Microbial and host factors contribute to bloodstream infection in a pediatric acute lymphocytic leukemia mouse model

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ABSTRACT

Background: Hematological malignancies are the most common cancers in the pediatric population, and T-cell acute lymphocytic leukemia (T-ALL) is the most common hematological malignancy in children. Bloodstream infection (BSI) is a commonly occurring complication in leukemia due to underlying conditions and therapy-induced neutropenia. Several studies identified the gut microbiome as a major source of BSI due to bacterial translocation. This study aimed to investigate changes in the intestinal and fecal microbiome, and their roles in the pathophysiology of BSI in a pediatric T-ALL mouse model using high-throughput shotgun metagenomics sequencing, and metabolomics.

Results: Our results show that BSI in ALL is characterized by an increase of a mucin degrading bacterium (Akkermansia muciniphila) and a decrease of butyrate producer Clostridia spp., along with a decrease in short-chain fatty acid (SCFA) concentrations and differential expression of tight junction proteins in the small intestine. Functional analysis of the small intestinal microbiome indicated a reduced capability of SCFA synthesis, while SCFA supplementation ameliorated the development of BSI in ALL.

Conclusions: Our data indicates that changes in the microbiome, and the resulting changes in levels of SCFAs contribute significantly to the pathogenesis of bloodstream infection in ALL. Our study provides tailored preventive or therapeutic approaches to reduce BSI-associated mortality in ALL.

1. Introduction

Leukemia is a group of cancers affecting the hematopoietic system. Over 400,000 people are diagnosed with leukemia worldwide, and approximately 309,000 people die of this disease annually [1]. Hematological malignancies are the most common cancers during childhood, and leukemia comprises 30% of all pediatric cancers. Ninety-seven percent of childhood leukemias are acute, and acute lymphocytic leukemia (ALL) is the most frequently occurring pediatric leukemia, comprising 80% of childhood leukemias, with the incidence highest between 2-5 years of age [2].

Bloodstream infection (BSI) is a severe, often fatal complication in leukemia due to underlying conditions and therapy-induced neutropenia. BSIs are also the most concerning complications in the treatment of leukemia, as they lead to increased mortality rates, extended hospital stays and delays or dose reductions in chemotherapy [3, 4]. Despite the recognized importance and severity of infectious complications, little is known about the pathophysiology of endogenous infections. The intestinal microbiome has been recognized as a major source of BSI, as ALL [5] and therapy-induced neutropenia leads to increased intestinal permeability and subsequent translocation of bacteria [6, 7].

Several studies describe microbiome changes [8, 9, 10] in feces from ALL patients. However, chemotherapy and antibiotics treatments, as part of the standard protocol for most ALL patients, heavily influence microbiome composition. That is one of the possible reasons why findings in microbiome compositions differ significantly between studies [11]. In addition, clinical studies only analyze feces, which may not reflect the complex development of the microbiome in the small intestine and the colon [12]. We hypothesized that the compositions and metabolites of microbiome in both intestine and feces contribute together to the pathophysiology of gut bacterial translocation and subsequent BSI in pediatric patients with ALL. In this study, we applied shotgun metagenomics sequencing to a pediatric T-ALL mouse model to investigate the changes in composition, microbial metabolites and metabolic processes of the bacterial, fungal and viral microbiome by analyzing intestinal contents and longitudinal investigation of feces.

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2. Methods

2.1. Mice

Three-week-old female Nod/Scid mice were used in this study (Jackson Laboratories, Bar Harbor, ME, USA). All experimental procedures were approved by the University of Illinois College of Medicine at Peoria Institutional Animal Care and Use Committee review board (protocol number #1643433) and all methods and procedures were performed in accordance with the relevant guidelines and regulations. Leukemia was induced (n = 8 leukemic and n = 8 controls) as previously described [13] using 10^6 CCL-119 cells (lymphoblasts from a pediatric acute lymphocytic leukemia patient, from ATCC) in 50 μL PBS (pH = 7.84) and equal volume of PBS without cells for controls per mice. The average weight of the mice were 17.5 ± 1 g for controls, and 17.1 ± 2.4 g for leukemic mice (p = 0.6). Mice were housed in a barrier room with a 12-hr dark–light cycle, in cages (four mice/cage) with UV-treated shredded bedding. Water and regular chow were provided ad-libitum. Fecal and blood samples were freshly collected weekly from arrival (week 1) until the end of the experiment (week 5) with the exception of the xenograft (week 2). Blood samples were collected using tail nicking. Fecal DNA was extracted from 50 mg fecal material using the QiAmp DNA Stool mini kit, DNA from blood was extracted using the QiAmp DNA Blood mini kit. Mice were sacrificed at the end of the 5th week, small intestines (n = 8 in the control group, n = 6 in the leukemic group) and colons (n = 8 in the control group, n = 6 in the leukemic group) were removed, and intestinal contents were thoroughly washed with 1ml sterile PBS (pH = 7.84). DNA from intestinal content was extracted using the QiAmp DNA Stool mini kit, while DNA from intestinal tissue was extracted using the DNeasy Blood and Tissue kit (all from Qiagen). One small intestinal content from a leukemic mouse did not have sufficient DNA for sequencing following extraction. RNA was extracted from intestinal tissue using the Zymo Quick RNA miniprep kit. Euthanasia was performed with cervical dislocation in anesthesia induced by 5% Isothesia in 1.0 L/min oxygen in compliance with the ARRIVE guidelines. The following humane endpoints were used for immediate euthanasia: severe weight loss (>15% from starting weight), body condition score 2, ascites, moribund condition, and dehydration. The mouse model was validated on mouse blood and intestinal samples using TPOX [14].

2.2. Sequencing

Intestinal contents and feces were subjected to shotgun metagenomics, performed on an Illumina HiSeq 4000 instrument using 150 base pair reads with an average read number of over 13M reads per sample. No template control (sterile water) did not result in a library. Reads shorter than 30 base pair or with quality score (>10% of bases in a read) below 30 were discarded. RTG Core 3.4 was used to filter reads against the mouse genome (GRCh38-p6) and to map against bacterial, fungal and viral genomes by using the composition-meta-pipeline command with default settings [15]. The assay sensitivity and specificity were verified using ZymoBIOMICS Microbial Community Standard II with minimum sensitivity detected at relative abundance of 0.00089 (Suppl Figure 1).

MEGAHGIT was used for genome assembly [16], with d = 2 minimum multiplicity for filtering, and minimum contig length was set to 200. Genes were predicted and annotated using Prokka [17], with locus tag counter increment set to 1 using gff3 Genbank-compliant output (--gffver 3, -compliant). Prokka GenBank outputs were converted to KEGMapper for pathway analysis [18] using Prokka2KEGG (https://github.com/SilentGene/Bio-py/tree/master/prokka2kegg).

2.3. RT-qPCR and qPCR

Real-time qPCR to quantify microbial DNA and RT-qPCR to quantify expression changes in intestinal tissues was performed using a Corbett RotorGene 6000 instrument. The PowerUp SYBR Green qPCR and RT-qPCR master mix was used for setting up qPCR and RT-qPCR reactions with 200 nM primer concentrations. Bacteria were quantified using the S30F-806R 16S primers [19], and fungi were quantified using the ITS3-ITS4 primers on DNA extracted from 10 μl blood. Ooccludin, zonulin and claudin genes were quantified as described by Ohtsuki et al. [20] and MUC2 gene was detected using Qiagen Quantitect MinMuMC2_2,SG primers with 50 ng of intestinal RNA as a starting material. All PCR reactions were subjected to 2 min denaturation, then cycled at 95 °C for 20 s), 50 °C for 30 s and 72 °C for 30 s. Mouse GAPDH was used for relative quantification of MUC2, claudin, zonulin and ooccludin genes, and fold change was determined using the ΔΔct method [21]. Standard curves were created using Staphylococcus aureus and Saccharomyces cerevisiae genomic DNA (Sigma, St. Louis, MI, USA) with known concentrations. Samples for short-chain fatty acid (SCFA) concentrations used 50 mg feces prepared in 50 μl 1xPBS and 8% acetic acid and centrifuged at 14,000 RPM for 30 min, and measured in LC-MS (University of Illinois Biotechnology Center, Urbana-Champaign, IL, USA) using the Sciex LC/MS—6500 + Triple Quadrupole Mass Spectrometer. pH was measured using an Accumet AE150 instrument (Fisher Scientific, Pittsburgh, PA, USA). The presence of A. muciniphila was confirmed in fecal and intestinal samples using 5'-CAGACGTTGAAGTGGGAGC-3' forward, and 5'-CCTTGGGTGTGGTTCAGAT-3' reverse primers as described [22].

2.4. Statistical analysis

Statistical analysis was performed using Student’s t-test or ANOVA, with significance set to 0.05. Phylogeny was performed using Mega 7 using UPGMA default settings, and PCA graphs were created using STAMP [23]. Linear regression analysis by using the least squares method was selected for independent variables, with ANOVA to determine significance with significance level set to 0.05. PERMANOVA was used with Bray-Curtis similarity index in Primer7 (v0.21) with 999 permutations.

2.5. SCFA treatment

An additional 12 NOD/Scid mice were used for SCFA experiments (4 controls, 4 leukemic, 4 leukemic + SCFA treated). A mixture of sodium salts of 100mM acetate, propionate and butyrate dissolved in 100 μl water was administered via oral gavage to leukemic mice (n = 4) every second day following xenograft until the end of the experiment. Untreated leukemic mice (n = 4) and untreated control mice (n = 4) received sterile water via oral gavage.

3. Results

3.1. Bacterial microbiome

3.1.1. Akkermansia muciniphila abundance increased in ALL feces

Fecal compositions have been investigated from week 3 to week 5. At week 3, there was no significant difference in the abundance of A. muciniphila between control and ALL groups (Figure 1). The relative abundance of A. muciniphila significantly increased (Figure 2) from 0.33 (week 3) to 0.71 by week 5 (p < 0.01) in ALL. In the control group, there was no significant change in the relative abundance of A. muciniphila between weeks (week 3: 0.43, week 5: 0.44, p = 0.47). Differences between ALL and control fecal microbiome were investigated with principal component analysis showing clustering of ALL and control groups in week 5 (PERMANOVA p < 0.05, Suppl. Figure 2). In order to identify microbial biomarker signatures in feces in week 5, we used the linear discriminant analysis (LDA) effect size method [24]. A. muciniphila and Bacteroides oelicluplus were identified as overrepresented in ALL feces, while Alistipes and Clostridium species were dominant in the control group (Figure 3).
Whole genome sequences were assembled by mapping reads to \textit{A. muciniphila} ATCC BAA-835 (GCF_000020225.1) as reference resulting in an average > 99% coverage, with average coverage depth >15. Phylogenetic tree constructed using the whole genome sequences of \textit{A. muciniphila} showed a clustering of ALL samples from weeks 4 and 5, indicating changes in the species sequence with disease progression (Suppl. Figure 3).

To analyze these sequence differences, we conducted a Pfam domain search \cite{25} to identify potential protein domains that were acquired throughout the disease development (from week 3 to weeks 4 and 5). The most common protein domain which \textit{A. muciniphila} genomes leukemic mice gained was alpha/beta hydrolase (5 samples out of 8), along with AMP-binding enzyme (2/8), and NADH dehydrogenase (1/8).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Bacterial compositions of control and ALL feces from week 3 to week 5 show a significant increase of \textit{Akkermansia muciniphila} in ALL. Taxa with the 10 highest abundances are shown.}
\end{figure}
3.1.2. Compositional changes in the bacterial microbiome in the small intestinal and colonic content

Intestinal compositions have been investigated at the end of the experiment (week 5). A compositional graph of the small intestinal content with the 10 highest abundance taxa is shown in Figure 4A as determined by LeSe [24]. An increase in *Clostridia* spp. were identified as differentially abundant species in the control group (C. *scindens*, C. *methylpentosum*, C. *symbiosum*, Figure 4B).

A compositional graph of the colonic content in ALL and control groups with the 10 highest abundance taxa is shown in Figure 5A. Differentially abundant taxa showed an increase in the relative abundance of *Edwardsiella ictaluri* in ALL, and *Clostridia* and *Alistipes* spp. among others in the control group (Figure 5B). Principal component analyses showed partial clustering between leukemic and control samples both in the colon (PERMANOVA *p* < 0.05, Suppl. Figure 4) but not in the small intestine (PERMANOVA *p* > 0.05).

3.1.3. Differences in alpha diversity of the bacterial microbiome

Alpha diversity was measured by the Shannon index. There was no significant difference between ALL (1.14 ± 0.2) and controls (1.43 ± 0.4) in the small intestinal contents (*p* = 0.08). However, a significant decrease was identified in the colonic contents of ALL mice compared with controls (1.43 ± 0.6 in ALL, 2.27 ± 0.5 in controls, *p* = 0.01). A significant decrease was also measured in ALL in feces in week 5 (1.87 ± 0.4 in controls, 1.05 ± 0.4 in ALL, *p* < 0.01), but not in weeks 3–4.

3.1.4. SCFA levels decrease in the small intestine in ALL

Acetic acid, formic acid, propanoic acid and butanoic acid were measured in feces (from week 3 to week 5) and in colonic and small intestinal contents. All SCFA levels were significantly reduced in ALL compared with the control group in the small intestine (Figure 6), while no significant changes were found in the colonic contents or feces. Acetic acid levels were 3.02 ± 0.88 mM (mean ± SD) in the control group, and 1.28 ± 1.25 mM (*p* = 0.02) in the ALL group. Formic acid levels were 1.71 ± 0.52 mM in controls, and 0.75 ± 0.26 mM in the ALL group (*p* = 0.01). Propanoic acid levels were 0.42 ± 0.17 mM in controls, and 0.15 ± 0.18 mM in the ALL group (*p* = 0.03). Butanoic acid levels were 0.13 ± 0.04 mM in the control group, and 0.09 ± 0.01 mM in the ALL group (*p* = 0.05). pH values were measured of small intestinal and colonic contents dissolved in PBS (pH = 7.84). There was a significant increase in pH values in the small intestinal contents in ALL mice (pH = 8.33) compared with the control group (pH = 8.12, *p* = 0.001). In the colonic contents, there was no difference in pH values (control: 8.16; ALL: 8.12, *p* = 0.112).

3.1.5. Correlation between SCFA levels and the intestinal and fecal microbiome

*A. muciniphila* is a major producer of acetic acid [26], and its abundance was correlated with acetic acid levels in feces in ALL mice throughout weeks 3–5 (*n* = 18, R2 = 0.22, *p* = 0.05), but not in controls (*n* = 20, R2 = 0, *p* = 0.73) or in small intestinal or colonic content. *A. muciniphila* abundance was also correlated with propionate levels in feces in ALL (R2 = 0.32, *p* = 0.02), but not in controls, or small intestine and colonic content.

Butanoic acid plays an important role in gut barrier homeostasis and is an important source of nutrition for gut epithelial cells [27]. Regression analysis showed a significant correlation between *C. methylpentosum* and butanoic acid levels (R2 = 0.55, *p* = 0.05) in the small intestinal contents. *C. methylpentosum*, *C. scindens* and *C. symbiosum* abundances significantly decreased in the small intestinal contents in ALL (Figure 4), and the sum of the 3 *Clostridia* spp. abundances were also correlated with increased butanoic acid level (R2 = 0.59, *p* = 0.04). However, *Clostridia* spp. abundances were not correlated with other SCFA levels.

3.1.6. Functional analysis of small intestinal contents

In order to characterize their ability to synthesize SCFAs, we performed a functional analysis on the microbiomes of small intestinal contents. Our analysis shows that the microbiome from small intestines...
of ALL mice lack key enzymes for de novo fatty acid biosynthesis (Suppl. Figure 5). Only 20% of ALL samples expressed 3-hydroxyacyl-dehydrogenase (63% of controls), an enzyme that catalyzes the synthesis of fatty acids from acetyl-CoA. Sixty percent of ALL samples lacked 3-oxoacyl-reductase (present in 88% of controls), another key enzyme in the biosynthesis of fatty acids from acetyl-CoA as it reduces beta-ketoacyl-ACP substrates to beta-hydroxyacyl-ACP. S-malonyltransferase, an enzyme participating in fatty acid synthesis by transferring malonate from malonyl-CoA to acyl-carrier protein was present in 40% of ALL, and 100% of control samples (Suppl. Figure 5).

3.2. Bacterial translocation

We evaluated bacterial levels in the intestine via 16S qPCR of small intestine and colon tissue samples from ALL and control groups (Figure 7A). There was no significant change in the small intestine in ALL compared with controls in bacterial levels measured by 16S qPCR (25,699.8 ± 2,961 in control group vs 52,767.3 ± 36,447.7 in ALL group, p = 0.2) or in the colon (58,635 ± 26,128.4 in controls and 57,826.3 ± 38,413.8 in ALL, p = 0.49).

We further evaluated translocation of bacteria to the bloodstream by 16S qPCR of blood samples (Figure 7B). In ALL mice, bacterial copy numbers significantly increased from week 3 throughout the experiment (531.5 ± 133.1, mean ± SEM) compared with controls (167.5 ± 26.9, p < 0.01), as a probable result of bacterial translocation in ALL as shown earlier [5]. Moreover, 16S copy number showed a positive correlation with fecal *A. muciniphila* abundances (R2 = 0.34, p = 0.02) from week 4.

3.2.1. Host factors in bacterial translocation

As parts of the intestinal barrier, the mucus layer and the epithelial tissue protect the gut microbiome from translocation to sterile body sites [6]. MUC2 is a major mucin component secreted by goblet cells [28], while occludin, zonulin and claudin are core components of tight junctions in epithelial tissues [29]. We measured MUC2, claudin, zonulin and occludin levels in small intestine and colon tissues from control and ALL mice using RT-qPCR. Mouse GAPDH was used to measure fold change.

There was no significant change in MUC2 and claudin expression levels in small intestine or colonic tissues (p > 0.05). There was a significant increase of zonulin expression levels in the small intestinal tissue in the ALL group (ddCt = -0.77 between ALL and control groups, p < 0.01), indicating a 1.7-fold change [21]. Occludin expression levels showed a >600-fold decrease in the ALL group in the small intestine (ddCt = 9.3, p = 0.02). There was no significant difference of zonulin and occludin expression levels in the colonic tissue.

3.3. SCFA treatment ameliorates bacterial translocation

SCFAs play an important role in maintaining gut barrier function to prevent bacterial translocation [27, 28, 29], while our results indicated a depletion of SCFAs in the small intestine in ALL (Figure 6). We tested the hypothesis that SCFA supplementation may prevent bacterial translocation and differential expression of occludin in ALL by using 4 SCFA treated leukemic (LS), 4 leukemic (L), and 4 control (C) mice. Confirming our previous result of bacterial translocation, 16S qPCR of blood samples on weeks 4-5 indicated bacteremia (p < 0.05) in L mice, but was not significantly different in LS mice (Figure 7C). Occludin expression levels
decreased in leukemic mice ($\Delta\Delta C_t = 7.4$) similarly to our previous findings, and slightly improved in LS mice ($\Delta\Delta C_t = 5.2, p > 0.05$).

3.4. Fungal microbiome

Even though >99% of the sequencing reads belonged to bacteria, shotgun sequencing identified fungal species in intestinal contents and feces.

In the small intestinal contents, *Aspergillus flavus* (in 3 samples out of 5) and *Malassezia globosa* (2/5) were only present in ALL mice. *Botryotinia fuckeliana* were present both in controls (4/5) and in ALL mice (5/5).

Similarly to the small intestine, *A. flavus* (3/5) and *M. globosa* (2/5) were only present in ALL mice, and *Botryotinia fuckeliana* were present both in controls (2/5) and in ALL mice (5/5) in the colonic contents. In feces, no fungal species was present in more than a single sample.

*Aspergillus* spp were shown to increase gut permeability [30], therefore we investigated the correlation between the abundance of *A. muciniphila* and the presence of *A. flavus* (Suppl. Figure 6). In the small intestine, where *A. flavus* was present, the abundance of *A. muciniphila* was significantly higher (0.64 ± 0.22) than in samples without *A. flavus* (0.12 ± 0.25, $p = 0.04$). There was no correlation between *Aspergillus* and *Akkermansia* in the colon. In comparison, we investigated the correlation between *M. globosa* and *A. muciniphila*, but there was no significant correlation in *A. muciniphila* abundance between samples with or without *M. globosa* ($p = 0.15$ in small intestinal content, and $p = 0.3$ in colonic content) in ALL mice.
3.4.1. Fungal translocation

We investigated the possibility of fungal translocation from the intestinal contents to the intestinal tissue using universal fungal (ITS 3–4) primers. No fungi have been detected in any of the samples from the small intestine and colon tissues.

3.5. Viral microbiome

Encephalomyocarditis virus (EMCV) is a member of the Picornaviridae family, and it has been isolated from over 30 animal species and can be spread with feces [31]. EMCV was detected in both control (n = 4) and ALL (n = 4) groups in the small intestinal contents, and it was also detected in colon in control (n = 2) and ALL (n = 5) mice. In the feces, no viral species were detected in more than one animal.

4. Discussion

BSI is a significant complication and a major source of mortality in ALL due to underlying immunosuppression. The gut microbiome has been identified as a source of BSI via bacterial translocation as a consequence of an increased intestinal permeability. Understanding the role of the microbiome in the development of BSI in ALL is crucial for the discovery of preventive and therapeutic treatments. Our study highlights the complex intertwining mechanisms between the host and the microbiome contributing to the pathophysiology of BSI in ALL. We have previously shown that the damage of the gut-associated lymphoid tissue is one of the elements of BSI pathogenesis in ALL [5]. In this work, we further investigated alterations in the compositions and microbial metabolites in intestinal and fecal microbiome and gut barrier functions, which may play a role in the pathophysiology of BSI in ALL. The current study determined that changes in the bacterial and fungal microbiome, in SCFA levels and in the intestinal barrier may potentially contribute to bacterial translocation and subsequent BSI.

4.1. Changes in the intestinal and fecal microbiome

Microbiome changes were characterized by a decrease in Clostridia spp. in the leukemic group in the small intestine, colon, and feces (Figures 3, 4, and 5), and an increase of A. muciniphila in feces (Figure 2). Clostridia spp. are commonly present in the gut microbiome [11], and have been reported to increase [8] and decrease [9, 10] in ALL. We found that C. methylpentosum has decreased in both intestinal contents and feces in the ALL group compared with control mice (Figures 3, 4, and 5). C. methylpentosum is part of the normal microbiome, and can utilize...
pentoses and methylpentoses as fermentable substrates to produce SCFAs 
[32]. *C. symbiosum*, a bacterium which produces butyrate in the intestine 
in physiological conditions [33] also decreased in the ALL group in all 
sites (Figure 6). Butyrate improves gut barrier function by facilitating 
tight junction assemblies [34], and through regulating nuclear NF-xB and 
inhibition of histone deacetylase [28]. We detected a decrease in butyrate 
levels in the small intestinal contents of the ALL group, and the 
relative abundance of *Clostridia spp.* were correlated with the decrease in butyrate 
level (R² = 0.59, p = 0.04). In agreement with this finding, it has been previously shown that butyrate regulates tight junction protein 
production in epithelial cells [29, 34, 35], and upregulation of zonulin 
[36, 37] and downregulation of occludin [38, 39] leads to an increase in intestinal 
permeability. Therefore, our results combined with other 
sources suggest that the ALL microbiome led to alterations in expression 
levels of tight junction proteins occludin and zonulin via butyrate, and 
possibly other factors.

Functional analysis of the microbiome indicated that the reduced 
SCFA levels in the small intestine of leukemic mice are a result of an 
incomplete pathway of fatty acid synthesis (Suppl. Figure 5). Our pre-
liminary data suggest that gut barrier integrity and bacterial trans-
location in ALL can be ameliorated, but not reversed by SCFA 
supplementation. Optimization of composition, dosage and frequency of 
administration may further enhance the protective effect of SCFAs on gut 
barrier function [40].

*A. muciniphila* is part of the normal microbiome, and can contribute to 
an increase in gut permeability by degradation of the mucus layer [41]. 
However, it has also been shown to be beneficial in obesity [42] as a 
probiotic. *A. muciniphila* produces acetic acid from mucin to supply 
nutrition to goblet cells [26]. This was supported in this work as 
*A. muciniphila* abundance showed a linear association with acetic acid 
levels in feces. Our work also shows that *A. muciniphila* gained protein 
domains throughout ALL development, such as hydrolase and dehydro-
genase which may also contribute to mucin degradation [34] and gut 
barrier disruption. Therefore, this study suggests that the gut microbiome 
changes in ALL, which weakens the intestinal barrier via decreased 
butyrate production by *Clostridia spp.* [35, 43], increased abundance of 
*A. muciniphila*, and mucus degradation [26].

In accordance with this finding, linear regression showed a strong 
correlation between bacterial copy numbers in the blood and fecal 
*A. muciniphila* abundance. In addition, our results show that the abund-
ance of *A. muciniphila* in feces was positively correlated with the pres-
ence or absence of *A. flavus* in small intestinal content. *A. flavus* also 
negatively impacts the gut barrier integrity via mycotoxins [31], and this 
effect may be enhanced when both microbes are present.

### 4.2. Bacterial translocation

The intestinal barrier prevents the gut microbiome from exiting the 
lumen and invading sterile body sites. The intestinal barrier is composed 
of a mucus layer containing antimicrobial peptides, a monolayer 
columnar epithelium with tight junctions, and the gut-associated lymphocytic 
tissue (GALT). Tight junction proteins are key components of 
the intestinal barrier as they form a penetrable layer between epithelial 
cells. SCFAs produced by the gut microbiome provide nutrition to 
epithelial cells and help maintaining gut barrier integrity. Failure in the intestinal 
barrier function is crucial in the pathogenesis of BSI in ALL [5, 
6, 44].

It has been demonstrated previously that acute leukemia can result in 
leaky blood brain barrier due to impaired tight junctions [45]. Our re-
sults show that ALL resulted in bacterial translocation, dominantly 
localized in the small intestine (Figure 7A), which led to bacteremia 
(Figure 7B). We showed expression differences in 2 key tight junction 
proteins, zonulin and occludin in the small intestine, which may have 
contributed to bacterial translocation [46, 47]. The small intestine is a 
primary location for nutrient absorption, as well as for bacterial trans-
location through a disruption in the gastrointestinal equilibrium [6, 48].
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