Supplemental Materials, Methods and Results

Materials and Methods

Animals, bacterial strain and experimental infections

C57BL/6 *gpt* delta mice were constructed as described previously (1). It has been documented that 129/SvEv *Rag2*$^{-/-}$*l10*$^{-/-}$ mice can be utilized to dissect the molecular mechanisms underlying bacteria-induced intestinal carcinogenesis, including the important role of Il-10 in inhibiting colon cancer of *H. hepaticus* infection (2). Thus 129/SvEv *Rag2*$^{-/-}$*Il10*$^{-/-}$ *gpt* delta (Rag2Il10gpt) mice were generated by back-crossing C57BL/6 *gpt* delta mice on 129/SvEv *Rag2*$^{-/-}$*l10*$^{-/-}$ mice for 10 generations. This *gpt* delta mouse strain was produced at the MIT breeding facility and then housed in groups of up to five in polycarbonate microisolator cages on hardwood bedding (PharmaServ, Framingham, MA) under specific pathogen free conditions (free of *Helicobacter* spp., *Citrobacter rodentium*, *Salmonella* spp., endoparasites, ectoparasites and exogenous murine viral pathogens). All mice were maintained in an Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility at 70±2°F, 30-70% relative humidity, a 12:12 hour light to dark cycle, fed standard rodent chow (Purina Mills, St. Louis MO) and given water *ad libitum*. Animal use was approved by the MIT Committee on Animal Care. The mice were dosed with *H. hepaticus* 3B1 organisms which were harvested from blood agar plates after incubation of 2-3 days under microaerobic conditions as described previously (3).

***gpt* Assay**

Briefly, phages rescued from murine genomic DNA were transfected into *E. coli* YG6020 expressing Cre recombinase. Two equal volumes of infected cells were spread on plates containing chloramphenicol (Cm) alone (calculating a total number of the *cat-gpt* plasmids rescued from

phages) or Cm and 6-TG (selecting the loss-of-function *gpt*$^-$ plasmid), respectively. The plates were cultured for approximately 3 days until 6-TG–resistant colonies appeared. The 6-TG–resistant colonies were restreaked on plates containing Cm and 6-TG to further confirm their 6-TG–resistant phenotype. Confirmed 6-TG–resistant colonies were sent for direct colony sequencing at Quintarabio (Allston, MA) with the primers targeting the entire *gpt* sequence as described previously (4). Sequences were aligned with the *E. coli gpt* gene (GenBank M13422.1) using the DNASTAR lasergene 13 software package (DNASTAR, Inc., Madison, WI).

qPCR analyses on intestinal cytokines

Total RNA from murine cecal tissues was prepared using Trizol Reagents following the supplier's instructions (Invitrogen). cDNA from tissue mRNA (2μg) was reverse-transcribed using the High Capacity cDNA Archive kit following the supplier's instructions (Life Technologies, Foster City, CA). Using the 7500 Fast Real-Time PCR System, mRNA expression of murine genes involved in innate immunity and oncogenesis, including *Ifnγ, Tnfα, iNos, Il-17A, Il-22, Atm* and *Atr,* were measured using primers and probes from Life Technologies. All target genes were normalized to the endogenous control glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) mRNA, and expressed as fold change in reference to sham-dosed control mice using the Comparative C_T method (Applied Biosystems User Bulletin no. 2).

qPCR Assays for bacteria

Colonization levels of cecal and colonic *H. hepaticus* 3B1 were measured using the *cdtB*-based primers and probe as described previously (5). Levels of *pks+ E. coli* in the cecum, colon and feces were enumerated using a *clbB*-based primer pair (ClbB-F: 5'-GCG CAT CCT CAA GAG TAA ATA-3' and ClbB-R: 5'-GCG CTC TAT GCT CAT CAA CC-3') with the SyBr green

master mixture (Thermo Fisher Scientific, Waltham, MA). The primer pair was designed to amplify a ~280 bps region of *clbB* as described previously (6). A standard curve was generated using serial 10-fold dilutions of the *H. hepaticus* 3B1 genomic DNA or the *E. coli* NC101 genome (~5 Mb, GenBank: NZ_AEFA00000000.1) representing 1×10^6 to 10 genome copies.

Immunohistochemistry

Briefly, the slides were incubated with mAb either for γH2AX (1:200 dilution) or for anti-iNos (1:500 dilution), followed by incubation with Alexa Fluor 488-conjugated anti-rabbit $F(ab')_2$ fragment (1:750, Cell Signaling). The cell nuclei were stained using 10 µl of Prolong Gold Antifade Reagent with DAPI (Cell Signaling). The tissue sections were visualized using a Zeiss Axioskop 2 Plus microscope (Zeiss, Germany). Levels of epithelial iNos expression and γH2AX foci-positive epithelial cells in the ceca and colons were graded for intensity, distribution and crypt-positivity of fluorescence signal with scores of ascending 0-4 respectively; an index of iNos expression and γH2AX foci-positive epithelial cells represents a total of scores of these 3 categories with a maximal number of 12.

Statistical Analysis

All statistical analyses were performed using the Prism 5 software Package (Graphpad, San Diego, CA). Intestinal scores of HAI, mutation frequencies in the lower bowel, iNos expression and γH2AX foci-positive epithelial cells were compared between groups by the Mann-Whitney U-test. Data on colonization levels of *H. hepaticus* and *pks+ E. coli*, and cytokine mRNA levels were examined for normality of the distribution using the Kolmogorov-Smirnov test. The data with and without the normality of distribution were analyzed using the two-tailed Student's *t* test and the Mann-Whitney U-test, respectively. Values of $P<0.05$ were considered significant.

Results

There was no significant difference in both cecal and colonic mutation frequencies between infected females and their controls, although the average level of colonic MFs was relatively higher in the infected females ($9.15\pm3.78 \times 10^{-6}$) compared to that in the control females ($5.86\pm3.26 \times 10^{-6}$) (Figure 2A, Table I) . MF data showed that the average level of the colonic MFs were relatively higher than that of the cecal MFs in the control males, whereas opposite was true for the control females (Figure 2A, Table 1). In addition, the average levels of cecal and colonic MFs were relatively lower and higher in the control males compared to the control females, respectively (Figure 2A, Table 1). It is worth noting that all these differences in MFs were statistically insignificant. In addition, the average G>T MF was significantly higher in the cecum ($3.66\pm2.97\text{x}10^{-6}$) than that in the colon ($0.39\pm2.97\text{x}10^{-6}$) in the control females (Figure 2B, P=0.048) and also was relatively higher compared to the cecum of the control males without statistical significance (Figure 2B).

Reference

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