Intestinal Bacteria Modify Lymphoma Incidence and Latency by Affecting Systemic Inflammatory State, Oxidative Stress, and Leukocyte Genotoxicity

Mitsuko L. Yamamoto, Irene Maier, Angeline Tilly Dang, David Berry, Jared Liu, Paul M. Ruegger, Jiue-in Yang, Phillip A. Soto, Laura L. Presley, Ramune Reliene, Aya M. Westbrook, Bo Wei, Alexander Loy, Christopher Chang, Jonathan Braun, James Borneman, and Robert H. Schiestl

Abstract

Ataxia-telangiectasia is a genetic disorder associated with high incidence of B-cell lymphoma. Using an ataxia-telangiectasia mouse model, we compared lymphoma incidence in several isogenic mouse colonies harboring different bacterial communities, finding that intestinal microbiota are a major contributor to disease penetrance and latency, lifespan, molecular oxidative stress, and systemic leukocyte genotoxicity. High-throughput sequence analysis of rRNA genes identified mucosa-associated bacterial phylotypes that were colony-specific. Lactobacillus johnsonii, which was deficient in the more cancer-prone mouse colony, was causally tested for its capacity to confer reduced genotoxicity when restored by short-term oral transfer. This intervention decreased systemic genotoxicity, a response associated with reduced basal leukocytes and the cytokine-mediated inflammatory state, and mechanistically linked to the host cell biology of systemic genotoxicity. Our results suggest that intestinal microbiota are a potentially modifiable trait for translational intervention in individuals at risk for B-cell lymphoma, or for other diseases that are driven by genotoxicity or the molecular response to oxidative stress.

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Introduction

Ataxia-telangiectasia is an autosomal recessive disorder associated with high incidence of lymphoid malignancies, neurological degeneration, immunodeficiency, radiation sensitivity, and genetic instability (1). Approximately 30% to 40% of all patients with ataxia-telangiectasia develop neoplasia during their life (2); more than 40% of tumors are non–Hodgkin B-cell lymphomas, about 20% acute lymphocytic leukemias, and 5% Hodgkin lymphomas (3). Ataxia-telangiectasia is caused by biallelic mutations in the ATM gene. More than 600 different ATM mutations have been described (www.LOVD.nl/ATM). The ATM gene encodes a ~350-kDa protein, phosphoinositide 3-kinase, which is expressed abundantly in multiple tissues (4) and plays an important role in cell-cycle checkpoint control as well as repair responses to DNA double-strand breaks (DSB; ref. 5). Absence of functional ATM protein results in chromosomal breakage and rearrangements, aberrant V(DJ) recombination, and heightened sensitivity to radiation and chemicals with radiomimetic and prooxidant activity.

Although investigations into ataxia-telangiectasia have been greatly enhanced by the development of mouse models, disease penetrance in genetically identical mouse colonies at different laboratories can vary widely. Some ATM-deficient (Atm−/−) mice develop early lymphomas and have short lifespans (2–5 months; ref. 6), whereas others display dramatically delayed phenotypes, where 50% of the mice remain viable after 7 to 12 months (7, 8). Lifespan studies on inbred and mixed background mice have failed to show phenotypic differences (9), suggesting that other factors besides genetic diversity are contributing to disease penetrance. Environmental factors such as housing conditions and diet have been postulated to be contributing factors (10). In this study, we examined another potential contributor—the intestinal microbiota.

Intestinal bacteria have been implicated in several types of cancer. In animal models of colorectal cancer, lower incidences in germ-free or antibiotic-treated animals point toward intestinal microbes playing a causative role (11, 12). Helicobacter species have been associated with enhanced carcinogenesis including liver cancer, colon cancer, and mammary carcinoma (13). Bacterial products have also been associated with...
increased immune activation and inflammation as well as hyperplasia (14, 15). Conversely, probiotic formulations containing lactic acid bacteria have been shown to reduce the incidence of chemically mediated hepatocellular carcinoma and colon cancer in rats (16).

The objective of this study was to examine the role of intestinal bacteria in the penetrance of lymphoma in $\text{Atm}^{-/-}$ mice. We first show the effects of housing and intestinal bacteria on lifespan, lymphoma latency, oxidative stress, and systemic DNA damage (or genotoxicity). Next, high-throughput sequence analysis was used to identify mucosa-associated bacteria from animals reared in 2 distinct housing conditions, differing in intestinal microbiota and the aforementioned ATM-deficient traits. Finally, after determining that $\text{Lactobacillus johnsonii}$ was higher in abundance in our more cancer-resistant mouse colony, we showed its ability to reduce systemic inflammation and genotoxicity when administered to animals from our more cancer-prone colony.

**Materials and Methods**

**Animal housing and husbandry**

$\text{Atm}^{-/-}$ mice were obtained by intercrossing $\text{Atm}^{+/+}$ mice and identified by genotyping (see Supplementary Materials and Methods for additional details). Mice were housed under standard conditions in accordance with the Animal Research Committee at University of California Los Angeles (UCLA, Los Angeles, CA). Mice were housed under 2 types of specific pathogen-free (SPF) conditions, where either sterile (SPF-S) or nonsterile (SPF-N) food, water, and bedding were used. $\text{Atm}^{-/-}$ mice harboring restricted microbiota (RM) and conventional microbiota (CM) were created by rederivation as described in the study by Fujwara and colleagues (17) and by antibiotic treatment (18) followed by orogastric gavage of CM feces, respectively.

**Mouse longevity studies**

For the longevity studies, $\text{Atm}^{-/-}$ mice were kept until they developed signs of tumors or became sick according to ARC protocols, at which time they were euthanized, or if nonsymptomatic kept until they were found dead. Mice were sent to a veterinary pathologist for necropsy. Differences in longevity and lymphoma latency were analyzed using the log-rank test.

**Pun reversion assay**

Pun reversions were counted in mice that were 10-days-old. Pun reversions can be seen as a black spot on the fur and were counted as described previously (19). Statistics were done using the $\chi^2$ test.

**Oxidative stress**

Frozen blood was prepared for enzymatic measurement of the oxidized form of glutathione using the Bioxytech GSH: GSSG-H12 Kit Assay (Oxis). Reduced GSH and GSSG were determined separately by reaction with glutathione reductase. The colorimetric assay was conducted in triplicate for each blood sample from all male experimental $\text{Atm}^{-/-}$ mice. Data were expressed as the ratio of free GSH to GSSG.

**Micronucleus assay**

Micronuclei were examined in peripheral blood erythrocytes collected from ~6-month-old mice and stained with Wright-Giemsa (Sigma-Aldrich). At least 2000 erythrocytes were counted at $\times 100$ magnification as described previously (20). Statistics were done using Student $t$ tests.

**Alkaline comet assay**

DNA strand breaks were measured in peripheral blood cells using the alkaline comet assay. Blood was collected from the facial vein of mice approximately 6-months-old and diluted 1:1 with RPMI + 20% dimethyl sulfoxide (DMSO) for storage at $-80^\circ$C until the assay was conducted. The comet assay was basically conducted as previously described (20). Statistical analyses were done using Student $t$ tests.

**Bacteria community analyses of SPF-N and SPF-S mice**

Fecal pellets from $\text{Atm}^{-/-}$ mice (SPF-N and SPF-S) were collected and immediately snap-frozen in liquid nitrogen and stored at $-80^\circ$C. Nucleic acids from fecal pellets were purified using a phenol–chloroform extraction with bead-beating and a fragment of the 16S rRNA gene targeting the V6–V9 region of most bacteria was amplified with PCR primers 909F (5’-ACT-CACCAKGAATWGACGG-3’) and 1492R (5’-NTACCTTGTTAC-GACT-3’). The template was amplified and tagged with a sample-specific 8-nt barcode sequence via a 2-step, low cycle number, barcoded PCR protocol. Pyrosequencing was conducted on a GS FLX 454 sequencer at the Norwegian Sequencing Centre. Pyrosequencing reads were quality-filtered using LUCY and clustered into phylotypes at 97% identity using UCLUST. Taxonomic classifications were assigned, and alpha and beta diversity metrics were produced using the QIIME software package (21). The VEGAN package was used for permutational multivariate analysis of variance and the indi- species package was used to identify phylotypes that were indicators for a colony type; identifying indicators involves analysis of occurrence and abundance. To focus on abundant indicators, we considered only indicators that had an elevated relative abundance of at least 1% (e.g., 0.5%–1.5%). See Supplementary Materials and Methods for additional details.

**Bacteria community analyses of CM and RM mice**

Intestinal mucosa samples of CM and RM mice were obtained as described in the study by Presley and colleagues (22). DNA was extracted from these samples using the PowerSoil DNA Isolation Kit (MO BIO Laboratories), and a 30-second bead-beating step using a Mini-Beadbeater-16 (BioSpec Products). One hundred microliter PCR amplification reactions were conducted in an MJ Research PTC-200 thermal cycler (Bio-Rad Inc.). PCR primers targeted a portion of the 16S and 23S rRNA genes and the hypervariable intergenic region, with the reverse primers including a 12-bp barcode (Supplementary Tables S1a and S1b). PCRs contained: 50 mmol/L Tris (pH 8.3), 500 µg/ml bovine serum albumin, 2.5 mmol/L MgCl$_2$, 250 µmol/L of each deoxynucleotide triphosphate (dNTP), 400 nmol/L of forward PCR primer, 200 nmol/L of each reverse PCR primer, 4 µL of DNA template, and 2.5 units JumpStart Taq DNA polymerase (Sigma-Aldrich). Thermal cycling parameters were
were 94°C for 5 minutes; 35 cycles of 94°C for 20 seconds, 56°C for 20 seconds, and 72°C for 40 seconds, and followed by 72°C for 5 minutes. PCR products were purified using a MinElute 96 UF PCR Purification Kit (Qiagen). DNA sequencing was conducted using an Illumina HiSeq 2000 (Illumina, Inc.). Clusters were created using a template concentration of 2.5 pmol/L. One hundred base sequencing reads of the 3′-end of the amplicons and 7 base barcode reads were obtained using the sequencing primers listed in Supplementary Table S1c. De-multiplexing, quality control, and operational taxonomic units (OTU) binning were conducted using QIIME (21). OTUs were binned at 97% identity.

**L. johnsonii isolation and oral inoculation experiments**

*L. johnsonii* (strain LJ-RS-I) was isolated from RM wild-type mouse feces using Lactobacillus Selection Agar (BD). For the oral inoculation experiments, this bacterium was grown on LB agar supplemented with 2% glucose and 0.05% (wt/vol) cysteine at 37°C under anaerobic conditions. The strain was grown overnight and suspended in PBS. Before inoculation with *L. johnsonii*, CM Atm−/− mice were treated with 1 g/L ampicillin (Sigma-Aldrich), neomycin (Thermo Fisher Scientific), and metronidazole (Baxter) and 500 mg/L vancomycin (Hospira) in their drinking water for 1 week as described previously (18). Then, 10⁸ colony-forming units (CFU) of *L. johnsonii* was administered every other day by orogastric gavage to a group of 8 animals for 4 successive weeks; in addition, the drinking water for same group of animals contained 10⁸ CFU/mL of *L. johnsonii*. Fecal population densities of *L. johnsonii* were measured before, during, and after *L. johnsonii* administration, using a previously described sequence-selective quantitative PCR (qPCR) assay (22). After 4 weeks, mice were euthanized using 3% isoflurane and analyzed as described below.

**Gene expression by real-time-PCR**

RNA from peripheral blood mononuclear cells (PBMC) or tissue was collected by phenol–chloroform extraction followed by ethanol precipitation. Pellets were resuspended in nucleotide-free water, and RNA was treated with 200 units of DNase I (Ambion) for 2 hours at 37°C. RNA levels of TGF-β, interleukin (IL)-1β, IL-4, IL-10, IL-17, IFN-γ, and myeloid differentiation primary response gene 88 (*MYD88*) were determined from cDNA and compared as relative expression changes in *L. johnsonii*-treated animals compared with PBS-treated animals. Real time (RT)-PCR of gene expression was carried out using the TaqMan Gene Expression Assays System (Applied Biosystems). The level of gene transcription was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**Flow cytometry**

PBMCs or tissue cell pellets were resuspended in staining buffer. Lymphocytes were stained with CD3, CD4, CD19, and CD335 antibodies (Biolegend). Cells were fixed in 4% paraformaldehyde-PBS and scanned on a Becton Dickinson FACS Calibur flow cytometer. A minimum of 25,000 events were collected. Flow cytometry data were analyzed using Tree Star FlowJo software. Statistical analyses (Student *t* test) were used to determine if mean values were different (*P* < 0.05).

**Sequence data**

DNA sequence data have been deposited at the NCBI Short Read Archive under accession numbers SRA059288 and SRX256360.

**Results**

**Housing affects genetic instability, lifespan, and lymphoma latency of Atm−/− mice**

When the Schiestl laboratory moved their mice from Harvard University to UCLA in the year 2000, the median lifespan of their Atm−/− mice began to increase over a 7-year period from approximately 4 to 12 months (unpublished observations). To examine the influence of housing conditions on traits associated with ATM deficiency, we first compared Atm−/− and wild-type mice from 3 housing conditions: (i) Harvard housing and (ii and iii) UCLA housing in SPF conditions with either sterile (SPF-S) or nonsterile (SPF-N) food, water, and bedding. Genetic instability was assessed using the *p*⁰ reversion assay, which measures DNA deletion events repaired by homologous recombination (19). Atm−/− mice at Harvard had a significantly higher level of genetic instability compared with their wild-type littermates (Fig. 1A; ref. 19). As lifespan increased in the Atm−/− mice over 7 years under SPF-S conditions, DNA deletion frequency decreased to 10%. These levels are comparable to the wild-type mice (Fig. 1A), indicating loss of the abnormally high genetic instability that has been recognized as a hallmark of Atm−/− mice and human cells from ataxiantelangiectasia subjects (19, 23). Conversely, DNA deletion frequencies of Atm−/− mice housed under SPF-N conditions were 43%, a level even higher than those observed in the Harvard colony (Fig. 1A). Atm−/− mice housed under SPF-N conditions also had an increased level (*P* < 0.05) of DNA deletions compared with their wild-type littermates (Fig. 1A).

Focusing on the 2 UCLA Atm−/− mouse colonies, we then compared lifespan and lymphoma latency. SPF-N mice exhibited significantly shorter lifespans than SPF-S mice (44 vs. 51 weeks; Fig. 1B). Atm−/− mice primarily die from lymphomas (6), we examined the cause of mortality or morbidity. Although the incidence of lymphomas was similar between mice housed in SPF-N (74%) and SPF-S (76.5%) conditions, time of onset was significantly shorter in the SPF-N mice (Fig. 1C). The median age at which SPF-N mice died of lymphoma was 25 weeks compared with 60 weeks for SPF-S mice. These results are consistent with the observed increase in SPF-N genetic instability (Fig. 1A), likely the primary driver of accelerated carcinogenesis. The decreased lymphoma latency in SPF-N mice was also the major cause of the decreased lifespan as the lymphoma-free mice in the SPF-N and SPF-S conditions had similar lifespans (data not shown).

After observing the striking effects of housing on ATM-deficient traits, we examined the intestinal bacteria from fecal pellets of Atm−/− mice in these 2 UCLA mouse colonies. High throughput sequence analysis of bacterial 16S rRNA genes revealed that these 2 colonies harbor distinct microbial communities (Fig. 1D). An indicator analysis identified several species-level phenotypes whose populations were higher in CM versus RM mice, suggesting a possible causative role in
carcinogenesis. One correlate is a member of the Helicobacteraceae, which are bacteria known to promote cancer (Supplementary Fig. S1).

**Atm**−/−** mice with RM have an increased lifespan and decreased systemic genotoxicity and oxidative stress**

To further investigate the role of intestinal microbiota in lymphoma penetrance, we created **Atm**−/− mice harboring RM and CM, which were created by redereivation as described by Fujiwara and colleagues (17) and by antibiotic treatment (18) followed by orogastric gavage of CM feces, respectively. These models were chosen because CM and RM harbor distinct intestinal microbiota (17, 22, 24), and RM mice possess immunologic traits that can potentially influence lymphoma penetrance, including unusually high levels of cytolytic central memory CD8+ T cells that target neoplastic cells. In addition, as RM mice are physically isolated from other colonies, their immunologic traits have persisted for many years, providing a stable platform for our investigations.

Lifespan in the 2 mouse colonies was significantly different, with median survival of CM mice (31.1 weeks) being shorter than RM mice (45.6 weeks; Fig. 2A). Examination of systemic genotoxicity using different metrics showed that **Atm**−/− mice with CM microbiota exhibited higher levels of DNA damage than those with RM microbiota. Similar differences were not found between SPF-N and SPF-S mice. Clastogenic DNA damage, determined by the presence of micronuclei in peripheral blood erythrocytes, was nearly 85% higher in CM versus RM mice (Fig. 2B). DNA strand breaks, measured by the alkaline comet assay, were also higher in CM than in RM mice (Fig. 2C). Levels of DNA strand breaks were determined by the Olive tail moment (Fig. 2C) and confirmed by analysis of percent tail DNA at the 80th percentile (data not shown).

As oxidative stress can be produced by intestinal microbiota both directly and indirectly (25, 26), and oxidative stress is associated with both genotoxicity and carcinogenesis (27), we also assessed the antioxidant state of **Atm**−/− mice harboring CM and RM microbiota. Glutathione (GSH) is a major cellular antioxidant linked to several diseases including cancer. A metric of oxidative stress can be expressed as the ratio of GSH to GSSG (the dimeric oxidized form) in peripheral blood. We found that **Atm**−/− mice harboring CM microbiota had higher levels of oxidative stress (lower GSH/GSSG ratios) than RM.
mice (Fig. 2D), suggesting that it may also play a role in lymphoma penetrance in this model system.

The results presented in Fig. 1 indicate that sterility may play a role in carcinogenesis in $Atm^{-/-}$ mice; the results in Fig. 2 suggest that the intestinal microbiota is a major contributor to the phenotypic differences observed within and among isogenic $Atm^{-/-}$ mouse colonies (9).

Microbiota in CM and RM mice are distinct

A high-throughput sequence analysis revealed broad taxonomic differences in bacterial rRNA gene composition from the intestinal mucosa of CM and RM mice. We conducted this analysis because all prior investigations of CM and RM microbiota were relatively shallow in depth (17, 22, 24). A UniFrac analysis showed that the bacterial communities between CM and RM mice are distinct (Fig. 3A and B). The majority of rRNA gene sequences were classified into the phyla Bacteroidetes/Chlorobi (purple, Fig. 3C) and Firmicutes (light green, Fig. 3C). However, the most consistent phyla-level difference between CM and RM was in the Proteobacteria (sage green with asterisks, Fig. 3C), with the most statistically significant differences occurring in the $Atm^{-/-}$ mice (Fig. 3D). Although compositional differences were detected between mouse genotypes ($Atm^{-/-}$ vs. $Atm^{+/+}$) and intestinal regions (small intestine vs. colon), CM versus RM provided the most distinct groupings (Fig. 3A and B). A more comprehensive analysis is provided in Supplementary Table S2.

An analysis of the rRNA gene sequences at a finer taxonomic level revealed several OTUs from the intestinal mucosa that were differentially abundant in CM and RM mice (Fig. 4A). We consider differentially abundant OTUs worthy of further study because they might represent individual bacterial species participating in the enhancement or inhibition of systemic genotoxicity or other pertinent CM-RM metrics. The closest BLAST hit of one of the most abundant OTUs in many RM habitats was *Porphyromonas asaccharolytica* (98% identity to CP002689, 43% coverage; light blue with black asterisks, Fig. 4A). This phylotype was significantly more abundant in RM than CM $Atm^{-/-}$ mice in both the small intestine and colon, and it comprised at least 50% of the total sequencing reads from these habitats (Fig. 4B), warranting further investigation as a potential inhibitor of systemic genotoxicity. Similarly, another OTU exhibiting higher populations in RM habitats of $Atm^{-/-}$ mice (Fig. 4C; and maroon with white asterisks, Fig. 4A) was identical to *L. johnsonii* (100% identity to CP002464, 100% coverage). A phylotype that was more abundant in CM than RM
mice and therefore a candidate for causing the observed genotoxocity, included a member of the Helicobacteriaceae (Supplementary Table S3). A more comprehensive OTU analysis is provided in Supplementary Table S3.

**L. johnsonii decreases systemic inflammation and genotoxicity in Atm<sup>−/−</sup> mice**

To verify whether individual bacteria were contributing to the differential systemic genotoxicity detected in CM and RM mice, we carried out oral inoculation experiments. The high-throughput sequence analysis had identified numerous phylotypes exhibiting higher populations in RM than CM mice—a feature suggesting a possible beneficial role. We attempted to culture 2 of the most abundant of these (Fig. 4B and C) and successfully isolated and grew *L. johnsonii* from RM mice in pure culture; the rRNA gene sequence of our *L. johnsonii* isolate is identical to the one identified by the high-throughput sequence analysis (Fig. 4C).

CM Atm<sup>−/−</sup> mice were orally gavaged with 10<sup>9</sup> CFU of *L. johnsonii* every other day for 4 weeks. Fecal qPCR targeting...
*L. johnsonii* showed again the deficiency of this phylotype in CM *Atm*\(^{−/−}\) mice and also that periodic administration resulted in successful establishment and maintenance of high enteric levels (Fig. 5A). After this 4-week period, *L. johnsonii* but not the vehicle control (PBS) resulted in reduced micronucleus levels (Fig. 5B); however levels of DNA strand breaks were not significantly changed as measured by the COMET assay or γH2AX focus formation (data not shown). This was a time-dependent effect, as no difference was observed after only 1 or 2 weeks of treatment (Supplementary Fig. S2a).

As systemic genotoxicity is induced by innate inflammatory mediators (20, 28), we analyzed the effect of *L. johnsonii* administration on basal systemic inflammatory parameters in these mice. In the liver, *L. johnsonii* significantly reduced the abundance of both hepatic natural killer (NK) and T cells (Fig. 5C and D). A comparable reduction of these leukocyte subsets was also observed in the splenic and blood compartments (Supplementary Fig. S2b and S2c).

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Similar changes were also observed in the blood compartment (Supplementary Fig. S2d).

These findings indicate that short-term administration of the single RM-associated bacterium, *L. johnsonii*, recapitulated the reduction of systemic micronucleus formation observed in RM mice, a host effect associated with reduced systemic inflammatory activity.

**Discussion**

Our results are the first to show a relationship between intestinal microbiota and lymphoma onset. In addition, these investigations generated a detailed catalog of bacterial phylotypes that are differentially abundant between CM and RM mice, thereby providing candidates that may influence a wide range of traits from systemic genotoxicity (this study), oxidative stress (this study), colitis resistance (29), pathogen clearance (30) and selective reduction of marginal zone (MZ) B cells (31), plasmacytoid dendritic cells (pDC; ref. 17), and invariant natural killer (iNK) T cells (24). Moreover, we isolated one highly enriched species, *L. johnsonii*, from RM mice and subsequently showed its ability to decrease systemic inflammation and micronucleus formation in *Atm*−/− mice via oral inoculation experiments. Below, we propose several mechanistic hypotheses of how *L. johnsonii* might influence these important host traits.

First, *L. johnsonii* may reduce systemic genotoxicity by inhibition of basal intestinal inflammatory activity and its systemic sequelae. Our recent work revealed that intestinal inflammation-associated genotoxicity occurs not only locally but also systemically. Using either a model chemical inflammatory agent, dextran sodium sulfate, or immune-mediated genetic models to induce local intestinal inflammation, we found that systemic genotoxicity was elevated in peripheral lymphocytes, an effect amplified in *Atm*−/− versus wild-type mice (20). Moreover, such lymphocyte genotoxicity is particularly abundant in the B lymphocyte subset, the progenitor cell type for B-cell lymphoma (the predominant cancer in patients with ataxia-telangiectasia; ref. 32). Genetic and intervention studies have revealed that one factor is systemic dispersal of the intestinal cytokine TNFα, which permits genotoxicity in TNFα receptor–bearing lymphocytes remote from the intestinal compartment, in a process dependent on cell-autonomous, NF-κB and AP-1 induction of reactive oxygen and nitrogen species (RONS; ref. 28).

The present study documents the reduction of several systemic measures of inflammatory activity after *L. johnsonii*
restoration in CM Atm−/− mice. There are several known anti-inflammatory mechanisms of Lactobacillus spp. that may account for this host response, including products that directly modulate the NF-kB inflammatory program of mucosal epithelial and hematopoietic cell types, or indirectly by ecologically changing the composition or functional activity of enteric microbial community (33, 34). Our study also documented that enteric L. johnsonii resulted in a reduction in the abundance of hepatic and migratory (blood, splenic) NK and T cells; a reduction also concordant with their reduced levels in CM colonies (17, 31). The control of NK and T cells in these compartments is complex, integrating a diversity of chemotaxtractant and trophic cytokines. Lactobacillus spp. may either augment or reduce the production of these key cytokines, by parenchymal epithelial and hematopoietic cells, depending on differences in host compartment and Lactobacillus spp. (34, 35). The present study suggests that enteric (dietary) administration of L. johnsonii may represent a viable strategy to reduce inflammation-induced genotoxicity.

The ability of L. johnsonii to reduce pathogen-associated inflammation has been demonstrated in several prior in vivo investigations, including 2 involving H. pylori, a known cancer-promoting bacterium. In experiments examining H. pylori infection in C57BL/6 mice, oral administration of L. johnsonii over a 3-month period reduced the amounts of lymphocytic and neutrophilic infiltration of the lamina propria as well as proinflammatory chemokines (36). Similarly, L. johnsonii reduced both H. pylori populations and gastritis in Mongolian gerbils (37). A single inoculation of 1-day-old chickens with L. johnsonii inhibited the colonization and persistence of Clostridium perfringens, a poultry pathogen that causes necrotic enteritis (38). Finally, prior inoculation of gerbils with L. johnsonii prevented a persistent infection by the protozoan parasite, Giardia intestinalis (39).

Second, analysis of the literature suggests that L. johnsonii reduces immune-mediated oxidative stress and systemic genotoxicity by decreasing NF-kB activation. Linked to cancer and various inflammatory disorders, NF-kB is involved in managing responses to a wide range of potentially deleterious stimuli. Consistent with an agent that downregulates NF-kB activation, L. johnsonii and other CM-RM–associated microbiota may affect lymphoma penetrance by altering systemic genotoxicity caused by immune-mediated oxidative stress. Our results and others show that oral administration of L. johnsonii reduces both oxidative stress (40, 41) and systemic genotoxicity (this study). Mechanistically, there are precedents that microbial environments modulate cancer formation, in part, through oxidative stress mediated by inflammatory or carcinogenic bacterial metabolites on local epithelial cells (15, 42). For example, inflammation that accompanies H. pylori infection, S. haematum infection, or human inflammatory bowel disease is associated with elevated risk of stomach, bladder, or colon cancer, respectively (42). In an experimental system examining vaginal infection of mice with the bacterium Gardnerella vaginalis, oral administration of L. johnsonii reduced levels of proinflammatory cytokines, oxidative stress (iNOS), and activation of NF-kB (41). An important factor linking inflammation to neoplasia is genotoxicity from inflammation-associated oxidative products, created either in trans (due to local oxidative products of inflammatory cells), and cell autonomously (due to endogenous intracellular oxidative products induced by receptors to TNFα and other cytokines; refs. 43, 44). In disease models, enteric colonization of H. hepaticus elicits innate immune activation, which via TNF-α and iNOS induction yields host oxidative products required for neoplasia.

Finally, genomic analysis of L. johnsonii revealed several features that may contribute to a superior colonizing ability in the mucosa and to its ability to outcompete pathogens and other proinflammatory organisms (45). Attachment to the host is often a key feature of mucosa-associated microbes. Putative cell surface proteins in L. johnsonii have similarity to the mucin-binding protein (MUB) from L. reuteri (46). In addition, similarities to Fap1 and GspB from Streptococcus species suggest L. johnsonii encodes adhesive and fimbrial proteins, respectively (47, 48). L. johnsonii also produces a putative cell surface protein with similarity to an IgA protease, which could enable it to avoid a key host defense mechanism. In addition, L. johnsonii may inhibit potential microbial competitors by producing the bacteriocin Lactacin F and by increasing Paneth cell numbers, which are a host cell type that produces antimicrobial compounds (49–50).

In sum, these investigations build on prior work linking intestinal microbiota, via control of basal levels of inflammation and oxidative stress, to systemic genotoxicity and carcinogenesis. Given that intestinal microbiota is a potentially modifiable trait, these and related insights hold considerable promise for translational intervention of B-cell lymphoma and other diseases driven by immune-mediated oxidative stress and its resulting systemic genotoxicity. The translational promise of this approach, exemplified by the present study, suggests that simple interventions such as sustaining enteric levels of L. johnsonii may favorably shift microbial composition and function to reduce basal levels of genotoxicity in a manner that may reduce cancer risk in susceptible individuals, such as those bearing the ataxia-telangiectasia genotype.

Disclosure of Potential Conflicts of Interest

J. Braun and J. Borneman have ownership interest (including patents) as the co-inventor on provisional patent application by the University of California related to this article. R.H. Schiestl has ownership interest (including patents) in Microbio, Pharma Com. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: L. Yamamoto, I. Maier, L.L. Presley, J. Braun, J. Borneman, R.H. Schiestl


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Yamamoto, I. Maier, A.T. Dang, D. Berry, J. Liu, R. Reliene, A.M. Westbrook, J. Borneman


Writing, review, and/or revision of the manuscript: L. Yamamoto, I. Maier, A.T. Dang, D. Berry, J. Liu, P.M. Ruegger, J.-I. Yang, P.A. Soto, R. Reliene, A.M. Westbrook, A. Loy, J. Braun, J. Borneman, R.H. Schiestl

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I. Maier, P.M. Ruegger, J.-I. Yang, B. Wei, J. Braun, J. Borneman, R.H. Schiestl

Study supervision: I. Maier, J. Borneman, R.H. Schiestl
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References

37. Isobe H, Nishiyama A, Takano T, Higuchi W, Nakagawa S, Taneike I, et al. Reduction of overall Helicobacter pylori colonisation levels in the stomach of Mongolian gerbil by Lactobacillus johnsonii La1 (LC1) and


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