Gastrointestinal, Hepatobiliary and Pancreatic Pathology

CD4⁺CD45RB^{Hi} Interleukin-4 Defective T Cells Elicit Antral Gastritis and Duodenitis

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We have analyzed the gastrointestinal inflammation which develops following adoptive transfer of IL-4 gene knockout (IL-4^{-/-}) CD4⁺CD45RB^{Hi} (RB^{Hi}) T cells to severe combined immunodeficient (SCID) or to T cell-deficient, T cell receptor β and δ double knockout (TCR^{-/-}) mice. Transfer of IL-4^{-/-} RB^{Hi} T cells induced a similar type of colitis to that seen in SCID or TCR^{-/-} recipients of wild-type (wt) RB^{Hi} T cells as reported previously. Interestingly, transfer of both wt and IL-4^{-/-} RB^{Hi} T cells to TCR^{-/-} but not to SCID mice induced inflammation in the gastric mucosa. Notably, TCR^{-/-} recipients of IL-4^{-/-} RB^{Hi} T cells developed a more severe gastritis with erosion, apoptosis of the antral epithelium, and massive infiltration of macrophages. This gastritis was partially dependent on the indigenous microflora. Recipients of both wt and IL-4^{-/-} RB^{Hi} T cells developed duodenitis with multinuclear giant cells, expansion of mucosal macrophages, and dendritic cells. Full B cell responses were reconstituted in TCR^{-/-} recipients of RB^{Hi} T cells; however, anti-gastric autoantibodies were not detected. We have now developed and characterized a novel model of chronic gastroduodenitis in mice, which will help in our understanding of the mechanisms involved in chronic inflammation in the upper gastrointestinal tract of humans. (*Am J Pathol* 2004, 165:1257–1268)

A number of murine models of colitis involve either aberrant T cell or cytokine expression. For example, spontaneous colitis develops in T cell receptor (TCR)- α gene knockout mice where aberrant T cells producing Th2-type cytokines actually mediate disease.^{1–3} Perhaps the

most useful murine model for both T cell and cytokine regulation of inflammatory bowel disease (IBD) has involved adoptive transfer of CD45RB^{Hi} T cells from normal mice to either SCID^{4–6} or to RAG 2 gene knockout (RAG^{-/-}) mice.^{7,8} The transfer of RB^{Hi} T cells into SCID mice results in development of a severe mononuclear cell infiltration, epithelial cell hyperplasia, and tissue damage. Strong evidence has emerged provided that colitis results from enteric bacterial antigen (Ag)-driven Th1 cell induction,⁹ since RB^{Hi} T cell transfer into SCID mice with reduced bacterial flora failed to develop large bowel disease.^{10,11} Interestingly, T cell subsets producing TGF- β ¹² and IL-10¹³ in the RB^{low} T cell population, when co-delivered with RB^{Hi} T cells, actually suppressed colitis. The RB^{low} T cell population was enriched in CD4⁺CD25⁺ T regulatory (Tr) cells.¹⁴ Despite this compelling evidence that RB^{Hi} T cells give rise to effector Th1 cells which mediate colitis, this model had not allowed study of either normal or abnormal B cell participation in the pathogenesis of CD4⁺ T cells. Since the majority of antibody forming cells (AFCs) in humans and higher mammals reside in the gastrointestinal (GI) tract mucosa,^{15,16} the impact of the presence of B cells and plasma cells in the mucosal pathology cannot be ignored. For example, we recently showed that dysregulated Th2 cells cause villus atrophy and goblet cell transformation in the small intestinal epithelium. Further, a wasting disease was seen and was mediated by excess IL-4 and the presence of B cells.¹⁷

Chronic GI tract inflammation is one of the most common types of inflammatory processes. For many years, gastric ulcers were thought to occur in susceptible individuals, especially those hypersecreting gastric hydrochloric acid.^{18,19} However, it has become clear that gas-

Supported by U.S. Public Health Service National Institutes of Health Grants DK 44240, AI 18958, AI 43197, DE 12242, AI 35932, and DC 04976, and by grants and contracts from International Health Cooperation Research, the Ministry of Health, Labor, and Welfare, the Ministry of Education, Culture, Sports, Science, and Technology; the Japan Health Sciences Foundation and Organization; and The Organization for Pharmaceutical Safety and Research.

Accepted for publication June 21, 2004.

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tritis, peptic ulcer disease, and gastric cancer are the result of infection with the bacterium *Helicobacter pylori*.^{19,20} Gastritis has been reproduced in experimental animals gastrically infected with either *H. pylori* or related bacteria.^{21–24} Thus, gastritis and duodenitis are directly related to *H. pylori* infection; however, surprisingly little is known about what causes chronic inflammatory responses in the upper GI tract. Although many groups have shown that disease severity is related to certain virulence factors associated with *H. pylori*, only a minority of individuals infected with virulent strains of *H. pylori* develop severe disease.²⁵ In animal models, IL-10-deficient mice infected with *H. pylori* exhibited enhanced gastritis, lower bacterial loads, and higher serum IgG antibody (Ab) titers when compared with control mice.²⁶ These results have suggested the importance of the host immune system in perpetuating the gastritis and duodenitis. Indeed, a gastritis model in mice with *H. pylori* infection showed that CD4⁺ T cells were both necessary and sufficient for gastritis, and IFN- γ contributed to this inflammation.²⁷ Further, oral immunization of mice with the *H. pylori* urease induced protection against *H. pylori* infection, but was often associated with corpus gastritis, which is now recognized as post-immunization gastritis.^{28–30} In addition, *H. pylori*-infected SCID mice reconstituted with splenic T cells from *H. pylori*-infected, C57BL/6 mice developed severe gastritis, however, host colonization of *H. pylori* did not correlate to the severity of gastritis.³¹ Thus, cell-mediated immunity appears necessary for gastritis development, and does not necessarily relate to the bacterial load. Although still controversial, there is significant evidence that CD4⁺ Th1-type responses contribute to *H. pylori*-induced gastritis in humans.³² For example, IFN- γ and TNF- α are the major cytokines produced by gastric T cells from *H. pylori*-infected subjects.^{33,34}

In the murine colitis models induced by adoptive transfer of RB^{Hi} T cells, the microflora is necessary for disease; however, no priming of donor T cells with pathogens was required.^{10,11} Furthermore, no specific colitogenic bacterial species has been identified to date. Nonetheless, regulation of colitis by Tr cells seems to occur in both innate and adaptive immunity.³⁵ These results indicate that T cells from normal mice have the potential to induce inflammation in response to the indigenous bacteria; however, the healthy host simultaneously develops Tr cells that attenuate the disease producing T cells. Similar mechanisms for upper GI tract inflammation may be present; however, there have been no reports of a gastritis model in the absence of infection or deliberate immunization, except for autoimmune gastritis induced by neonatal thymectomy^{36,37} or ionizing radiation.³⁸

In this study, we hypothesized that normal T cells prepared from pathogen-free mice would contain a subset, which potentially could cause inflammation in the upper GI tract, as well as colitis. We describe a new type of RB^{Hi} T cell transfer model in TCR^{-/-} mice that allows assessment of RB^{Hi} T cells in the presence of responsive B cells. We discovered that transfer of RB^{Hi} T cells from IL-4 gene knockout (IL-4^{-/-}) mice resulted in gastroduodenitis in the absence of infection by pathogenic bacteria. Our results suggest the CD4⁺ Th1-type T cells

mediate both colitis and gastroduodenitis in TCR^{-/-} mice reconstituted with RB^{Hi} T cells.

Materials and Methods

Mice

Normal wild-type (wt), IL-4^{-/-}, IFN- γ gene knockout (IFN- γ ^{-/-}) TCR β and δ chain-defective (TCR^{-/-}) and SCID mice on the C57BL/6 background were originally obtained from the Jackson Laboratory (Bar Harbor, ME). This mouse colony was maintained under pathogen-free conditions in flexible Trexler isolators at the University of Alabama at Birmingham (UAB) Immunobiology Vaccine Center Mouse Facility. A separate colony was also maintained in the Immunocompromised Mouse Facility of the Research Institute, International Medical Center of Japan (IMCJ, Tokyo, Japan). We periodically and extensively performed health surveillance on these colonies. These tests were performed in laboratories with expertise in laboratory animal health care (Jackson Laboratories, Charles River Laboratories, Wilmington, MA and Central Laboratories for Experimental Animals, Kawasaki, Japan). This analysis included serological testing for viral infections, bacterial cultures of the nasopharynx, stomach and cecum, ecto- and endoparasitic examinations, and histology of all major organs and tissues. Feces and the stomach were also tested for *Helicobacter spp.* in these laboratories, including *H. hepaticus*, *H. bilis*, *H. muridarum*, and "*H. rappini*", by PCR.^{39–42} No lesions or pathogens including *Helicobacter spp.* and intestinal parasites have ever been detected in these two separate mouse colonies. We obtained essentially identical results in the mouse facilities of both UAB and the IMCJ, and these experiments are quite reproducible. Although original reports indicated that TCR^{-/-} mice develop intestinal inflammation,³ they did not develop histologically obvious gastritis, duodenitis, or colitis in our mouse facility until they were 24 weeks of age, which is well beyond the time frame of our experiments.

Purification of T Cells

Splenic T cells for adoptive transfer were purified as described previously.¹⁷ In brief, following lysis of erythrocytes, splenic T cells were enriched by passage through a nylon wool column, and then stained with phycoerythrin (PE)-conjugated anti-CD45RB (23G2), FITC-labeled anti-B220, anti-CD11b, and anti-CD8 monoclonal antibodies (mAbs) (BD PharMingen, San Diego, CA). The CD45RB^{Hi} T cell subset was separated by flow cytometry using a FACS Vantage (Becton-Dickinson Co., Sunnyvale, CA). Sorted, CD45RB^{Hi} subsets were > 99% pure by the reanalysis using anti-CD4 Ab, anti-TCR $\gamma\delta$ Ab, anti-NK1.1 Ab, and anti-Gr-1 Ab.

Adoptive Transfer of T Cells

In these studies, purified populations of RB^{Hi} T cells were adoptively transferred to 6- to 8-week-old TCR^{-/-} mice. We routinely transferred 1.0×10^6 T cells by the intrave-

nous (i.v.) route. Body weight was monitored weekly, and mice were taken for analysis when their weight became less than 75% of the initial weight, or at 12 weeks after adoptive transfer of RB^{Hi} T cells.

Histological Analysis

The stomach was removed by excising the esophagus and the anal side of the gastro-duodenal junction, opened along the greater curvature, washed and extended. Next the stomach was cut longitudinally in the middle first, and a 3- to 4-mm-wide strip was prepared. The lateral side of this strip included the fundus, antrum, and gastro-duodenal junction. This side was cut for preparation of sections for histological examination. At least one such section from each mouse prepared in this manner was examined. Duodenal tissues were obtained from 0.5 cm to 3 cm from the gastro-duodenal junction. Colonic tissue was taken from the middle and distal parts of the large intestine. The tissues were opened, fixed in 5% glacial acetic acid in ethanol, and paraffin-embedded. Tissue sections (4 μ m) were prepared and stained with hematoxylin and eosin (H&E). In some experiments, 6 μ m-frozen sections were prepared, dried and fixed with cold acetone for 10 minutes, and subjected to histological analysis. The immunohistochemical staining was performed using FITC- or biotin-labeled anti-CD3, anti-CD4, anti-B220, anti-CD11b, and anti-CD11c mAbs, respectively (all from BD PharMingen, San Diego, CA), followed by FITC- or TRITC-labeled streptavidin (BD PharMingen). For the detection of IgA⁺, IgG⁺ or IgM⁺ plasma cells, sections were reacted with biotin-labeled anti-mouse IgA Ab, TRITC-labeled anti-mouse IgG Ab and FITC-labeled anti-mouse IgM Ab (BD PharMingen) followed by aminocoumarin-labeled streptavidin. Apoptotic cells were detected by TdT-mediated dUTP nick end labeling (TUNEL) using the Apoptosis *in Situ* Detection Kit Wako (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) on paraffin-embedded sections, according to the vender's protocol. To detect myeloperoxidase in granulocytes, sections were directly incubated with a substrate solution, 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) in the presence of H₂O₂. Histological scores for gastritis were based on a summation of scores for cell infiltration (0, none; 1, moderate; 2, severe), epithelial hypertrophy (0, none; 1, moderate; or 2, severe), erosion (0, none; 1, focal; or 2, diffuse), giant cells (0, none; 1, less than 2; or 2, more than 3 per section), and deformity of pits (0, none; 1, moderate; or 2, severe). Histological scores for duodenitis and colitis were determined by severity of the inflammation (0, none; 1, mild; 2, moderate; or 3, severe). For histological examination, two observers performed all of the scoring. One was a pathologist who was not involved in this research, and for this examiner, the samples were provided in blinded fashion.

Purification of Lamina Propria Lymphocytes

Peyer's patches were excised from the intestinal wall, and small intestinal lamina propria lymphocytes (LPLs)

were prepared as described previously.⁴³ Briefly, the intestinal tissue was treated with 1 mmol/L EDTA in phosphate-buffered saline (PBS) for 20 minutes to remove the epithelium. The tissue was then digested with type V collagenase (Sigma) for 20 minutes, and this step was repeated once more. The mononuclear cells were further purified by using a discontinuous Percoll gradient of 75% and 40%.

ELISPOT Assay

Enumeration of antibody forming cells (AFCs) was performed as described previously.⁴⁴ In brief, 96-well nitrocellulose plates (Millititer HA, Millipore, Bedford, MA) were coated with goat anti-mouse Ig (H+L) [Southern Biotechnology Associates (SBA), Birmingham, AL]. After blocking with 1% bovine serum albumin (BSA) in PBS, the LPLs were incubated for 4 hours at 37°C in moisturized atmosphere of 5% CO₂ in an incubator. After washing the plates, the captured Ig was visualized using peroxidase-labeled anti-mouse IgG, IgA or IgM (SBA). The spots in the individual wells were counted with the aid of a stereomicroscope.

Quantification of Plasma Immunoglobulin Levels

Plasma immunoglobulin (Ig) levels were determined by a sandwich ELISA using the combination of anti-mouse Ig (H+L) (SBA) and peroxidase-labeled anti-mouse IgG, IgA, or IgM (SBA) as described previously.¹⁷

RT-PCR for mRNA Analysis

Total RNA was prepared from gastric tissue or from separated cells using an RNA-Bee RNA isolation reagent (Tel-Test, Inc., Friendswood, TX). Complementary DNA was synthesized from RNA by reverse transcription (RT). The PCR primers for murine GAPDH used were 5'-AGC-CAAACGGGTCATCATCTC and 5'-TGCCTGCTTCACCACCTTCTT; for TNF- α , 5'-TTCTGTCTACTGAACCTCGGGGT-CATCGGTCC-3' and 5'-GTATGAGATAGCAAATCGGCT-GACGTGTGCC. The step-cycle program was set for denaturing at 95°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 45 seconds for a total of 40 cycles. Expression of mRNA was assessed by quantitative PCR using a SYBR Green PCR Master Mix (Applied Biosystems, Warrington, United Kingdom) and ABI PRISM 7700 Sequence Detector (Applied Biosystems). Quantification of mRNA for IFN- γ and IL-10 was performed using ABI Taqman probes (Applied Biosystems). The PCR primers for IFN- γ were 5'-TGATCCTTTGGACCCTCTGA and 5'-GCAAAGCCAGATGCAGTGT, for IL-10, 5'-GCTCTTGAC-TACCAAAGCCAC and 5'-CATGCCAGTCAGTAAGAG-CAGG. The Taqman probes used for IFN- γ were 5'-CCTCCTGCGGCCTAGCTCTGAGAC and for IL-10, AAGAGAGCTCCATCATGCCTGGCTCA. Threshold cycle numbers (C_T) were determined with Sequence Detector Software (version 1.7; Applied Biosystems) and transformed using the $\Delta C_T/\Delta \Delta C_T$ method as described by the manufacturer, with GAPDH used as the calibrator gene.

Quantification of Cytokine Production and Intracellular Cytokine Analysis

For quantification of cytokines, CD4⁺ T cells were purified from freshly prepared LPLs by staining with FITC-labeled anti-CD4 mAb (BD PharMingen) followed by flow cytometry with a FACS Vantage system. The purified CD4⁺ T cells were added to wells of plates coated with anti-CD3 mAb (10 μ g/ml, clone 145-2C11; BD PharMingen) and cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS), sodium pyruvate, L-glutamine, HEPES, and 50 μ mol/L 2-mercaptoethanol (complete medium). Cultures were incubated for 48 hours at 37°C in 5% CO₂ in a moist air incubator. Cells in culture were removed, separated by centrifugation, and the supernatants were then subjected to a cytokine-specific ELISA, as described previously.⁴³ Briefly, microtiter plates were coated with mAbs to individual cytokines and blocked with 3% BSA in PBS at 37°C for 2 hours, and then diluted samples were added to well and incubated overnight at 4°C. Captured cytokines were detected using biotinylated detection mAbs and peroxidase-labeled anti-biotin mAb (Vector Laboratories Inc., Burlingame, CA). The following mAbs were used for coating and detection, respectively: anti-IFN- γ , R4-6A2 and XMG 1.2; anti-IL-2, JES6-1A12 and JES6-5H4 mAbs; anti-IL-4, BVD4-1D11 and BVD6-23G2. For intracellular cytokine analysis, LPLs were cultured in plates coated with anti-CD3 mAb in complete medium with anti-CD28 mAb. After 48 hours of culture, the cells were subjected to intracellular cytokine staining as described previously.⁴⁵ In brief, cells were stained with FITC-labeled anti-CD4 mAb (BD PharMingen), fixed, permeabilized, and then stained with PE-labeled anti-IFN- γ mAb (BD PharMingen) for analysis using flow cytometry.

Treatment with Antibiotics and Bacterial Culture

In some experiments, recipient mice were given a combination of antibiotics in their drinking water. Metronidazole (0.6 g/L, Wako Pure Chemical Industries Ltd.), neomycin (0.35 g/L, Wako), streptomycin (0.2 g/L, Wako), and bacitracin (0.35 g/L, Wako) were added to the drinking water, and the mice were continuously given this water *ad libitum* until the histological and cytological analyses were performed. To determine total colony forming units (CFU), mice were treated with antibiotics as above for 6 weeks, fasted for 6 hours and then the entire stomach was removed, washed three times with PBS, minced and homogenized with 2 ml of PBS in a Teflon-glass homogenizer. This homogenate was then subjected to serial dilution and spread over culture plates. Anaerobic bacteria were cultured on GAM agar plates (Nissui Pharmaceuticals, Tokyo, Japan) in culture jars (GasPack System BBL, Becton Dickinson) with AneroPac (Mitsubishi Gas Chemical Co, Inc., Tokyo, Japan). For aerobic cultures, brain-heart infusion agar (Difco, Becton Dickinson), chocolate agar, and sheep blood agar plates (both from Nissui) were used. To examine for the presence of indigenous microflora in the oral cavity, the mucosal surfaces of

Figure 1. Histological scores of gastritis, duodenitis and colitis. TCR^{-/-} or SCID mouse recipients were given RB^H T cells prepared from splenocytes of wt (blank column, *n* = 8), IL-4^{-/-} (solid column, *n* = 10) or IFN- γ ^{-/-} (hatched column, *n* = 7) mice. The results shown are the mean and SEM. *, Difference between wt and IFN- γ ^{-/-} mice was statistically significant.

oral cavity were swabbed, and the swabs were placed in 300 μ l of PBS. Then 100 μ l of this suspension was spread over a culture plate. Anaerobic cultures were initiated within 20 minutes after taking samples. After 24 hours of incubation at 37°C, numbers of CFU were enumerated.

Statistics

The results were compared by the Mann-Whitney test using the Statview II statistical program (Abacus Concepts, Berkeley, CA) adapted for Macintosh computers. The results were considered to be statistically significant if *P* values were less than 0.05.

Results

Inflammation of the GI Tract in TCR^{-/-} and SCID Mouse Recipients

Previous studies have shown that the transfer of RB^H T cells resulted in severe colitis in either SCID or RAG^{-/-} mouse recipients, and the colitis was mediated by CD4⁺ Th1-type cells.^{4-6,9,46} A similar type of colitis was induced in TCR^{-/-} recipients of wt RB^H T cells. Recipients of IL-4^{-/-} RB^H T cells also developed a severe colitis (Figure 1). We noted that TCR^{-/-} recipients of either wt or IL-4^{-/-} RB^H T cells developed a duodenitis with a heavy cell infiltration, which was rarely described in SCID recipients previously. These changes were limited to the duodenum within 5 cm of the pylorus, and both the jejunum and ileum remained normal. We also found that these mouse recipients developed an inflammation of the stomach, especially in the antral region. When recipients of wt, IL-4^{-/-}, or IFN- γ ^{-/-} RB^H T cells were compared, recipients of IL-4^{-/-} T cells developed the most severe gastritis and those mice receiving IFN- γ ^{-/-} T cells showed only minimal changes (Figure 1). Interestingly, SCID recipients of either wt or IL-4^{-/-} RB^H T cells showed mild lesions in the stomach, with only slight cell infiltration. Adoptive transfer of wt or IL-4^{-/-} CD45RB^{low} T cells did not result in gastritis, duodenitis, or colitis (data not shown).

Pathological Features of Gastritis

In all 10 TCR^{-/-} recipients of IL-4^{-/-} RB^H T cells, the gastric antral and duodenal mucosa were increased in

Figure 2. Macroscopic and histological features of murine gastritis. **A:** Normal stomach tissue taken from naïve TCR^{-/-} mice. **B:** Inflamed stomach from TCR^{-/-} mice after adoptive transfer of IL-4^{-/-} RB^{H1} T cells. **C:** The gastroduodenal junction of naïve TCR^{-/-} mice. **D:** The gastroduodenal junction of the SCID recipients given IL-4^{-/-} RB^{H1} T cells. **E:** The gastroduodenal junction of the TCR^{-/-} recipients given IL-4^{-/-} RB^{H1} T cells. **F:** The inflamed gastroduodenal junction with erosion. **G and H:** Antral gastritis with surface erosion and elongation of pits of TCR^{-/-} recipients of IL-4^{-/-} RB^{H1} T cells. **Arrows** indicate a multinuclear giant cell. **I:** The fundic lesion with mild changes was taken from the same specimen as shown in **G**. **J:** Cell infiltration and dilated pits including migrating cells in the surface of the gastric fundic region in TCR^{-/-} recipients of IL-4^{-/-} RB^{H1} T cells. **K:** Histochemistry for peroxidase of the antral region TCR^{-/-} recipients of IL-4^{-/-} RB^{H1} T cells. As positive control, a section from a mouse with colitis induced by dextran sulfate with neutrophil infiltration was used (**L**). Sections were counterstained with methylgreen. Images were captured using a $\times 4$ objective lens (**C**, **D** and **E**), $\times 20$ lens (**G**, **I**, **K**, and **L**) or $\times 40$ lens (**F**, **H**, and **J**).

thickness and exhibited an overall turbid appearance. In five recipients of IL-4^{-/-} RB^{H1} T cells, the gastric fundic area was also edematous, and erosions with petechiae were seen (Figure 2, A and B). Histological examination revealed that the inflammation was accompanied by hypertrophy of

glands and elongation of pits, and these changes were most evident at the gastro-duodenal junction (Figure 2, C to F). Most typically, the antral glands were elongated more than twofold longer than their normal length with pit dilatation in some parts, and mononuclear cell infiltration in the

Figure 3. Immunohistochemistry and detection of apoptosis. CD4⁺ T cells in the antrum of a SCID recipient of IL-4^{-/-} RB^H T cells (A). CD4⁺ T cells (B), B220⁺ cells (C), and CD11b⁺ cells (D) in the inflamed antrum of TCR^{-/-} mouse recipients of IL-4^{-/-} RB^H T cells. Apoptotic cells were detected by TUNEL in TCR^{-/-} recipients of wt RB^H T cells (E) or IL-4^{-/-} RB^H T cells (F and G). Images were captured using a ×20 objective lens (A–F) or ×100 lens (G).

recipients of wt and IL-4^{-/-} RB^H T cells was seen (Figure 2G). In addition to these findings, recipients of IL-4^{-/-} RB^H T cells developed surface erosions with multinuclear giant cell infiltration (Figure 2H). Neutrophil infiltration was not frequent in the stomach or the duodenum as shown in the histochemical reaction of myeloperoxidase (Figure 2, K and L). In 50% of IL-4^{-/-} T cell recipients, a fundic inflammation with mononuclear cell infiltration and deformity of glandular pits with cells migrating into the dilated pits were seen (Figure 2J), while the other 50% of mice showed only mild changes (Figure 2I). In both cases, destruction specific for parietal cells was not seen. In the inflamed stomach, a massive infiltration of CD4⁺ T cells occurred (Figure 3B), an event scarcely seen in SCID recipients (Figure 3A). Furthermore, B cell aggregates were also detected by staining with anti-B220 mAb (Figure 3C). We also assessed the presence of plasma cells in the inflamed areas; however, no IgG-, IgA- or IgM-containing cells were seen in the gastric mucosa by immunostaining (data not shown). The infiltrating cells were mostly macrophage-like cells as shown by staining with anti-CD11b Ab (Figure 3D). On the other hand, there was no expansion of CD11c⁺ dendritic cells in the inflamed stomach (data not shown). Since surface erosion was enhanced in the gastric mucosa of recipients of IL-4^{-/-}

RB^H T cells, we also assessed apoptosis. In recipients of wt RB^H T cells, although there was elongation of glands and a cell infiltration, apoptotic cells were rare in the epithelium (Figure 3E). In contrast, in recipients of IL-4^{-/-} RB^H T cells, the numbers of apoptotic cells were clearly increased. Apoptosis was readily detected in the infiltrating cells in all layers of the mucosa and especially in the surface epithelium (Figure 3, F and G).

Pathological Features of Duodenitis

Duodenal inflammation was seen in TCR^{-/-} mouse recipients of both wt and IL-4^{-/-} RB^H T cells. The villi and crypts were both remarkably elongated, and the villi were dilated due to the cell infiltration (Figure 4, B and C). In the lamina propria, multinuclear giant cells were frequently seen; however, granulomas were absent (Figure 4D). Immunohistological analysis revealed an infiltration of CD4⁺ cells (Figure 4E), expansion of dendritic cells and an infiltration of macrophages (Figure 4F), which were not seen in naive TCR^{-/-} mice.

Figure 4. Histological features of murine duodenitis. **A:** H&E staining of duodenum of $TCR^{-/-}$ mice without cell transfer. **B:** Inflamed duodenum of $TCR^{-/-}$ recipients of wt RB^{Hi} T cells. **C and D:** Inflamed duodenum of $TCR^{-/-}$ recipients of $IL-4^{-/-}$ RB^{Hi} T cells. Dilated villus of duodenal tissue was stained with anti-CD4 (green, **E**), and anti-CD11b (green) and anti-CD11c (red) mAbs (**F**). Some $CD11c^{+}$ cells also express CD11b. **G:** IgA AFCs in the duodenum of recipients of wt RB^{Hi} T cells. **H:** Peyer's patches from recipients of wt RB^{Hi} T cells stained with anti-B220 (green) and anti-CD3 (red) mAbs. **I:** Peyer's patches of $TCR^{-/-}$ mice without T cell transfer. Images were captured using a $\times 10$ objective lens (**A-C**), $\times 20$ (**E**), or $\times 40$ lens (**D, F-I**)

Production of Cytokines

To assess cytokine production by T cells that infiltrated the mucosa of adoptive hosts, $CD4^{+}$ T cells were isolated from LPLs of the small intestine. The $CD4^{+}$ T cells recovered from recipients of wt or $IL-4^{-/-}$ RB^{Hi} T cells released both IFN- γ and IL-2 (Figure 5A). It should be noted that T cells from the recipients of wt RB^{Hi} T cells also produced high levels of IL-4 (Figure 5A). We also noted that T cells infiltrating the gastric mucosa produced IFN- γ in the $TCR^{-/-}$ recipients of $IL-4^{-/-}$ T cells (Figure 5B), although T cells from recipients of $IL-4^{-/-}$ T cells tended to contain slightly more IFN- γ producing cells than recipients of wt RB^{Hi} T cells, as determined by flow cytometry (Figure 5B). Thus, IFN- γ release by T cells recovered from recipients of wt or $IL-4^{-/-}$ RB^{Hi} T cells were, for the most part, comparable. However, the lack of IL-4 secretion resulted in a remarkable phenotype, which contributed to the distinct and severe gastritis in $TCR^{-/-}$ recipients of $IL-4^{-/-}$ RB^{Hi} T cells. Further, to prove that the milder gastritis in SCID than in $TCR^{-/-}$ recipients of $IL-4^{-/-}$ RB^{Hi} T cells, we quantitatively assessed TNF- α , IFN- γ , and IL-10 by RT-PCR from total RNA extracts taken from the gastric antrum. Expression of IFN- γ and TNF- α in $TCR^{-/-}$ recipients was higher than those seen in SCID recipients (Figure 5C).

Mucosal and Systemic B Cell Responses in $TCR^{-/-}$ Recipients of RB^{Hi} T Cells

It should be noted that $TCR^{-/-}$ recipients of either wt or $IL-4^{-/-}$ RB^{Hi} T cells exhibited IgA-positive cells in the small intestine, including the duodenum (Figure 4G). Peyer's patches were also reconstituted with distinct T and B cell zones (Figure 3H), which were filled with B220 $^{+}$ cells in $TCR^{-/-}$ mice before T cell transfer (Figure 3I). The plasma Ig levels were also elevated following adoptive transfer of either wt or $IL-4^{-/-}$ RB^{Hi} T cells. The IgG levels were comparable in both groups; however, the IgA levels were lower in recipients of $IL-4^{-/-}$ RB^{Hi} T cells than recipients of wt RB^{Hi} T cells (Figure 6A). The numbers of AFCs in the small intestine in $TCR^{-/-}$ recipients were also reconstituted by adoptive transfer of RB^{Hi} T cells. Numbers of IgG and IgA secreting cells were much less frequent in $TCR^{-/-}$ recipients of $IL-4^{-/-}$ T cells than those mice given wt T cells (Figure 6B). Thus, systemic IgG responses were fully reconstituted in both groups of mice; however, recipients of $IL-4^{-/-}$ T cells exhibited little class switching to the IgG or IgA isotypes. Plasma from these mice did not contain autoreactive Abs when assessed by the binding capacity to sections prepared from the stomach of naïve mice.

Figure 5. Production of cytokines by CD4⁺ T cells infiltrating the GI tract. **A:** CD4⁺ T cells were isolated from the small intestine of TCR^{-/-} recipients of wt (blank column) or IL-4^{-/-} (solid column) RB^{Hi} T cells and stimulated with anti-CD3 mAb for 48 hours. Culture supernatants were subjected to a cytokine ELISA. Values shown are the mean of three experiments obtained from pooled cells from three mice in each group. The results shown are the mean and SD. The difference was statistically significant. **B:** Production of IFN-γ by infiltrating cells. LPLs were prepared from the inflamed gastric mucosa of TCR^{-/-} recipients of IL-4^{-/-} (left) or wt (right) RB^{Hi} T cells, stimulated with anti-CD3 and anti-CD28 mAbs for 48 hours, and subjected to intracellular cytokine analysis. **C:** Relative expression of mRNA for cytokines in the stomach in TCR^{-/-} and SCID recipients of IL-4^{-/-} RB^{Hi} T cells determined by quantitative RT-PCR. Total RNA was extracted from the antral mucosa, and mRNA for individual cytokines and GAPDH were analyzed. Based on the average ΔC_T value of four SCID recipients, data from individual mice were shown as relative expression to SCID mice. Results from four mice are shown and the results indicated as (+) are the average of relative expression for each group. *, Statistically significant difference from SCID recipients.

Gastritis Was Dependent on the Presence of a Microflora

To this point, we found that gastritis was induced in the TCR^{-/-} recipients of RB^{Hi} T cells without infection of pathogenic bacterial strains. However, the indigenous microflora in the upper GI tract or bacteria in ingested food may play a role in the induction of gastritis. To clarify this, we gave mice neomycin, streptomycin, bacitracin, and metronidazole in their drinking water following transfer of IL-4^{-/-} RB^{Hi} T cells. This treatment essentially eliminated the indigenous flora in the oral cavity and the stomach (Table 1), and efficiently suppressed the gastritis. Interestingly, the effect on gastritis was efficient but

Figure 6. Reconstitution of systemic and mucosal Ig production. **A:** Plasma Ig levels in TCR^{-/-} recipients of wt RB^{Hi} T cells (blank column), IL-4^{-/-} RB^{Hi} T cells (solid column) or naïve TCR^{-/-} mice (shaded column). **B:** Numbers of IgG, IgA, or IgM secreting cells in the small intestinal LPLs isolated from TCR^{-/-} recipients of wt (blank column), IL-4^{-/-} RB^{Hi} (solid column) or naïve TCR^{-/-} mice (hatched column) was determined by ELISPOT assay. Values shown are the mean and a SD of each group containing 4 to 7 mice.

only partial, whereas colitis was completely blocked in these mice (Figure 7). These results indicated that induction of gastritis was partially dependent on the indigenous microflora, while colitis essentially required its presence.

Discussion

We have established a new model for gastritis that is induced by adoptive transfer of RB^{Hi} T cells incapable of production of IL-4 (Th1-prone T cells). The gastritis and duodenitis develops in the absence of a particular bacterial pathogen, such as *Helicobacter* spp. and is also distinct from autoimmune models of gastritis. The first major finding was that this pathogen-free gastritis developed after adoptive transfer of IL-4^{-/-} RB^{Hi} T cells into TCR^{-/-} mice. Gastritis did not occur after transfer of IL-4^{-/-} RB^{Hi} T cells into SCID mice, clearly suggesting a requirement for B cell responses for full-blown gastric inflammation. Finally, adoptive transfer of IL-4^{-/-} RB^{Hi} T cells yielded greater mucosal damage when compared with TCR^{-/-} recipients of wt RB^{Hi} T cells. Each of these significant new findings is discussed in more detail below.

Numerous studies have attempted to establish *in vivo* models for gastric inflammation following infection with *H. pylori*. In our model, we successfully induced gastritis without bacterial infection or specific immunization. Of course, several models for autoimmune gastritis are induced in the absence of pathogens. Autoimmune disease induced by thymectomy^{36,37} or ionizing radiation³⁸ have both resulted in gastritis. These types of gastritis were associated with damage of parietal cells or loss of parietal and chief cells by autoantibodies to the gastric H⁺/K⁺-ATPase.^{47,48} A similar type of autoimmune gastritis occurs spontaneously in C3H/He mice.⁴⁹ "Autoimmune gastritis" and "colitis induced in SCID/Rag2^{-/-} recipients of RB^{Hi} T cell transfer" are both caused by the absence of CD4⁺CD25⁺ regulatory T cells, because gastritis was induced in nu/nu mice recipients of CD25⁻ T cells prepared from CD25⁺ cell-depleted mice.⁵⁰ However, these two models have not been compared with each other very often. Importantly, autoimmune gastritis

Table 1. Total Viable Aerobic and Anaerobic Counts of the Whole Stomach and the Oral Mucosa of Antibiotic-Treated and Nontreated TCR^{-/-} Mice (or mean CFU $\pm$ SD of 3 mice)

	Untreated	Treated
Aerobes		
Stomach (10 ³ CFU/a whole stomach)		
Brain-heart infusion agar	159.3 $\pm$ 67.2	1.1 $\pm$ 0.7
Chocolate agar	167.0 $\pm$ 98.7	1.0 $\pm$ 0.6
Blood agar	80.3 $\pm$ 25.5	1.0 $\pm$ 0.5
Oral mucosa (CFU/swab)		
Brain-heart infusion agar	1969 $\pm$ 309	35 $\pm$ 36
Anaerobes		
Stomach (10 ³ CFU/a whole stomach)	305.6 $\pm$ 119.7	1.2 $\pm$ 0.4
Oral mucosa (CFU/swab)	1444 $\pm$ 287	39 $\pm$ 30

induced by neonatal thymectomy was not dependent on a microflora.⁵¹ Autoimmune gastritis was seen in germ-free mice with similar severity of inflammation, and auto-antibody levels were comparable to those seen in conventional mice. In contrast, as shown in Figure 7, our gastritis model was dependent, in a significant way, on the microflora. Further, no anti-parietal cell autoantibody could be detected. Besides a requirement for a microflora and the absence of autoantibody, our model is different from autoimmune gastritis in several important ways. For example, although neonatal thymectomy caused gastritis as well as autoimmune oophoritis, orchitis, thyroiditis, pancreatitis, and prostatitis,^{37,52} colitis has not been described previously. In contrast, the colon was a major organ affected by RB^{Hi} T cell transfer in SCID/RAG2^{-/-}/TCR^{-/-} recipients, which requires the presence of a microflora. Further, in autoimmune gastritis, lesions are limited to the gastric corpus, and parietal cell destruction was the main histological feature. On the other hand, our model showed more severe inflammatory changes in the antral mucosa. Since other autoimmune disease models such as spontaneous gastritis in MRL-lpr mice were also independent of a microflora or infection,^{37,52} it seems that different subsets of T regulatory cells are affected in autoimmune models and RB^{Hi} T cell-induced colitis/gastritis models. Thus, our model is quite distinct from autoimmune gastritis reported previously.

Figure 7. Treatment of recipient mice with antibiotics. A preparation of IL-4^{-/-} RB^{Hi} T cells was transferred to eight TCR^{-/-} mice (including two sets of male littermates in each experimental group). Four of these mice were given a combination of antibiotics in their drinking water (blank squares), and after 10 weeks histological scores were compared with control mice without treatment (solid squares).

TCR^{-/-} recipients of wt or IL-4^{-/-} T cells developed gastroduodenitis as well as colitis; however, no obvious changes in the jejunum or ileum were seen. This anatomical localization suggests the possible involvement of luminal foreign antigens in the development of this type of inflammation. Colitis induced in SCID or RAG2^{-/-} mice by adoptive transfer of RB^{Hi} T cells does not occur in the absence of an indigenous flora.^{10,11} Through extensive testing for *Helicobacter* spp., no pathogenic strains (including *Helicobacter* spp.) were detected in mice, which developed gastritis and duodenitis. In addition, neutrophil infiltration, which generally indicates bacterial infection, was not seen in the stomach or the duodenum in our model. On the other hand, TCR^{-/-} recipients of IL-4^{-/-} RB^{Hi} T cells developed a milder form of gastritis when they were treated with antibiotics. Of note, colitis was totally blocked in this group of mice. These results indicate that gastritis was partially dependent on an indigenous microflora, while colitis essentially required its presence. We speculate that orally ingested microbes or indigenous microflora in upper GI tract, in addition to food antigens, which have not been fully degraded in the stomach or duodenum, may play a similar role in this type of inflammation.

Another novel aspect of our model is the use of TCR^{-/-} mice, as opposed to SCID or RAG^{-/-} mice. Thus, TCR^{-/-} mice lack T cells but have a fully responsive B cell repertoire. Indeed, after adoptive transfer of RB^{Hi} T cells, TCR^{-/-} mice exhibit increased plasma IgG levels, and AFCs were seen in the mucosal tissues. The fact that transfer of RB^{Hi} T cells resulted in colitis and duodenitis but not gastritis in SCID mice clearly suggests the involvement of B cells in the pathogenesis of gastritis. In this regard, a different colitis model has also shown that B cells play protective roles from inflammation.⁵³ However, no AFCs were seen in the inflamed stomach tissues themselves, although small B cell aggregates were detected. Further, mucosal IgA production and IgA AFCs were actually lower in TCR^{-/-} recipients of IL-4^{-/-} RB^{Hi} T cells when compared with recipients of wt RB^{Hi} T cells, despite the more significant gastritis which characterize IL-4^{-/-} RB^{Hi} T cell recipients. On the other hand, plasma IgG levels in IL-4^{-/-} RB^{Hi} T cell recipients were comparable to recipients of wt RB^{Hi} T cells. The role of B cells and Ab production in our model certainly needs to be further investigated. We speculate that the presence of B cells and antibody production increases the sensitivity of

T cells to be triggered into an inflammatory expansion as well as the activation of macrophage-type cells. Our results suggest that TCR^{-/-} recipients were more sensitive than SCID recipients and the former mice would be able to fully respond to the foreign antigens in the upper GI tract. To increase this sensitivity, T/B cell interactions are likely involved, although this interaction may not necessarily occur in the local mucosa but could occur in any lymphoid tissue, such as spleen or mesenteric lymph nodes. In the case of *H. pylori* infection, it is known that the presence of anti-*H. pylori* Abs are not required for the exclusion of bacteria,⁵³ but rather the Abs are actually involved in the pathogenesis of gastritis in humans⁵⁴ and mice due to antigenic mimicry.⁵⁵ Although we could not detect autoantibodies against the gastric parietal cells or H⁺/K⁺-ATPase, the presence of elevated Ig levels of particular idiotypes could play a role in the gastritis. In this regard, for our model of gastritis in the absence of infection or immunization, one could implicate molecular mimicry between any bacterial LPS and host blood group determinants^{56–59} as an attractive hypothesis, although there is no direct evidence that this phenomenon actually occurs.

There is now clear evidence for a central role of T cell-mediated immunity in gastric inflammation. In the RB^{hi} T cell transfer model described here, Th1-type cytokine production was required for the induction of colitis and gastroduodenitis, since transfer of IFN- γ ^{-/-} RB^{hi} T cells resulted in much milder gastroduodenal inflammation (Figure 1) and colitis.¹⁷ On the other hand, it is well known that IL-4 suppresses cytokine gene expression induced by IFN- γ and IL-2 in murine peritoneal macrophages.^{60,61} The severe tissue damage in recipients of IL-4^{-/-} RB^{hi} T cells was likely caused by enhanced IFN- γ and IL-2 production by IL-4^{-/-} T cells due to their predisposition toward a Th1-phenotype. However, the levels of IFN- γ release by isolated T cells were comparable in these mice. These quantitative differences in Th1 cytokines may not fully explain the fact that the epithelial cell apoptosis and surface erosion was much more frequent in IL-4^{-/-} than in wt RB^{hi} T cell recipients. Since IL-4 is a cytokine that has pleiotropic effects on a variety of cell types, including epithelial cells and other non-hematopoietic cells, a lack of IL-4 production by infiltrating T cells may have an impact on tissue repair in addition to a cytokine imbalance. Gastritis induced by infection with pathogenic *H. pylori* in IL-4^{-/-} mice was more severe than that in IFN- γ ^{-/-} mice.⁶² In a rat acute gastric ulcer model, healing was accompanied by a rapid rise in tissue IL-4 levels.⁶³ IL-4^{-/-} mice were more susceptible to the colitis induced by administration of trinitrobenzene sulfonic acid, and tended to develop focal but penetrating ulcers, which were not frequently seen in IFN- γ ^{-/-} mice.⁶⁴ It is also known that fibroblasts express the IL-4 receptor, and Th2-type cells activate lung fibroblasts with resultant increase in deposition of collagen and fibronectin.^{65,66} In the airway or ileal epithelium, IL-4 induces mucin gene expression⁶⁷ and goblet cell metaplasia.^{17,67} Thus, IL-4 may be significantly involved in the epithelial cell turnover and tissue protection required for the maintenance of the gastrointestinal tract architecture, in addition

to its role as a mediator for allowing immunological homeostasis in the gut.

In summary, we have established a novel murine model for the upper gastrointestinal tract, which does not require pathogen infection or deliberate immunization. The inflammation was mediated by Th1-type immune responses restricted to a particular subset of T cells isolated from normal mice. This model also points to the significance of the host immune system in gastric lesions and should be of importance to help better understand the pathophysiology of chronic gastroduodenitis seen in *H. pylori* infection of humans.

Acknowledgments

We thank Dr. Teruo Kirikae, Research Institute, International Medical Center of Japan, for his expert advice for bacterial culture.

References

- Mizoguchi A, Mizoguchi E, Chiba C, Spiekermann GM, Tonegawa S, Nagler-Anderson C, Bhan AK: Cytokine imbalance and autoantibody production in T cell receptor- α mutant mice with inflammatory bowel disease. *J Exp Med* 1996, 183:847–856
- Mizoguchi A, Mizoguchi E, Tonegawa S, Bhan AK: Alteration of a polyclonal to an oligoclonal immune response to cecal aerobic bacterial antigens in TCR α mutant mice with inflammatory bowel disease. *Int Immunol* 1996, 8:1387–1394
- Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S: Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell* 1993, 75:274–282
- Morrissey PJ, Charrier K, Braddy S, Liggitt D, Watson JD: CD4⁺ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice: disease development is prevented by cotransfer of purified CD4⁺ T cells. *J Exp Med* 1993, 178:237–244
- Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL: Phenotypically distinct subsets of CD4⁺ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol* 1993, 5:1461–1471
- Leach MW, Bean AG, Mauze S, Coffman RL, Powrie F: Inflammatory bowel disease in C.B-17 scid mice reconstituted with the CD45RB^{high} subset of CD4⁺ T cells. *Am J Pathol* 1996, 148:1503–1515
- Corazza N, Eichenberger S, Eugster HP, Mueller C: Nonlymphocyte-derived tumor necrosis factor is required for induction of colitis in recombination activating gene (RAG)2^{-/-} mice upon transfer of CD4⁺CD45RB^{hi} T cells. *J Exp Med* 1999, 190:1479–1492
- De Jong YP, Comiskey M, Kalled SL, Mizoguchi E, Flavell RA, Bhan AK, Terhorst C: Chronic murine colitis is dependent on the CD154/CD40 pathway and can be attenuated by anti-CD154 administration. *Gastroenterology* 2000, 119:715–723
- Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL: Inhibition of Th1 responses prevents inflammatory bowel disease in SCID mice reconstituted with CD45RB^{hi} CD4⁺ T cells. *Immunity* 1994, 1:553–562
- Powrie F, Mauze S, Coffman RL: CD4⁺ T-cells in the regulation of inflammatory responses in the intestine. *Res Immunol* 1997, 148:576–581
- Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M: Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4⁺, CD45RB^{high} T cells to SCID recipients. *J Immunol* 1997, 158:3464–3473
- Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL: A critical role for transforming growth factor- β but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB^{low} CD4⁺ T cells. *J Exp Med* 1996, 183:2669–2674
- Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F: An essential

- role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999, 190:995–1004
14. Read S, Malmstrom V, Powrie F: Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25⁺CD4⁺ regulatory cells that control intestinal inflammation. *J Exp Med* 2000, 192:295–302
15. McGhee JR, Kiyono H: Mucosal immunology. *Fundamental Immunology*. Edited by Paul WE. San Diego, Academic Press, 1999, pp 909–945
16. McGhee JR, Lamm ME, Strober W: Mucosal immune responses: an overview. *Mucosal Immunology*. Edited by Ogra P. San Diego, Academic Press, 1999, pp 485–506
17. Dohi T, Fujihashi K, Koga T, Shirai Y, Kawamura YI, Ejima C, Kato R, Saitoh K, McGhee JR: T helper type-2 cells induce ileal villus atrophy, goblet cell metaplasia, and wasting disease in T cell-deficient mice. *Gastroenterology* 2003, 124:672–682
18. Blum AL: Treatment of acid-related disorders with gastric acid inhibitors: the state of the art. *Digestion* 1990, 47(Suppl 1):3–10
19. Czinn SJ, Blaser MJ, Nedrud JG: Peptic ulcers and gastritis. *Mucosal Immunology*. Edited by Ogra P. San Diego, Academic Press, 1999, pp 1035–1046
20. Marshall BJ, Armstrong JA, McGeece DB: Attempt to fulfill Koch's postulates for pyloric *Campylobacter*. *Med J Aust* 1985, 142:436–439
21. Fox JG, Lee A: The role of *Helicobacter* species in newly recognized gastrointestinal tract diseases of animals. *Lab Anim Sci* 1997, 47: 222–255
22. Eaton KA, Radin MJ, Krakowka S: An animal model of gastric ulcer due to bacterial gastritis in mice. *Vet Pathol* 1995, 32:489–497
23. Czinn SJ, Cai A, Nedrud JG: Oral immunization protects germ-free mice against infection from *Helicobacter felis*. *Gastroenterology* 1992, 102:A611
24. Lee A, Fox JG, Otto G, Murphy J: A small animal model of human *Helicobacter pylori* active chronic gastritis. *Gastroenterology* 1990, 99:1315–1323
25. Nedrud JG, Blanchard SS, Czinn SJ: *Helicobacter pylori* inflammation and immunity. *Helicobacter* 2002, 7:24–29
26. Chen W, Shu D, Chadwick VS: *Helicobacter pylori* infection: mechanism of colonization and functional dyspepsia: reduced colonization of gastric mucosa by *Helicobacter pylori* in mice deficient in interleukin-10. *J Gastroenterol Hepatol* 2001, 16:377–383
27. Eaton KA, Kersulyte D, Mefford M, Danon SJ, Krakowka S, Berg DE: Role of *Helicobacter pylori* cag region genes in colonization and gastritis in two animal models. *Infect Immun* 2001, 69:2902–2908
28. Michetti P, Cortes-Thoulaz I, Davin C, Haas R, Vaney A-C, Heitz M, Bille J, Kraehenbuhl J-P, Saraga E, Blum AL: Immunization of BALB/c mice against *Helicobacter felis* infection with *Helicobacter pylori* urease. *Gastroenterology* 1994, 107:1002–1011
29. Pappo J, Thomas WDJ, Kabok Z, Taylor NS, Murphy JC, Fox JG: Effect of oral immunization with recombinant urease on murine *Helicobacter felis* gastritis. *Infect Immun* 1995, 63:1246–1252
30. Lee CK, Weltzin R, Thomas Jr WD, Kleanthous H, Ermak TH, Soman G, Hill JE, Ackerman SK, Monath TP: Oral immunization with recombinant *Helicobacter pylori* urease induces secretory IgA antibodies and protects mice from challenge with *Helicobacter felis*. *J Infect Dis* 1995, 172:161–172
31. Eaton KA, Ringer SR, Danon SJ: Murine splenocytes induce severe gastritis and delayed-type hypersensitivity and suppress bacterial colonization in *Helicobacter pylori*-infected SCID mice. *Infect Immun* 1999, 67:4594–4602
32. Tushi MF, Sorensen RU, Czinn SJ: Cell-mediated immune responsiveness to *Helicobacter pylori* in healthy seropositive and seronegative adults. *Immun Infect Dis* 1992, 2:133–136
33. D'Elios MM, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, Telford JL, Romagnani S, Del Prete G: T helper 1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. *J Immunol* 1997, 158:962–967
34. D'Elios MM, Amedei A, Manghetti M, Costa F, Baldari CT, Quazi AS, Telford JL, Romagnani S, Del Prete G: Impaired T-cell regulation of B-cell growth in *Helicobacter pylori*-related gastric low-grade MALT lymphoma. *Gastroenterology* 1999, 117:1105–1112
35. Maloy KJ, Salan L, Cahill R, Dougan G, Saunders NJ, Powrie F: CD4⁺CD25⁺ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med* 2003, 197:111–119
36. Nishizuka Y, Sakakura T: Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science* 1969, 166:753–755
37. Fukuma K, Sakaguchi S, Kuribayashi K, Chen WL, Morishita R, Sekita K, Uchino H, Masuda T: Immunologic and clinical studies on murine experimental autoimmune gastritis induced by neonatal thymectomy. *Gastroenterology* 1988, 94:274–283
38. Sakaguchi N, Miyai K, Sakaguchi S: Ionizing radiation and autoimmunity: induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells. *J Immunol* 1994, 152:2586–2595
39. Shames B, Fox JG, Dewhirst F, Yan L, Shen Z, Taylor NS: Identification of widespread *Helicobacter hepaticus* infection in feces in commercial mouse colonies by culture and PCR assay. *J Clin Microbiol* 1995, 33:2968–2972
40. Riley LK, Franklin CL, Hook RR, Besch-Williford C: Identification of murine *Helicobacters* by PCR and restriction enzyme analyses. *J Clin Microbiol* 1996, 34:942–946
41. Goto K, Ohashi H, Takakura A, Itoh T: Current status of *Helicobacter* contamination of laboratory mice, rats, gerbils, and house musk shrews in Japan. *Curr Microbiol* 2000, 41:161–166
42. Battles JK, Williamson JC, Pike KM, Gorelick PL, Ward JM, Gonda MA: Diagnostic assay for *Helicobacter hepaticus* based on nucleotide sequence of its 16S rRNA gene. *J Clin Microbiol* 1995, 33:1344–1347
43. Dohi T, Fujihashi K, Rennert PD, Iwatani K, Kiyono H, McGhee JR: Hapten-induced colitis is associated with colonic patch hypertrophy and T helper 2-type responses. *J Exp Med* 1999, 189:1169–1180
44. Fujihashi K, McGhee JR, Kweon M-N, Cooper MD, Toneyawa S, Takahashi I, Hiroi T, Mestecky J, Kiyono H: γ/δ T cell-deficient mice have impaired mucosal immunoglobulin A responses. *J Exp Med* 1996, 183:1929–1935
45. Yoshino N, Ami Y, Terao K, Tashiro F, Honda M: Upgrading of flow cytometric analysis for absolute counts, cytokines and other antigenic molecules of cynomolgus monkeys (*Macaca fascicularis*) by using anti-human cross-reactive antibodies. *Exp Anim* 2000, 49:97–110
46. Simpson SJ, Shah S, Comiskey M, YP d, Wang B, Mizoguchi E, Bhan AK, Terhorst C: T cell-mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/signal transducer and activator of transcription (Stat)-4 pathway, but is not conditional on interferon γ expression by T cells. *J Exp Med* 1998, 187:1225–1234
47. Jones CM, Callaghan JM, Gleeson PA, Mori Y, Masuda T, Toh BH: The parietal cell autoantigens recognized in neonatal thymectomy-induced murine gastritis are the α and β subunits of the gastric proton pump. *Gastroenterology* 1991, 101:287–294
48. Toh BH, Gleeson PA, Simpson RJ, Moritz RL, Callaghan JM, Goldkorn I, Jones CM, Martinelli TM, Mu FT, Humphris DC, JM P, Mori Y, Masuda T, Sobieszczuk P, Weinstock J, Mantamadiotis T, Baldwin G: The 60- to 90-kDa parietal cell autoantigen associated with autoimmune gastritis is a β subunit of the gastric H⁺/K⁺-ATPase (proton pump). *Proc Natl Acad Sci USA* 1990, 87:6418–6422
49. Alderuccio F, Toh BH: Spontaneous autoimmune gastritis in C3H/He mice: a new mouse model for gastric autoimmunity. *Am J Pathol* 1998, 153:1311–1318
50. McHugh RS, Shevach EM: Cutting edge: depletion of CD4⁺CD25⁺ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J Immunol* 2002, 168:5979–5983
51. Murakami K, Muruyama H, Hosono M, Sinagawa K, Yamada J, Kuribayashi K, Masuda T: Germ-free condition and the susceptibility of BALB/c mice to post-thymectomy autoimmune gastritis. *Autoimmunity* 1992, 12:69–70
52. Maldonado MA, Kakkanaiah V, MacDonald GC, Chen F, Reap EA, Balish E, Farkas WR, Jennette JC, Madaio MP, Kotzin BL, Cohen PL, Eisenberg RA: The role of environmental antigens in the spontaneous development of autoimmunity in MRL-lpr mice. *J Immunol* 1999, 162:6322–6330
53. Garhart CA, Nedrud JG, Heinzel FP, Sigmund NE, Czinn SJ: Vaccine-induced protection against *Helicobacter pylori* in mice lacking both antibodies and interleukin-4. *Infect Immun* 2003, 71:3628–3633
54. Sugiyama T, Yabana T, Yachi A: Mucosal immune response to *Helicobacter pylori* and cytotoxic mechanism. *Scand J Gastroenterol Suppl* 1995, 208:30–32

55. Negrini R, Savio A, Poiesi C, Appelmelk B, Buffoli F, Paterlini A, Cesari P, Graffeo M, Vaira D, Franzin G: Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. *Gastroenterology* 1996, 111:655–665
56. Appelmelk BJ, Simoons-Smit I, Negrini R, Moran AP, Aspinall GO, Forte JG, De Vries T, Quan H, Verboom T, Maaskant JJ, Ghiara P, Kuipers EJ, Bloemena E, Tadema TM, Townsend RR, Tyagarajan K, Crothers Jr JM, Monteiro MA, Savio A, De Graaff J: Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. *Infect Immun* 1996, 64:2031–2040
57. Aspinall GO, Monteiro MA: Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. *Biochemistry* 1996, 35:2498–2504
58. Simoons-Smit IM, Appelmelk BJ, Verboom T, Negrini R, Penner JL, Aspinall GO, Moran AP, Fei SF, Shi BS, Rudnica W, Savio A, de Graaff J: Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. *J Clin Microbiol* 1996, 34:2196–2200
59. Wirth HP, Yang M, Karita M, Blaser MJ: Expression of the human cell surface glycoconjugates Lewis x and Lewis y by *Helicobacter pylori* isolates is related to *cagA* status. *Infect Immun* 1996, 64:4598–4605
60. Gautam S, Tebo JM, Hamilton TA: IL-4 suppresses cytokine gene expression induced by IFN- γ and/or IL-2 in murine peritoneal macrophages. *J Immunol* 1992, 148:1725–1730
61. Ohshima Y, Yang LP, Avic MN, Kurimoto M, Nakajima T, Sergerie M, Demeure CE, Sarfati M, Delespesse G: Naive human CD4⁺ T cells are a major source of lymphotoxin α . *J Immunol* 1999, 162:3790–3794
62. Smythies LE, Waites KB, Lindsey JR, Harris PR, Ghiara P, Smith PD: *Helicobacter pylori*-induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFN- γ , gene-deficient mice. *J Immunol* 2000, 165:1022–1029
63. Slomiany BL, Piotrowski J, Slomiany A: Down-regulation of endothelin-1 by interleukin-4 during gastric ulcer healing. *Biochem Biophys Res Commun* 1999, 263:591–595
64. Dohi T, Fujihashi K, Kiyono H, Elson CO, McGhee JR: Mice deficient in Th1- and Th2-type cytokines develop distinct forms of hapten-induced colitis. *Gastroenterology* 2000, 119:724–733
65. Derdak S, Penney DP, Keng P, Felch ME, Brown D, Phipps RP: Differential collagen and fibronectin production by Thy 1⁺ and Thy 1[−] lung fibroblast subpopulations. *Am J Physiol* 1992, 263:L283–L290
66. Roche WR, Beasley R, Williams JH, Holgate ST: Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989, 1:520–524
67. Dabbagh K, Takeyama K, Lee HM, Ueki IF, Lausier JA, Nadel JA: IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. *J Immunol* 1999, 162:6233–6237