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8.01 Susceptibility of NF-kappaB-deficient mice to Helicobacter hepaticus-induced colitis results from a defect within cells of the innate immune system

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Our previous experiments showed that infection of NF-κB deficient mice (p50−/− or p50−/−p65−/−) with Helicobacter hepaticus leads to the development of typhlocolitis. The observation that H. hepaticus is able to induce colitis in Rag-2-deficient mice, but not wild type (WT) mice, suggests that in normal mice the primary role of lymphocytes is to suppress the innate inflammatory response to Helicobacter infection. This raised the question of whether disease in p50−/−p65−/− (3x) mice was caused by a defect within suppressive lymphocyte populations, or within cells of the innate immune system that respond to these suppressive lymphocytes. To address this question, we adoptively transferred the splenocytes from WT or 3x mice into Rag-2−/− and Rag-2−/− p50−/−p65−/− mice. Parallel in vitro studies were done in which bone marrow derived macrophages (BMDM) from WT and 3x mice were cultured with H. hepaticus. Our results showed that H. hepaticus-induced disease in Rag-2−/− or Rag-2−/− p50−/−p65−/− was very similar, as measured by histopathology score and levels of inflammatory gene expression. However, while adoptive transfers of splenocytes from either WT or 3x mice were able to suppress Helicobacter-induced disease in Rag-2−/− mice, they were unable to suppress disease in Rag-2−/−p50−/−p65−/− mice. Moreover, H. hepaticus-infected 3x BMDM showed significant up-regulation of inflammatory genes and proteins in contrast to infected WT BMDM. The most striking differences were in the mRNA and protein level of IL-12p40. These results suggest that there is a defect within the innate immune system of 3x mice which abrogates the ability of lymphocyte populations to suppress the innate inflammatory response to infection with H. hepaticus.

8.02 Human plasmacytoid dendritic cells (pDC) recognize and induce interleukin-12 production in Th1 cells in vitro

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Introduction. The current study determined the antigenic potential of several H. pylori proteins. Urea A/B and OMP18 had been previously found to interact specifically with sera from H. pylori infected patients (Voland et al., Alim Pharm 2001) and were also expressed in HK293 cells. PCR revealed that these OMPs were highly conserved and found in virtually all H. pylori strains tested.

Results. (1) All recombinant proteins (rUreaB, rHpaA, Omp18, UreL-peptide) were incubated with human peripheral blood mononuclear cells (PBMC, isolated from H. pylori infected patients) at 1-30 μg/ml. A 2- to 4-fold stimulation of BDU incorporation was observed. rHpaA, rOmp18, rUreaB and UreL-peptide stimulated a mitogenic response in 10-15% of the mononuclear cells. rHpaA, rOmp18 and UreL-peptide stimulated IFN-gamma secretion from human PBMC, indicating a Th1-polarized response. In contrast, no significant stimulation of IFN-gamma secretion was observed with rUreaB, suggesting a Th2-polarized response.

(2) In order to determine the antigen presenting cells that reacted with these proteins, pDCs were isolated using magnetic bead sepration kits and incubated with the proteins. Furthermore, human blood macrophages were isolated, but did not show significant stimulation of IL-8 release after incubation with the recombinant proteins. In contrast, HpaA and OMP18 expressed in HK293 cells potently stimulated MHC-II and CD83 expression on isolated human plasmacytoid dendritic cells.

Conclusion. rHpaA, rOMP18 and UreL-peptide stimulated the proliferation of human PBMC, presumably Th-1 cells. pDCs recognize HpaA and Omp18 and react with the expression of co-stimulatory molecules such as CD83 and MHC-II. These proteins as well as Urel appear to have a potential for vaccination since they favor a Th-1 polarized response and are expressed on the surface of H. pylori.

8.03 Appendectomy at an early age is not protective against typhlocolitis in Helicobacter hepaticus infected B6.129 IL-10−/− mice

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Appendectomy in young adults has been reported to be potentially protective against the incidence of inflammatory bowel disease (IBD) later in life, potentially by altering the priming of host responses to enteric flora. Typhlocolitis with associated proctitis and rectal prolapse spontaneously develops in conventionally maintained immune dysregulated IL-10−/− mice and there is clear evidence from germ-free studies that disease results from an aberrant host response to intestinal flora. IL-10−/− mice have been used to model human IBD and studies have demonstrated the reproducibility of the model when promoted by infection with several helicobacter species. We used this paradigm to test the hypothesis that appendectomy in weaning mice (4 weeks of age) would decrease the severity of typhlocolitis in B6.129p2-Il10tm1Cgcr/mice naturally infected with Helicobacter hepaticus (Hh). IL-10−/− mice born to helicobacter-free or Hh-infected IL-10−/− mice (n=55) were randomized to experimental groups that underwent appendectomy at 4 weeks of age or did not have surgery. Maintenance of helicobacter-free status or horizontal transmission of Hh from dam to pups were confirmed by PCR of feces. At 6 months of age, serum antibody levels and fecal IgA against Hh as well as levels of Hh shedding in feces estimated by quantitative PCR were similar across experimental groups of infected mice, suggesting that appendectomy did not affect immune priming or Hh colonization of the cecum and colon. As clinical predictors of IBD-like disease, rectal prolapse was highest in Hh-infected males that were appendectomized at 4 weeks of age and the same cohort of female mice had the lowest body weights (p<0.03). These clinical indicators indicating that appendectomy did not affect immune priming or Hh colonization of the cecum and colon were similar among all groups that underwent appendectomy at 4 weeks of age or did not have surgery. Gender interacted significantly with time (all p<0.0001) as well as significant interactions of infection status with diet for all fundic and antral pathology parameters (p<0.001) except intestinal metaplasia. In infected animals, there was a highly significant effect for Helicobacter infection status for all fundic and antral pathology parameters scored. Gender interacted significantly with time (all p<0.03), and H. pylori colonization increased quantitatively over the course of the experiment. The Th1-associated serum IgG2a responses to H. pylori increased over time of experimental infection and were similar among all

8.04 Helicobacter pylori associated gastric cancer in INS-GAS mice is gender-specific

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Hypergastrinemia in mice can synergize with Helicobacter felis infection to induce gastric carcinoma. C57BL/6 mice fed high salt diet during H. pylori infection develop hypergastrinemia and preneoplastic gastric lesions. To address the relationship between gastric and salt and whether H. pylori can also lead to gastric cancer, we undertook a longitudinal study involving 86 INS-GAS mice. The mice were fed either a high salt (7.5%) or basal (0.25%) diet, and half were infected with H. pylori. Necropsies at 5 and 7 months post infection included histopathological examination, quantitative culturing for bacterial colonization levels and serology. Lesions consistent with in situ and intramucosal carcinoma were seen in H. pylori infected male mice only. There was a highly significant effect for Helicobacter infection status for all fundic and antral pathology parameters (p<0.0001) as well as significant interactions of infection status with diet for all the fundic parameters (all p<0.03) except intestinal metaplasia. In infected animals, there was a highly significant main effect for time, diet and gender (all p<0.03) on all of the fundic pathology parameters scored. Gender interacted significantly with time (all p<0.03), and H. pylori colonization increased quantitatively over the course of the experiment. The Th1-associated serum IgG2a responses to H. pylori increased over time of experimental infection and were similar among all
8.05 Isogenic mutants of *H. mustelae* lacking Helicobacter surface ring (Hsr) proteins only transiently colonize the ferret stomach

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Helicobacter mustelae, the gastric pathogen of ferrets, produces an array of abundant surface ring structures which have not been described in any other member of the genus. The unique ring structures are composed of a protein named Hsr (Helicobacter surface ring protein) and may be important in protection of the organism from the host immune response. Preliminary studies demonstrated transient colonization of the ferret stomach by 2 different Hsr mutants. To amplify and corroborate these findings, H. mustelae-free ferrets were inoculated with one of the Hsr-deficient strains (α/β), the wild-type *H. mustelae* strain (α/β) or sterile broth (α/β). Anti- and Hsr-specific IgG antibodies were taken by oral gavage and quantified at 3, 6, 9, 12 and 15 weeks after inoculation; quantitative culture and complete necropsies were performed at 18 weeks. Antral biopsies obtained at 3, 6 and 9 weeks postinoculation demonstrated no significant difference between bacteria numbers in the ferrets that received the Hsr-negative strain compared to the ferrets inoculated with the parent strain. At 12, 15, and 18 weeks after infection, however, the Hsr-negative strain was substantially reduced or absent. Quantitative cultures from gastric biopsies of the body demonstrated reduced bacterial numbers in the Hsr-negative strain group compared to wild-type. There was no colonization in the sham group. Histological examination of the study ferrets at necropsy revealed no appreciable gastric inflammation in the animals that received the mutant *H. mustelae* strain, whereas lesions characteristic of Helicobacter infection were present in the wild-type infected ferrets. These results demonstrate that the Hsr protein is not required for initial colonization but appears required for persistent colonization. Late elimination of the Hsr-negative *H. mustelae* strain following successful early colonization suggests that the Hsr protein may have a role in antigenic variation that allows the organism’s escape from immune surveillance.

8.06 Acute Helicobacter pylori infection and H,K-ATPase expression: Digital immunounquantitation of proton pumps in non-human primates

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H. pylori infection may cause gastric atrophy but outcome varies, possibly due to genetic differences in host response. Previous studies have shown loss of parietal cells in long term human *H. pylori* infection, but few studies have addressed the effects of acute *H. pylori* infection on proton pump (H.KATPase) expression.

**Aim:** To assess H.K-ATPase expression in acute *H. pylori* infection.

**Methods:** *H. pylori* (109 CFU, from humans) were inoculated into the stomach of 12 non-related Rhesus monkeys at gastroscope. Gastric biopsies were obtained preinoculation and 7, 14 days, 2, 4, 7 and 10 months postinoculation. *H. pylori* status was determined using culture, histology and PCR. Gastric body biopsies were stained with H&E and immunohistochemistry (monoclonal antibody, HB 12.18) against an H. KATPase epitope. Immunostaining of body sections was quantitated by digital imaging.

**Results:** Persistent infection was observed to 10 months (5 animals), transient infection (1 animal) and was not established in 3 animals. In established infection, H.KATPase expression declined significantly from baseline values in 35 animals at 7 days. In the absence of persistent infection, H.KATPase expression fell transiently in body mucosa but had returned to normal by 10 months. No body atrophy was apparent on H&E sections.

8.07 The effects of chronic Schistosomiasis on murine Helicobacter gastric epithelial cell proliferation

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Helicobacter gastric epithelial cell proliferation is increased in *Helicobacter* infection but the effect of concurrent parasitic infection is unknown.

**Aim:** To assess the effects of Schistosoma mansoni infection on Helicobacter induced gastric epithelial cell proliferation and cytokine expression in a mouse model.

**Methods:** Female C57BL/6 mice were infected with 25 S. mansoni cercariae. Three weeks later S. mansoni infected mice and controls were gavaged 3 times with *H. felis*. At 10-12 weeks animals were sacrificed following IP injection of bromodeoxyuridine (BrdU). Gastric epithelial cell proliferation was determined immunohistochemically. Infection was confirmed by urease test and histology. Gastric cytokines were analysed by RT-PCR.

**Results:** Gastric pathology in *H. felis* versus *H. felis/S. mansoni* infected animals showed no significant differences in basal epithelial cell proliferation, activity and chronic inflammatory scores were not significantly different, but antral atrophy and mucus cell hyperplasia were more frequent in co-infected mice. In the antrum epithelial cell proliferation in *H. felis* infected mice (mean labelling index (LI%) ± SEM) 16.1 ± 1.13) was significantly greater than uninfected controls (10.3 ± 1.41, p < 0.03) and mice with *S. mansoni* alone (7.7 ± 1.4, p < 0.01). Antral epithelial cell proliferation in mice with *S. mansoni/H. felis* (8.2 ± 1.46) was significantly lower (p < 0.01) than in mice with *H. felis* alone. In the corpus no significant changes in epithelial proliferation were associated with single or dual infection compared to uninfected controls. Gamma interferon mRNA was significantly increased (p < 0.02) in the gastric mucosa of both *H. felis* and *S. mansoni/H. felis* infected animals compared to uninfected controls. *S. mansoni* co-infection was associated with a significant (p < 0.01) reduction in gastric IL-12p40 mRNA compared to mice infected with *H. felis* alone.

**Conclusions:** These studies show that co-infection with *S. mansoni* increases Helicobacter-induced atrophy and surface mucus cell hyperplasia in the antrum and is associated with decreased antral epithelial cell proliferation.

8.08 Genetic modifications in polyphosphate kinase gene of *Helicobacter pylori* during a murine experimental infection

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**Aims:** Helicobacter pylori isolates present a high polymorphism which could be the consequence of adaptive changes during colonization making it a strain better able to survive, to evade the immune system and to develop a chronic infection. To investigator the in vivo acquisition of this genetic polymorphism, we developed a mouse model of *H. pylori* infection and looked for genetic modifications of the strains.

**Materials and Methods:** Three *H. pylori* strains were used: the SS1 and SS2 strains freshly isolated from infected patients. H141, and H145. C57BL6 mice were orally inoculated and sacrificed at day 3, 8, 15, 21, 45, 90, 150 and 360 post-infection. Ten randomly selected colonies of the emerging strains were studied at each time for genetic modification assessed by RAPD using 18 primers.

**Results:** Modifications of the genetic structure were detected with one primer after 365 days of infection by H141. They consisted in the the appearance of an additional fragment of about 950 pb. Cloning and sequencing of the fragment showed that it contained the polyphosphate

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**Results:** Acute *H. pylori* infection reduced H,KATPase expression without parietal cell ablation in 3 animals and in some without established infection, suggesting inhibition of H,KATPase gene transcription and/or translation. However H,KATPase expression increased in two other animals with persistent infection. The variable expression may be related to genetic differences in response to infection as the environment was controlled and the bacteria were of the same strain. Results in animals with persistent infection indicate that a similar infection can lead to opposite effects on acid secretion, which is similar to the variability of response in patients with peptic ulcer disease.
Helicobacter pylori infection affects growth rate in Mongolian gerbils

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The impact of H. pylori on the nutritional status of infected subjects is not well understood yet. Intriguingly, H. pylori infection has been epidemiologically related to either obesity or growth retardation.

Aim: To determine if H. pylori infection may affect the weight gain (WG) in Mongolian gerbils (MG).

Methods: 40 4-week old male MG were infected with the H. pylori strain C20; 40 control animals were used as controls. All MG were housed in polycarbonate cages and had free access to rodent food and water. Gastroscopy revealed that 5 non-infected MG and 5 infected MG were killed within 1, 3, 6 and 10 months after inoculation. The stomach was processed for histology. The body weight of MG was assessed before inoculation and at the time of sacrifice.

Results: The average WG of each group of animals at different times of follow up were: Hp+: 27.4±4.5 g vs Hp−: 23.4±4.8 g at 1 month (p=ns); Hp+: 30.8±5.2 vs Hp−: 30.2±4.9 g at 3 months (p=ns); Hp+: 28.2±4.3 g vs Hp−: 41.8±4.6 g at 6 months (p<0.05); Hp+: 23.8±4.6 vs Hp−: 49.0±5.1 g at 10 months (p<0.001). Interestingly, WG of infected animals was inversely related to the severity of the histological damage. In particular, at 1 and 3 months all animals showed severe chronic active gastritis, at 6 months they also showed mucosal erosions and at 10 months 3 of 5 MG showed penetrating ulcers. None of the control animals showed either H. pylori infection or inflammation.

Conclusions: Infected animals show lower WG compared to the uninfected. This difference becomes statistically significant 6 months after inoculation and correlates with the severity of gastric mucosal damage. Pain and ulcer-related anorexia may explain this phenomenon. An increase of gastric leptin induced by the infection or an enhanced cytokine-related cachexia may possibly also be involved.

Use of Bacillus subtilis strain CU1 as a vaccine delivery system for mucosal immunization against Helicobacter pylori infection in mice

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The lack of a suitable adjuvant has limited mucosal vaccine development. Probiotic bacteria of the genus Bacillus may have immunostimulating properties and can be used as antigen delivery vehicles for mucosal immunization. Bacillus subtilis strain, CU1 was selected and Helicobacter pylori infection was chosen as an experimental vaccine model.

Two different recombinant B. subtilis strains expressing H. pylori urease subunit B (UreB) were constructed. Constitutive expression of UreB by a pPPLUreB vector, derived from pPPL08, was detected by Western blot analysis. Soluble extra- and intracellular UreB accumulation was observed for both recombinant strains. Nevertheless, greater UreB production was obtained in the B. subtilis CU1UreB, which is defective in protease activity.

Mice (BALB/c) were given oral prophylactic and therapeutic immunization. They were immunized 5 weeks before or 3 weeks after an H. pylori SS1 challenge (for prophylactic or therapeutic immunization, respectively). The immunization regimens consisted of the 7 doses of 107 B. subtilis CU1UreB with an interval of approximately 2 days between each immunization. The protective effect of prophylactic and therapeutic immunization was measured 4 weeks after the last H. pylori challenge or last immunization, respectively. The efficacy of the constructed vaccine strain was compared with that of H. pylori saponite in the presence of cholera toxin, taken as reference. H. pylori was detected by microscopy, urease test, ELISA, and PCR. The protective effect as well as the therapeutic effect of the B. subtilis constructed vaccine were comparable (~75%) than that of the reference. These findings suggest that the B. subtilis system of mucosal immunization is an alternative to the current Helicobacter pylori challenge or colonization model.

Pathological changes in the gastric mucosa of Mongolian gerbils infected by different strains of Helicobacter pylori

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The presence of the cag PAI was documented by PCR. The positive proportion of the PAI+ and PAI- strains resembles the natural infection occurring in humans.

Aim: To characterize the effect of different H. pylori strains on the gastric mucosal changes induced in Mongolian gerbils (MG).

Methods: Ten H. pylori isolates from infected individuals, the strain ATCC-43504 and the CagA and VacA-positive strain C20 isolated from a patient with gastric carcinoma were tested for their ability to infect MG. Five animals were infected with each isolate. H. pylori infection was assessed at 4 weeks after inoculation by histology.

Results: Only the C20 strain resulted in consistent infection while another isolate showed mucosal colonization without inflammation. For this reason 40 4-week old male MG were infected with the C20 strain, while 40 controls were used as controls. Groups of 5 infected and 5 non-infected MG were sacrificed 1, 3, 6 and 10 months after inoculation. The stomach of each animal was processed for histology.

One month after inoculation, all infected animals showed moderate to severe antral-restricted or -predominant active gastritis with lymphoid aggregates. At 3 months they showed severe chronic active gastritis, involving both antral and oxyntic mucosa. At 6 months they also showed multiple erosions. Three of 5 animals at 10 months showed penetrating ulcers. Intestinal metaplasia was focally present in the antrum of a few animals after 6 months. Neither dysplasia nor carcinomas developed.
Conclusions: Successful H. pylori infection of MG results in a progressive disease of the antrum with later extension to the oxyntic mucosa accompanied by mucosal erosion and ulceration. Interestingly, only some H. pylori strains are able to induce an inflammatory response in MG; this finding suggests that the severity of the gastric mucosal lesions may be related to unique H. pylori strain virulence factors.

8.14 Effect of H. pylori infection on gastric mucin synthesis in Mongolian gerbils


The surface mucous gel layer (SMGL) of gastric mucosa is formed by two distinct types of mucins derived from surface mucous cells and gland mucous cells. In the present study, we first identified cDNA encoding Mongolian gerbil a1,4-N-acetylgalactosaminyltransferase (a4GnT) responsible for the biosynthesis of GlcNAcα-4Gal-B. This glycan is specifically present in the gastric gland mucous cells. Using this cDNA, we next evaluated the effect of H. pylori-infection on the gastric mucin biosynthesis in Mongolian gerbils using real-time RT-PCR targeted to a4GnT gene.

H. pylori-infected Mongolian gerbils were sacrificed at 4 weeks after inoculation, and then the stomach was immediately removed. Total RNA was extracted from the pyloric as well as fundic mucosa of stomach. After DNaseI treatment, the total RNA extracted was subjected to reverse transcription. As results, the expression level of a4GnT mRNA in gastric mucosa was quantitatively analyzed using real-time RT-PCR. The expression level of a4GnT mRNA tended to be increased in the pyloric mucosa of infected gerbils compared to controls. This suggests that H. pylori infection enhances the biosynthesis of gland mucous cell-type mucin.

8.15 Experimental reproduction of Candidatus Helicobacter suis infection in pigs


Hyperkeratoses and ulcers of the gastric pars oesophagea occur worldwide both in grower-finisher pigs and sows. These lesions have been associated with spiral bacteria in the stomach, which are hitherto uncharacterizable in vitro. Based on 16S ribosomal DNA sequence data, these bacteria have been placed in the genus Helicobacter and named "Candidatus Helicobacter suis" (H. suis) (1). There are indications that H. suis also causes disease in humans (2). The purpose of the present study was to experimentally reproduce the infection in pigs.

Five stomachs with ulcerative lesions of the pars oesophagea were collected in the abattoir. These tested positive for H. suis in PCR (3). Six 5 week-old SPF pigs were inoculated intragastrically with 10 ml antrum pyloricum mucosa homogenate containing 10⁴ mouse infectious doses 50% (MID50) H. suis per ml. 3h after intramuscular administration of cimetidine. The animals were killed from 1 to 5 weeks following inoculation. Two other pigs were used to confirm the presence of H. suis. The control pigs were negative. These experiments show that H. suis infection can be reproduced experimentally in SPF pigs. The pigs had no ulcers and only a mild superficial gastritis was present. This is similar to what is observed with other Helicobacters in other hosts. Probably additional factors are required for the development of ulcers or the used H. suis strain(s) is (are) of low virulence.

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References

8.16 Eradication of Candidatus Helicobacter suis infection in a murine model by antibiotic treatment

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In the past decade, spiral organisms morphologically distinct from Helicobacter pylori were observed in the human stomach in association with chronic gastritis and gastric lymphoma. These bacteria were assigned as "H. heilmannii" (1). Recent taxonomic data revealed 99.9% 16S rDNA homology between "H. heilmannii" type 1 and "Candidatus H. suis" (H. suis), a gastric helicobacter of pigs (2). No data are available on the antibiotic sensitivity of H. suis, mainly due to its hitherto unculturability.

In the present work, we have exploited a previously developed mouse model of infection by H. suis to assess the efficacy of two antibiotic treatment schemes on its eradication.

Balb/c mice were inoculated by gastric gavage of H. suis. After two weeks, amoxicillin, bicitarbaron and omeprazole or antibiotic X, omeprazole and bismuth subsalicylate was administered intragastrically, three times daily for 14 days. Control animals received no treatment. A PCR test specifically designed to detect H. suis (3) was carried out on daily collected faecal material and antrum, which were removed from the killed animals one week following the last treatment.

Starting one week after the onset of treatment involving amoxicillin, the excretion of H. suis markedly declined. PCR yielded a negative result on the antrum, claiming the absence and hence extinction of H. suis in case of amoxicillin treatment. In the animals subjected to antibiotic X and the control animals, H. suis remained present in the faeces and the antrum likewise tested positive, indicating the failure of this scheme to eradicate H. suis.

Acknowledgements: This work was supported by the Ministry of Agriculture (DG6, Brussels, Belgium) and by Farmacia Animal Health (Puurs, Belgium).

References

8.17 Hyperimmune colostroal preparation enhances the effect of antibiotic treatment of Helicobacter felis infection in mice


Aim: Efficacy of immune colostroal preparation (IP) containing specific antibodies against Helicobacter felis (HF) combined to amoxicillin trihydrochloride was studied in the treatment of experimental HF infection in mice.

Methods: IP was produced by immunizing pregnant cows successively with a vaccine containing HF strain CS1 inactivated with formalin in Al3OH adjuvant. IgG fraction of colostrum was enriched by chromatographic and membrane techniques. 45 SJL mice were infected orally with HF on three consecutive days. After 2 months lagtime 15 mice were given IP three times a day for 28 days. 7 mice were given amoxicillin (40 µg/kg) for 6 days. 9 animals first received IP for 22 days, and IP and amoxicillin for the last 6 days. Six mice served as healthy controls, and 14 mice as infected controls. Mice were killed immediately after the treatment. The HF status was assessed on the basis of bacteriological stainings and gastric histology of the stomachs.

Results: 11 out of 14 infected controls were HF positive whereas healthy control group remained HF negative. The administration of IP did not eradicate HF infection (13/15 HF positive). Amoxicillin decreased the grade of HF colonization in antrum with a trend setting (P=0.056) significance (5 out of 7 were eradicated). IP combined to amoxicillin was found to lower the level of inflammation and colonisation (all 9 HF negative) (p<0.0005; infected mice as controls).

Conclusion: The IP combined with amoxicillin treatment lowered the level of inflammation and colonisation more than the treatment with amoxicillin alone, and the difference to infected controls was statistically significant. The potential function of IP combined with antibiotics could be in the prevention of reinfections and inhibition of spreading of Helicobacter in gastric mucus during the antibiotic treatment, the IP thus contributing beneficially to the effect of antibiotics.
Bacterial factor of Helicobacter pylori is important for developing gastric mucosal lesions in Mongolian gerbil

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Background: Hirayama et al. established an animal model infected with H. pylori using Mongolian gerbils. H. pylori infection persisted over a long term and caused gastric lesions in this model. In addition, the processes of gastric mucosal changes resemble those of humans. This model seems an appropriate animal model to investigate pathogenesis of H. pylori. However, identical H. pylori strains, which had +ve cagA and vacA phenotype s1/m1, were used in previous reports.

Aim: The aim of this study is to evaluate a role of bacterial factor using Mongolian gerbils (MGS/sec) model.

Materials and Methods: H. pylori TN2GF4 and SS1 were used. TN2GF4 is a Japanese strain and a carcinogenic strain for MGS/sec. SS1 is an Australian strain. High levels of colonization were observed in mice. SS1 had a tendency to inhibit chemical gastric carcinogenesis in SPF C57BL/6 mice. Mongolian gerbil (MGS/sec) 6 week-old infected with each H. pylori strain. Pathological and bacterial examinations were performed at 18 months after inoculation.

The characteristic of TN2GF4 and SS1

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<th>TN2GF4</th>
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<tr>
<td>vacA</td>
<td>s1/m1</td>
<td>s2/m2</td>
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<tr>
<td>CagPAI</td>
<td>There are some differences.</td>
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<td>Lipid-A</td>
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Results and Conclusion: TN2GF4 strain induced ulceration and atypical gland in the stomach. On the other hand, SS1 strain induced hypertrophic gastritis (Menetrier’s disease). No ulcer and no atypical gland were detected. This result suggested that bacterial factors had an important role for developing gastric mucosal lesion. The differences between TN2GF4 and SS1 seems critical in evaluating roles of bacterial factor in gastric pathogenesis.

Five months persistence of H. pylori gastritis in guinea pigs

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Purpose: To evaluate a five month Helicobacter pylori infection in guinea pigs.

Methods: Male Dunkin-Hartley guinea pigs were orally inoculated with H. pylori strain SS1 and control animals with sterile TSB. Blood samples were collected prior to inoculation. Animals were sacrificed after five months and samples were taken for culture, PCR, histopathology and serology. Gastritis was graded on a four grade scale (no gastritis, mild, moderate or severe) and considered severe when several heavy inflammatory infiltrates spreading through the whole mucosal layer was present. Serum was analyzed by an enzyme immunoassay (EIA) and immunoblot towards H. pylori cell surface antigens.

Results: H. pylori was cultured from 3 of 5 challenged animals and a fourth animal was positive in Helicobacter genus-specific PCR and identified as H. pylori in denaturing gradient gel electrophoresis. Three of the four infected animals displayed severe antral gastritis. Control animals and the challenged animal that was negative in culture and PCR showed no or mild gastritis. No other changes were observed. The four infected animals displayed elevated EIA titers, whereas the challenged animal negative in culture and PCR showed no elevation. Infected animals displayed H. pylori specific antibodies towards proteins with approximate molecular weights of 15.5-, 18.5-, 24.5-, 28- and 33-kDa in immunoblot.

Conclusions: Experimental H. pylori infection persisted for five months in guinea pigs. Inflammation was severe, but gastric atrophy or premalignant changes did not occur within five months. A specific immune response was directed towards H. pylori cell surface antigens ranging from 15.5 to 33 kDa. The persistent infection, the severe gastritis and the prominent antibody response further supports the guinea pig as a useful model in H. pylori research. Further experiments with longer term infection will evaluate possible development of premalignant changes.