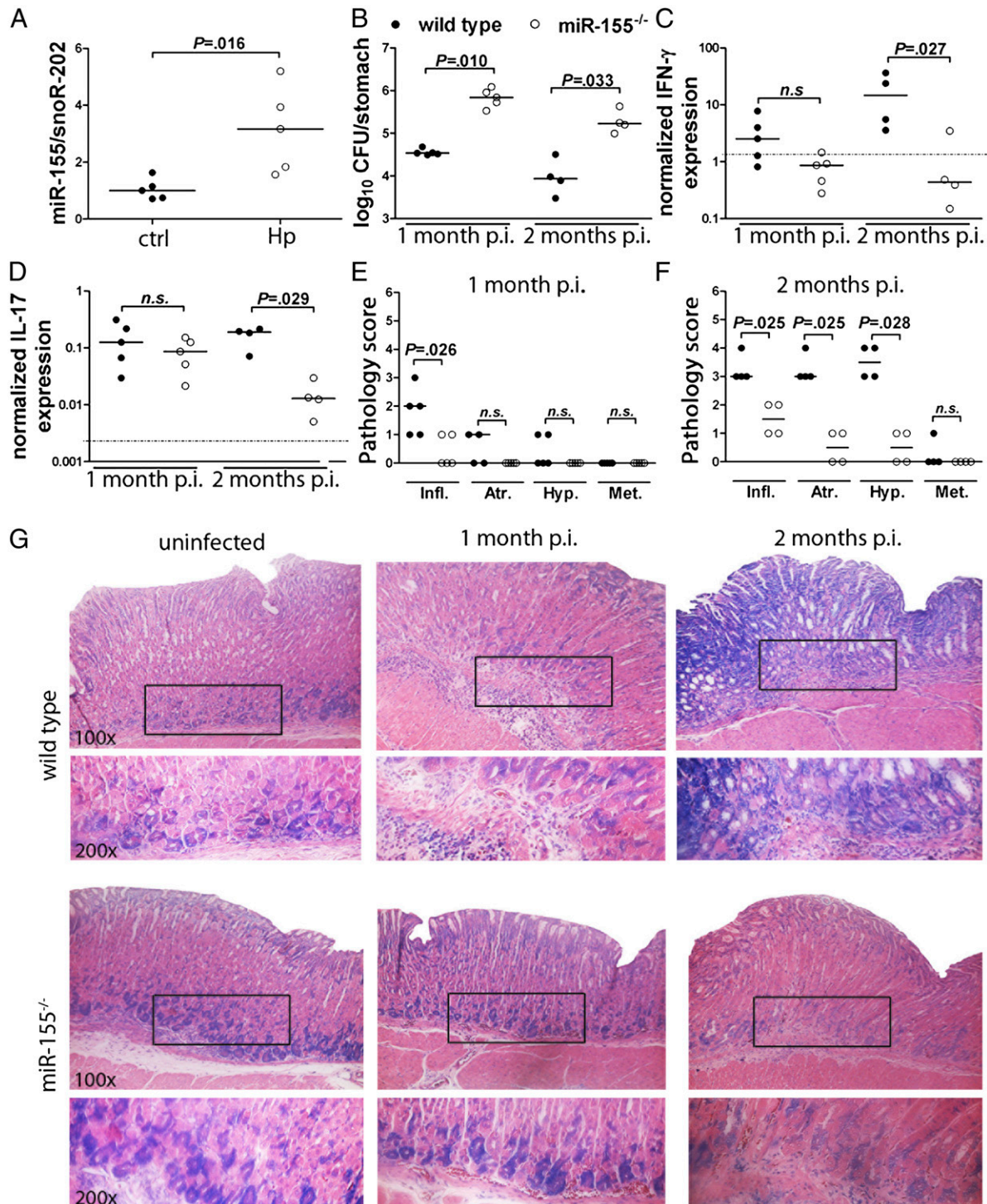
## Results

### miR-155 $^{-/-}$ mice fail to control experimental *H. pylori* infection because of their inability to generate Th1 and Th17 responses

Experimental murine *H. pylori* infections are controlled by MHC class II-restricted, Th1- and Th17-polarized Th cells (16, 18, 24). To examine a possible role for miR-155 in spontaneously reducing

*H. pylori* colonization upon experimental infection, wt and miR-155<sup>-/-</sup> mice were infected with the virulent *H. pylori* patient isolate PMSS1 for 1 and 2 mo and analyzed with respect to colonization, gastric cytokine levels, and gastric histopathology (Fig. 1). The expression of miR-155 was induced by ~3-fold in the

gastric mucosa of wt mice infected with *H. pylori* for 1 mo (Fig. 1A). miR-155<sup>-/-</sup> mice were colonized by at least 1 order of magnitude more densely than wt animals at both time points (Fig. 1B) and exhibited significantly lower levels of gastric IFN- $\gamma$  and IL-17 production at 2 mo postinfection (Fig. 1C, 1D). In line with



**FIGURE 1.** miR-155<sup>-/-</sup> mice are colonized more densely than wt controls, produce less IFN- $\gamma$  and IL-17, and do not develop preneoplastic gastric pathology. *A*, wt C57BL/6 mice were infected for 1 mo with *H. pylori* PMSS1, and the gastric mucosal expression of miR-155 was assessed by quantitative PCR. miR-155 expression was normalized to snoR-202. *B–G*, C57BL/6 wt and miR-155<sup>-/-</sup> mice were infected for 1 or 2 mo with *H. pylori* PMSS1 as indicated. *B*, Gastric *H. pylori* colonization as determined by plating and colony counting. *C* and *D*, Gastric mucosal IFN- $\gamma$  and IL-17 production as determined by qRT-PCR and normalized to GAPDH. The dotted line indicates the average baseline cytokine expression in uninfected controls of both genotypes (three to five mice each). *E–G*, Gastric histopathology at 1 mo (*E*, *G*) and 2 mo (*F*, *G*) postinfection. Scores in *E* and *F* were assigned independently for the parameters inflammation, atrophy, epithelial hyperplasia, and intestinal metaplasia. Representative micrographs of Giemsa-stained sections are shown in *G* at  $\times 100$  and  $\times 200$  final magnification in relation to uninfected controls of both genotypes. In *A–F*, each data point represents one mouse.

a role for both Th1 and Th17 cells in inducing *H. pylori*-associated gastric inflammation and gastric preneoplastic lesions, miR-155<sup>-/-</sup> mice were almost completely protected against the histopathological changes manifesting as gastritis, gastric atrophy, epithelial hyperplasia, and intestinal metaplasia that are a hallmark of *H. pylori*-infected wt animals (Fig. 1E–G). In conclusion, miR-155 is crucially involved in the generation of Th1 and Th17 responses to experimental *H. pylori* infection, which control the infection on the one hand, and mediate infection-associated gastric immunopathology on the other.

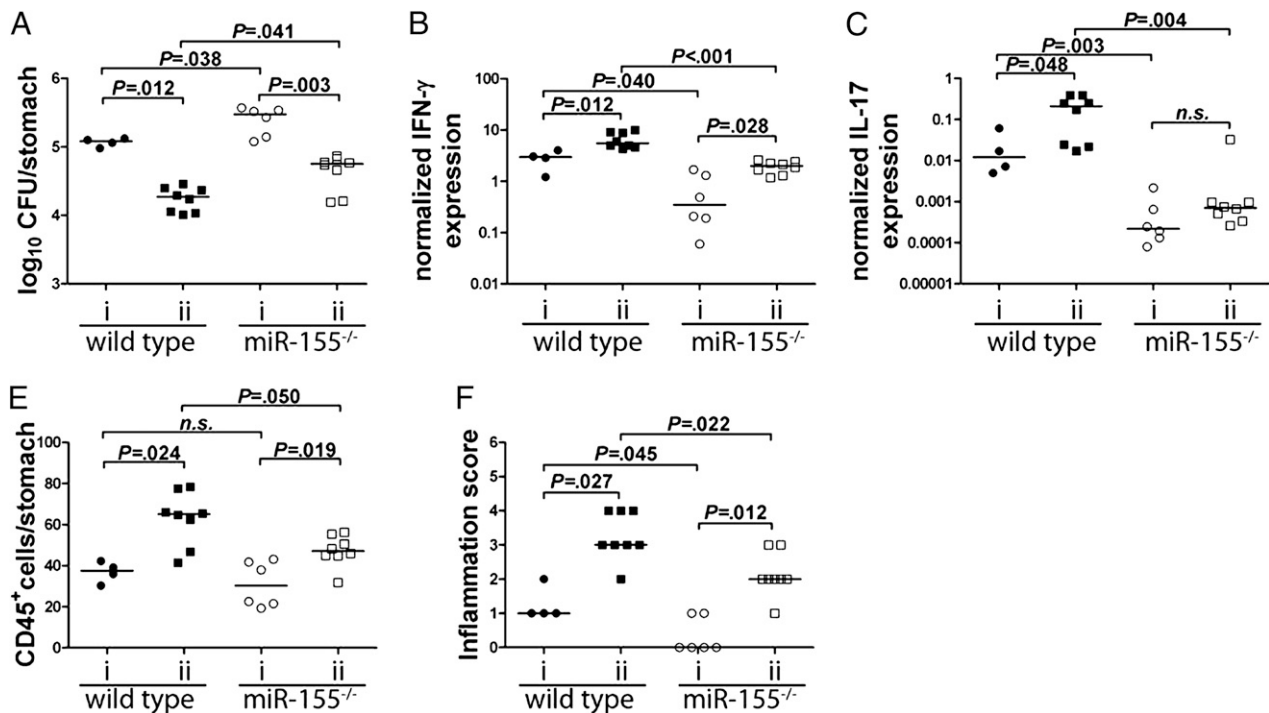
*miR-155<sup>-/-</sup> mice are less well protected than wt mice upon H. pylori-specific vaccination and challenge*

wt mice that receive three doses of an orally administered whole-cell *H. pylori* vaccine adjuvanted with cholera toxin develop protective immunity against challenge infection with the pathogen (16, 18). To assess whether miR-155<sup>-/-</sup> mice are protected as well as wt mice, groups of both genotypes were vaccinated three times at weekly intervals prior to autologous challenge infection with the commonly used *H. pylori* vaccine strain SS1. Although both strains were able to reduce *H. pylori* burdens by ~1 order of magnitude as a consequence of their prior immunization, the colonization levels in vaccinated miR-155<sup>-/-</sup> mice were significantly higher than those of their vaccinated wt counterparts (Fig. 2A). The colonization levels of naive (nonvaccinated) mice showed a similar trend (Fig. 2A), reproducing the findings obtained by experimental infection with the virulent isolate PMSS1 (Fig. 1B). Vaccinated miR-155<sup>-/-</sup> mice produced lower gastric levels of IFN- $\gamma$  and IL-17 than vaccinated wt animals (Fig. 2B, 2C). Their gastric mucosal infiltration of CD45<sup>+</sup> leukocytes, a reliable correlate of vaccine-induced protection (16), was signifi-

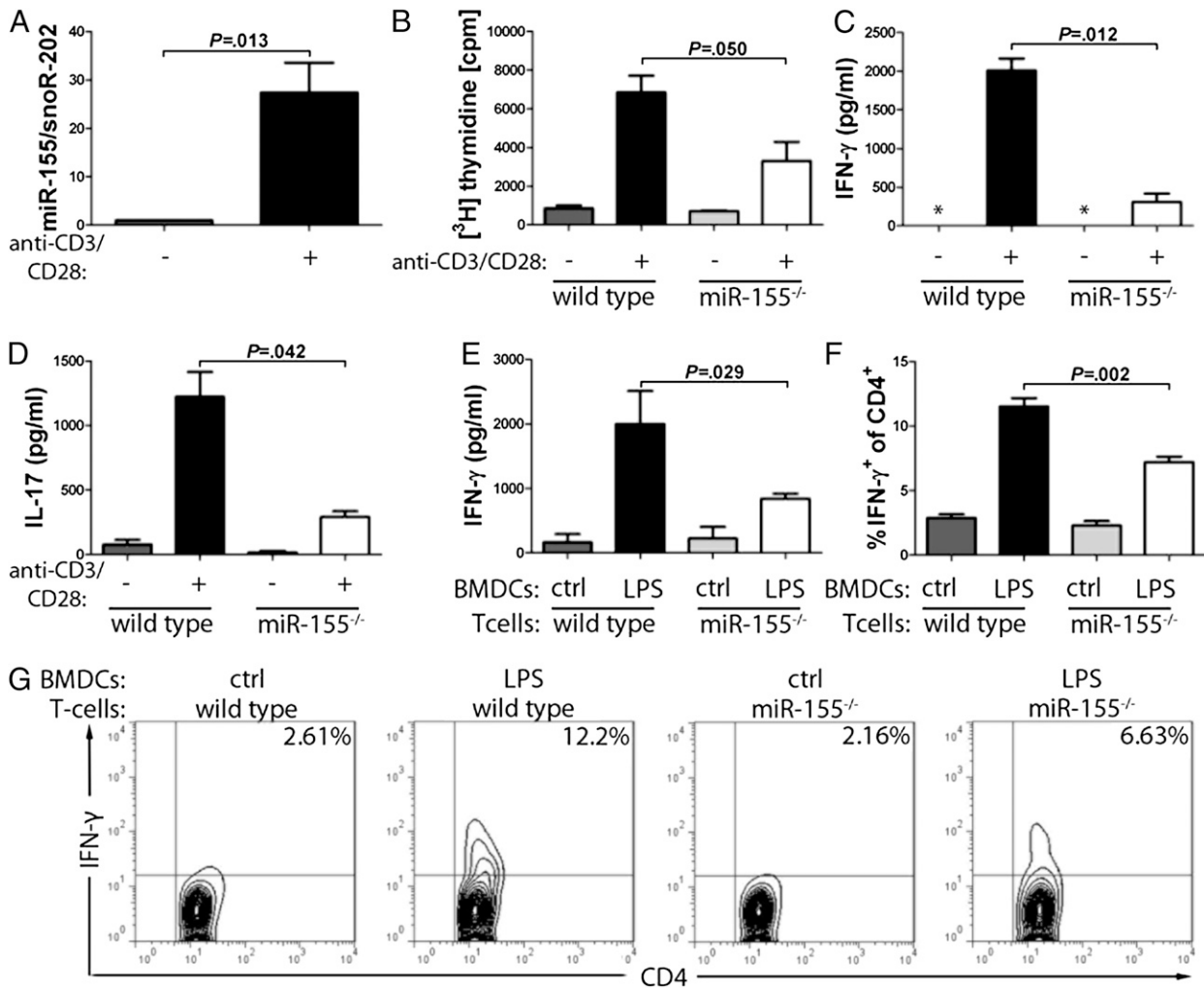
cantly reduced compared with wt mice (Fig. 2D). As a consequence of their lower cytokine expression and leukocyte infiltration, miR-155<sup>-/-</sup> mice exhibited less postchallenge gastritis than their vaccinated wt counterparts (Fig. 2E). In summary, miR-155 expression is essential for the optimal development of Th1/Th17-driven vaccine-induced protective immunity to *H. pylori* and contributes to the T cell-driven gastritis that is a hallmark of vaccinated, challenged wt mice.

*miR-155<sup>-/-</sup> T cells proliferate less and produce less IFN- $\gamma$  and IL-17 than wt T cells upon activation in vitro*

We and others (16–19) have shown that the spontaneous as well as the vaccine-induced control of *Helicobacter* infection depends on MHC class II-restricted, Th1- and Th17-polarized T cells. Speculating that the T cell-intrinsic expression of miR-155 is required to activate T cells and to generate IFN- $\gamma$ - and/or IL-17-producing effector cells, we treated immunomagnetically purified CD4<sup>+</sup>CD25<sup>-</sup> T cells with anti-CD3/anti-CD28 in vitro. In wt T cells, the expression of miR-155 was strongly induced upon anti-CD3/anti-CD28 treatment (Fig. 3A). miR-155<sup>-/-</sup> T cells proliferated less than wt cells upon anti-CD3/anti-CD28 stimulation as determined by thymidine incorporation assay (Fig. 3B) and produced significantly less IFN- $\gamma$  and IL-17 than miR-155-proficient T cells (Fig. 3C, 3D). In a complementary model, in which LPS-treated BMDCs provided costimulatory signals, miR-155<sup>-/-</sup> T cells produced less IFN- $\gamma$  than miR-155-proficient T cells as determined by ELISA and intracellular cytokine staining (Fig. 3E–G). IL-17 production was negligible under these circumstances (data not shown). Hypothesizing that regulatory T cells (Tregs) might also rely on miR-155 for proper function, we added immunomagnetically isolated CD4<sup>+</sup>CD25<sup>+</sup> Tregs to the T cell



**FIGURE 2.** Vaccinated miR-155<sup>-/-</sup> mice are less protected than their wt counterparts upon challenge infection with *H. pylori*. C57BL/6 wt and miR-155<sup>-/-</sup> mice were vaccinated three times with *H. pylori* SS1 sonicate adjuvanted with cholera toxin prior to challenge infection with the autologous strain. All vaccinated, challenged mice were sacrificed along with naive but challenged controls at 2 wk postchallenge infection. *A*, Gastric *H. pylori* colonization as determined by plating and colony counting. *B* and *C*, Gastric mucosal IFN- $\gamma$  and IL-17 production as determined by qRT-PCR and normalized to GAPDH. *D*, Gastric mucosal infiltration of CD45<sup>+</sup> leukocytes, as determined by flow cytometric analysis of mucosal single-cell preparations. *E*, Inflammation scores as assigned to all mice included in the study. In *A*–*E*, each data point represents one mouse. i, infected; ii, immunized infected.



**FIGURE 3.** miR-155<sup>-/-</sup> T cells proliferate less and produce less IFN- $\gamma$  and IL-17 than wt T cells upon activation. *A–D*, wt (and miR-155<sup>-/-</sup>) splenic CD4<sup>+</sup>CD25<sup>-</sup> T cells were treated with anti-CD3/CD28-coated beads for 72 h. *A*, miR-155 expression with and without anti-CD3/CD28 activation as determined by quantitative PCR and normalized to snoR-202. *B*, Proliferation as determined by [<sup>3</sup>H]thymidine incorporation assay. *C* and *D*, IFN- $\gamma$  and IL-17 production as quantified by ELISA. *E–G*, BMDCs were activated overnight with 10  $\mu$ g/ml *E. coli* LPS prior to coculturing with wt or miR-155<sup>-/-</sup> splenic CD4<sup>+</sup>CD25<sup>-</sup> T cells (1:2 ratio) for 72 h in the presence of anti-CD3 cross-linking Ab. *E*, IFN- $\gamma$  production as quantified by ELISA. *F* and *G*, IFN- $\gamma$  expression as determined by intracellular cytokine staining. Representative FACS plots of IFN- $\gamma$  versus CD4 are shown in *G*; averages of triplicate cocultures are shown in *F*. Vertical bars in *A–F* indicate SEM; \*, not detectable. The *p* values were calculated by Student *t* test.

activation system shown in Fig. 3*A–D*. Both wt and miR-155<sup>-/-</sup> Tregs prevented the CD3/C28 cross-linking–induced proliferation of T cells efficiently (Supplemental Fig. 1), indicating that miR-155 is not required for the suppressive activity of Tregs in vitro. Taken together, the results suggest that miR-155 is essential for the generation of Th1 and Th17 effector T cells subsets but dispensable for Treg function.

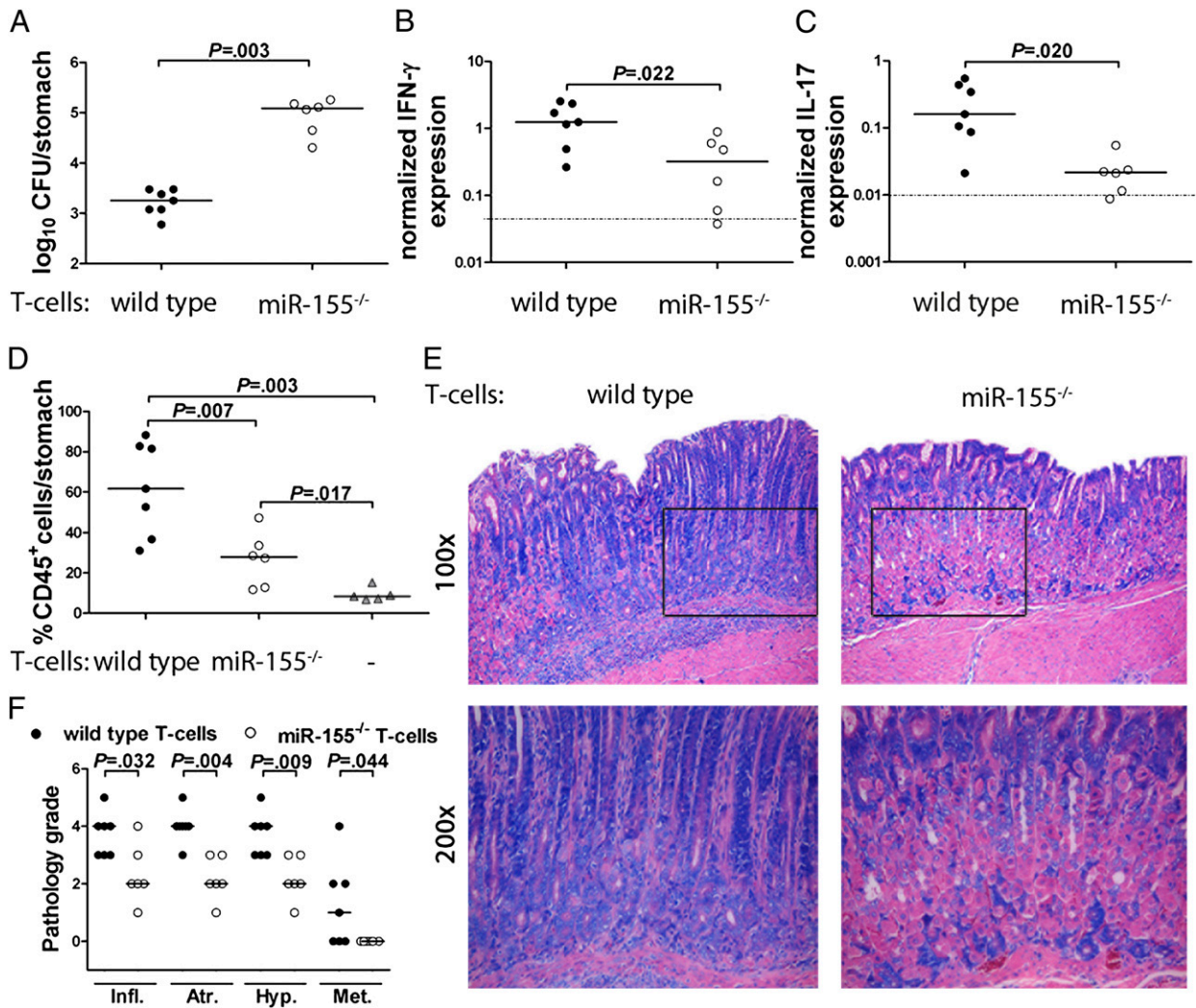
*miR-155 is required for clearance of H. pylori and the induction of gastritis and gastric preneoplastic pathology in an adoptive T cell transfer model*

We have shown earlier that the adoptive transfer of purified CD4<sup>+</sup>CD25<sup>-</sup> T cells from *Helicobacter*-infected donors is sufficient to induce infection-associated gastric preneoplastic pathology in T cell-deficient recipients (18, 24). The development of gastric immunopathology in this model is infection dependent on the part of the donor as well as the recipient (18). To examine a possible effect of miR-155 gene targeting in this model, we infected wt or miR-155<sup>-/-</sup> donors with *H. pylori* PMSS1 for 1 mo prior to the immunomagnetic isolation of splenic CD4<sup>+</sup>CD25<sup>-</sup> T cells. Rag-1<sup>-/-</sup> recipients were i.v. injected with either 300,000 wt or miR-

155<sup>-/-</sup> T cells and were experimentally infected on the day of adoptive transfer. Rag-1<sup>-/-</sup> recipients of miR-155<sup>-/-</sup> T cells were significantly less capable of clearing the *H. pylori* infection than recipients of wt cells (Fig. 4*A*) and produced lower levels of gastric IFN- $\gamma$  and IL-17 (Fig. 4*B*, 4*C*). Total leukocyte infiltration into the gastric mucosa was reduced in the recipients of miR-155<sup>-/-</sup> T cells (Fig. 4*D*), which also exhibited significantly less evidence of gastritis, atrophy, hyperplasia, and metaplasia than the recipients of wt T cells (Fig. 4*E*, 4*F*). In summary, the results suggest that the deficiency of miR-155<sup>-/-</sup> mice in clearing experimental *H. pylori* infections and in generating vaccine-induced protective immunity is due to a T cell-intrinsic defect. The inability of miR-155<sup>-/-</sup> T cells to optimally produce IFN- $\gamma$  or IL-17 upon in vitro activation (Fig. 3) lends further support to this model.

*T cell-intrinsic expression of miR-155 is required for the induction of chronic inflammation in a T cell-driven model of colitis*

Because of the mechanistic and molecular similarities between *H. pylori*-associated gastritis and T cell-driven models of chronic



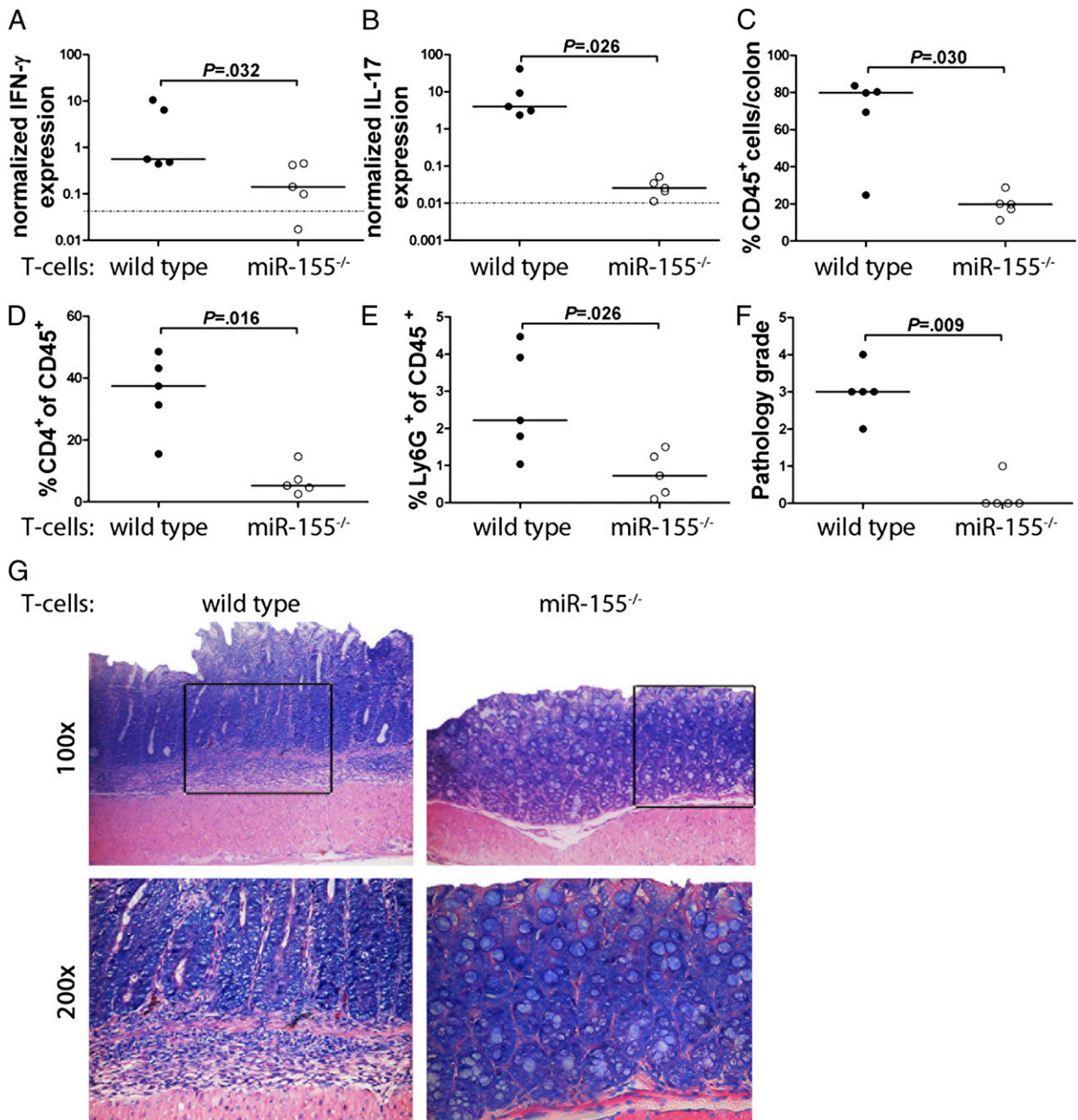
**FIGURE 4.** miR-155<sup>-/-</sup> T cells fail to clear *H. pylori* and to induce gastric pathology upon adoptive transfer into Rag-1<sup>-/-</sup> recipients. Rag-1<sup>-/-</sup> mice were infected with *H. pylori* PMSS1 and i.v. injected with 300,000 splenic CD4<sup>+</sup>CD25<sup>-</sup> T cells isolated from *H. pylori*-infected wt or miR-155<sup>-/-</sup> donors. Recipients were sacrificed 1 mo postinfection/adoptive transfer. **A**, Gastric *H. pylori* colonization as determined by plating and colony counting. **B** and **C**, Gastric mucosal IFN- $\gamma$  and IL-17 production as determined by qRT-PCR and normalized to GAPDH. The dotted line indicates the average baseline cytokine expression in uninfected controls that have not received cells. **D**, Gastric mucosal infiltration of CD45<sup>+</sup> leukocytes, as determined by flow cytometric analysis of mucosal single-cell preparations. **E** and **F**, Gastric histopathology as assessed on Giemsa-stained sections; representative micrographs are shown in **E** at  $\times 100$  and  $\times 200$  final magnification. Scores in **F** were assigned independently for the parameters inflammation, atrophy, epithelial hyperplasia, and intestinal metaplasia. In **A–D** and **F**, each data point represents one mouse.

inflammatory conditions of the colon, we hypothesized that the T cell-intrinsic expression of miR-155 should also influence disease outcome in murine colitis models. To test this notion, we adoptively transferred naive wt or miR-155<sup>-/-</sup> T cells into TCR- $\beta$ <sup>-/-</sup> recipients and assessed the development of colitis symptoms 4 wk posttransfer (Fig. 5). The recipients of miR-155<sup>-/-</sup> T cells exhibited significantly lower colonic expression of IFN- $\gamma$  and IL-17 than the recipients of wt T cells (Fig. 5A, 5B). The colonic mucosa of miR-155<sup>-/-</sup> T cell recipients was further infiltrated by substantially lower numbers of CD45<sup>+</sup> leukocytes, CD4<sup>+</sup> T cells, and Ly6G<sup>+</sup> neutrophils than their wt-recipient counterparts (Fig. 5C–E). The pathology scores of miR-155<sup>-/-</sup> T cell recipients—integrating the parameters inflammation, appearance of erosions and ulcerations, and epithelial hyperplasia—were significantly lower than those of wt T cell recipients (Fig. 5F, 5G). Whereas the latter group presented with on average grade 3 lesions (mild to moderate inflammatory cell infiltrates, sometimes transmural, often associated with ulceration, moderate epithelial hyperplasia, and mucin depletion), only mild, if any, colitis was detected in the

recipients of miR-155<sup>-/-</sup> T cells. In summary, the efficient induction of Th1- and Th17-driven colitis by adoptively transferred, naive T cells requires T cellular miR-155 expression. To assess the susceptibility of miR-155<sup>-/-</sup> mice in an alternative colitis model, wt and miR-155<sup>-/-</sup> animals were subjected to three cycles of DSS administration via the drinking water to induce chronic colitis. miR-155<sup>-/-</sup> mice exhibited lower levels of colonic IFN- $\gamma$  and IL-17 expression and also developed somewhat milder colitis than wt controls (Supplemental Fig. 2), suggesting that chronic colitis induced by an alternative mechanism also depends at least partially on miR-155.

## Discussion

A role for miRNAs in immunity to bacterial infections was initially demonstrated in plants with the discovery that resistance of *Arabidopsis thaliana* to the pathogen *Pseudomonas syringae* depended on miR-393 (26). A first indication that mammalian miRNAs are required for the defense of mammalian cells and tissues against bacterial infectious agents was provided by the finding that



**FIGURE 5.** miR-155<sup>-/-</sup> T cells fail to induce IFN- $\gamma$  and IL-17 and to cause colitis upon adoptive transfer into TCR- $\beta$ <sup>-/-</sup> recipients. TCR- $\beta$ <sup>-/-</sup> mice were i.v. injected with 300,000 splenic CD4<sup>+</sup>CD25<sup>-</sup> T cells isolated from naive wt or miR-155<sup>-/-</sup> donors and sacrificed 1 mo postadoptive transfer. *A* and *B*, Colonic mucosal IFN- $\gamma$  and IL-17 production as determined by qRT-PCR and normalized to GAPDH. The dotted line indicates the average baseline cytokine expression in uninfected controls that have not received cells. *C–E*, Colonic infiltration of CD45<sup>+</sup> leukocytes, CD4<sup>+</sup> T cells, and Ly6G<sup>+</sup> neutrophils as determined by flow cytometric analysis of mucosal single-cell preparations. *F* and *G*, Colonic histopathology as assessed on Giemsa-stained sections; representative micrographs are shown in *G* at  $\times 100$  and  $\times 200$  final magnification. Scores in *F* were assigned on a scale of 0–5. In *A–F*, each data point represents one mouse.

TLR4 ligands induce the NF- $\kappa$ B-dependent expression of miR-146a/b and miR-155 (2, 4, 6). miR-155<sup>-/-</sup> mice have since been shown to be hypersusceptible to *Salmonella typhimurium* infection and also cannot be vaccinated protectively against the pathogen (8), despite exhibiting no apparent immunological defects under steady-state conditions. The increased susceptibility of miR-155<sup>-/-</sup> mice to infectious agents has been attributed to their failure to generate proper Ab responses, which in turn may be due to defective class switching and plasma cell differentiation (7, 8). Additional immune deficiencies of miR-155<sup>-/-</sup> mice have been linked to DC-intrinsic defects and to imbalanced Th1/Th2 polar-

ization (8). More recent reports using noninfectious disease models have confirmed an involvement of miR-155 in governing Th subset differentiation. Specifically, O'Connell et al. (11) showed that miR-155<sup>-/-</sup> mice are highly resistant to experimental autoimmune encephalomyelitis because of their defective development and function of Th17 cells.

miR-155 is strongly induced in various cell types upon coculture with *H. pylori* in vitro and is highly elevated in the gastric mucosa of chronically infected patients and of human volunteers experimentally infected with *H. pylori* (21, 22). In gastric epithelial cell lines, *H. pylori* was shown to induce miR-155 expression in an



NF- $\kappa$ B- and AP-1-dependent manner; in *H. pylori*-infected epithelial cells, overexpressed miR-155 appears to downregulate proinflammatory responses such as IL-8 production (22). T cells respond particularly strongly to *H. pylori* exposure (21). We confirm in this study using a model of virulent *H. pylori* infection that miR-155 expression is induced in the gastric mucosa of experimentally infected mice. Naive miR-155-deficient mice are incapable of controlling *H. pylori* and, as a consequence, are protected from the preneoplastic gastric immunopathology that is a hallmark of wt mice infected with virulent *H. pylori* (24). We attribute both the inability of the mice to restrict bacterial growth and their relative lack of pathology to strongly decreased gastric levels of IFN- $\gamma$  and IL-17. Both cytokines are known to be required for *H. pylori* control and to contribute to gastric preneoplastic pathology (18–20). Interestingly, miR-155<sup>-/-</sup> mice that have received several doses of an orally administered *H. pylori* vaccine are clearly better able than their nonvaccinated littermates to control a challenge infection. Nevertheless, colonization levels in vaccinated miR-155<sup>-/-</sup> mice are significantly higher than in vaccinated wt mice, suggesting that optimal protective immunity requires miR-155. Again, decreased gastric levels of IFN- $\gamma$  and IL-17 in the miR-155<sup>-/-</sup> mice pointed to defective Th1 and Th17 differentiation and/or function. We propose that T cell-intrinsic differences are responsible for the infection- and vaccination-associated phenotypes of miR-155<sup>-/-</sup> mice, based on adoptive transfer experiments in which wt but not miR-155<sup>-/-</sup> T cells effectively cleared the infection in the recipients and at the same time triggered gastric preneoplastic pathology.

Our observation that miR-155<sup>-/-</sup> T cells proliferate less than wt cells and fail to produce IFN- $\gamma$  or IL-17 upon CD3/CD28 cross-linking in vitro suggests that miR-155 regulates one or multiple pathways central to T cell activation. Among the targets identified for miR-155 in T cells are the Th1-promoting macrophage-activating factor c-MAF (8) and the negative regulator SOCS1 (5). SOCS1 functions downstream of cytokine receptors and participates in a negative feedback loop to attenuate cytokine signaling. The connection between miR-155 and SOCS1 was initially discovered in Tregs, which require the Foxp3-dependent expression of miR-155 for development (27) and competitive fitness (5). We did not detect a functional defect of miR-155<sup>-/-</sup> Tregs in an in vitro suppression assay in this study, even though miR-155 is regulated by this Treg-specific transcription factor (21); moreover, the overall phenotype of the miR-155<sup>-/-</sup> strain is more consistent with a dominant role for miR-155 in effector T cells rather than Tregs in the context of *H. pylori* infection. Elucidating the T cell lineage-specific targets of miR-155 and the relative importance of these targets in Treg and effector T cell functions remains a challenge for future work.

Our T cell adoptive transfer experiments designed to induce *H. pylori* infection-dependent gastritis and infection-independent colitis illustrate the strong defect of miR-155<sup>-/-</sup> T cells in producing IFN- $\gamma$  and IL-17 in vivo. The experiments further illustrate the mechanistic similarities of chronic inflammatory conditions of the stomach and lower gastrointestinal tracts, which in both cases are associated with T cellular production of IFN- $\gamma$  and IL-17. In contrast to O'Connell et al. (11), we do not observe a differential defect in Th17 over Th1 cytokine production, because both cytokines are similarly reduced as a consequence of miR-155 gene deletion in our gastritis and colitis models. Rather, our data point toward a broader and more general role for miR-155 in Th cell differentiation and function. It will be interesting to examine in this context how miR-155<sup>-/-</sup> mice behave in Th2-driven models of asthma and allergies. In conclusion, the T cell-intrinsic expression of miR-155 is required for the Th1/Th17 cell differenti-

ation and function that underlies immunity to *H. pylori* infection and elicits *Helicobacter* infection-associated immunopathology.

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## Disclosures

The authors have no financial conflicts of interest.

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