The role of the intestinal microbiome in GVHD

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Acknowledgements

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Bone Marrow Transplant in Numbers

• 66,000 global transplants\(^1\) (2011-2012):
  - 36,000 autologous
  - 30,000 allogeneic

• 1 millionth patient transplanted in December 2012

• 22,222,377 donors\(^2\) currently registered worldwide

• 1,400 transplant centers worldwide

• Mostly for patients with leukemia, lymphoma or myeloma
Allogeneic Hematopoietic Stem Cell Transplantation

- High dose therapy with hematopoietic stem cell rescue
- Only established stem cell therapy
- Immunotherapy of cancer (graft-versus-tumor)
- Personalized/precision medicine
- Adoptive cell therapy
Causes of death after allogeneic BMT (2010-2011):

- **Primary disease**: 46-60%
- **GVHD**: 19-23%
- **Infection**: 17-21%
- **Organ failure**: 7-9%
- **Second malignancy**: 1%

Source: CIBMTR

Center for International Blood & Marrow Transplant Research
Pathophysiology of Graft-versus-host disease

Chemotherapy → Radiation → Antibodies → Graft → Host conditioning → Immune activation → Effector phase

Bacterial products

Donor T cell

Cytolytic granules

Donor T cell

Death ligands

Death receptors

Target cell

Inflammatory cytokines

Innate effector cell (NK/macrophage)

Host APC activation

Cell death

Acute GVHD

Skin

Digestive tract

Liver

Hematopoietic system

Graft-versus-tumor

Jenq and van den Brink, Nature Reviews Cancer, 2010
Pathophysiology of Graft-versus-host disease

Bacterial products

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Innate effector cell (NK/macrophage)

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Radiation
Antibodies

Host conditioning

Immune activation

Effector phase

Skin
Digestive tract
Liver
Hematopoietic system

Acute GVHD

Graft-versus-tumor

Jenq and van den Brink, Nature Reviews Cancer, 2010
An old question – can the flora impact on GVHD

Mortality and Gross Pathology of Secondary Disease in Germfree Mouse Radiation Chimeras

J. MIRIAM JONES, RAPHAEL WILSON, AND PATRICIA M. BEALMEAR

Mitigation of Secondary Disease of Allogeneic Mouse Radiation Chimeras by Modification of the Intestinal Microflora

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GRAFT-VERSUS-HOST DISEASE AND SURVIVAL IN PATIENTS WITH APLASTIC ANEMIA TREATED BY MARROW GRAFTS FROM HLA-IDENTICAL SIBLINGS

Beneficial Effect of a Protective Environment

Rainer Storb, M.D., Ross L. Prentice, Ph.D., C. Dean Buckner, M.D., R. A. Clift, F.I.M.I.L.S., Fred Appelbaum, M.D., Joachim Deeg, M.D., Kristine Doney, M.D., John A. Hansen, M.D., Mark Mason, Jean E. Sanders, M.D., Jack Singer, M.D., Keith M. Sullivan, M.D., Robert P. Witherspoon, M.D., and E. Donnalli. Thomas, M.D.
## Bacterial Taxonomy Guide

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Order</th>
<th>Genus</th>
<th>Gram stain</th>
<th>Facultative or obligate anaerobe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>Lactobacillales</td>
<td>Lactobacillus</td>
<td>+</td>
<td>facultative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridiales</td>
<td>Clostridium</td>
<td></td>
<td>obligate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blautia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Enterobacteriales</td>
<td>Escherichia</td>
<td>−</td>
<td>facultative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Klebsiella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidales</td>
<td>Bacteroides</td>
<td></td>
<td>obligate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barnesiella</td>
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</tr>
</tbody>
</table>
Comparison of baseline flora of mice and allo BMT patients

Mice

- Lactobacillales
- Other Firmicutes
- Bacteroidales
- Other Bacteria
Comparison of baseline flora of mice and allo BMT patients

Mice

Humans

Legend:
- Lactobacillales
- Other Firmicutes
- Bacteroidales
- Other Bacteria
Studying effects of GVHD on the flora: MHC-mismatched model B10BR into B6

1100 cGy

C57BL/6 (H2b)

B10.BR (H2k)

5 × 10^6 BM T cell-depleted ± 1 × 10^6 T cells

Harvest intestinal contents for analysis, in particular 16S deep sequencing
Effects of TBI/transplant and GVHD on the microbiota

- Day 14 ileal flora bacteria density (16S qPCR)
- No difference was seen in the large bowel

- TBI/transplant, without or with GVHD, results in a small increase in ileal flora bacterial density

- 3 experiments indicated by number
- Day 14 ileal flora
- Unweighted UniFrac

- TBI/transplant alone in absence of GVHD has only minor effects on the flora

- Ileal flora diversity at indicated time points during GVHD

- GVHD leads to reduced flora diversity
Despite variability in baseline flora, GVHD flora “signature” is highly reproducible and distinct: expansion of Lactobacillales
Effects of flora manipulation pre-BMT on GVHD

Overall survival (%)

Days post transplant

Allo BM (n=10)
Allo BM+T (n=19)

Day +14

Untreated
Ampicillin
Amp+Lacto

B10BR→B6
1100 cGy day 0
5 × 10^6 T cell depleted-BM day 0
2 × 10^6 CD5+ splenic T cells day 0
Effects of flora manipulation pre-BMT on GVHD

B10BR→B6
1100 cGy day 0
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2 × 10^6 CD5+ splenic T cells day 0
Ampicillin treatment days -21 to -14
Effects of flora manipulation pre-BMT on GVHD

B10BR→B6
1100 cGy day 0
5 × 10^6 T cell depleted-BM day 0
2 × 10^6 CD5^+ splenic T cells day 0

Ampicillin treatment days -21 to -7
Lactobacillus treatment days -12 to -2

Day +14
Untreated, Ampicillin, Amp+Lacto

Overall survival (%) vs. Days post transplant

p<0.02
p<0.004

Allo BM (n=10)
Allo BM+T (n=19)
Amp+Allo BM+T (n=18)
Amp+Lacto+Allo BM+T (n=19)
Does GVHD produce changes in humans as well as in mice?
GVHD vs non-GVHD patient selection

• 8 patients developed gut GVHD during transplant hospitalization, between days +18 to +21
• 10 control patients had no evidence for GVHD at day +100
# Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>non-GVHD</th>
<th>GVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patients</strong></td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>4 (40%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>6 (60%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td><strong>Median age (range)</strong></td>
<td>58 (32-70)</td>
<td>52 (26-64)</td>
</tr>
<tr>
<td><strong>Peripheral blood stem cell transplant</strong></td>
<td>4 (40%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td><strong>Cord blood stem cell transplant</strong></td>
<td>6 (60%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td><strong>Myeloablative conditioning</strong></td>
<td>7 (70%)</td>
<td>7 (87.5%)</td>
</tr>
<tr>
<td><strong>Non-myeloablative conditioning</strong></td>
<td>3 (30%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td><strong>NHL</strong></td>
<td>2 (20%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td><strong>Leukemia/MDS/MPD</strong></td>
<td>8 (80%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td><strong>Received therapy for febrile neutropenia</strong></td>
<td>10 (100%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td><strong>Received vancomycin</strong></td>
<td>10 (100%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td><strong>Received fluoroquinolone</strong></td>
<td>5 (50%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td><strong>Received metronidazole</strong></td>
<td>2 (20%)</td>
<td>2 (25%)</td>
</tr>
</tbody>
</table>
Human GVHD increases Lactobacillales representation within stool Firmicutes

18 recipients of T-cell replete allo BMT
8 developed GVHD ~D21, and 10 did not

Analysis of stool flora composition before and after onset of GVHD symptoms

Are there changes in the intestinal microflora that can predict the risk for GVHD?
Hierarchical clustering of allo-BMT patient samples shows biodiverse flora pre-transplant, followed by marked changes following transplant.

Taur, et al., CID, 2012
Decreased overall survival is associated with low diversity of intestinal microbial flora.
Diversity is associated with protection from lethal GVHD

- 64 patients from MSKCC
- Underwent allo BMT 9/09 to 10/12
- Ex vivo or in vivo TCD excluded
- Analyzed day 12 (+/- 4 days) stool by V1-V3 (454)
- Stratified by Shannon diversity index
Intestinal flora diversity is associated with protection from lethal GVHD

- 64 patients from MSKCC
- Underwent allo BMT 9/09 to 10/12
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- Analyzed day 12 (+/- 4 days) stool by V1-V3 (454)
- Stratified by Shannon diversity index

![Graph showing cumulative incidence of GVHD over years after BMT. The graph compares the bottom half (n=32) and top half (n=32) stratified by Shannon diversity index. The median incidence is 2.6 years with a p-value of 0.005.]
Bacterial genus associated with protection from lethal GVHD

- 64 patients from MSKCC
- Underwent allo BMT 9/09 to 10/12
- Ex vivo or in vivo TCD excluded
- Analyzed day 12 (+/- 4 days) stool by V1-V3 (454)

**Favors no GVHD-related mortality**
- Blautia
- Bacteroides
- Enterococcus
- Lactobacillus

**Favors GVHD-related mortality**
- Veillonella

**p value**
- p=0.05
Bacterial genus Blautia

- Named in 2008 in honor of Michael Blaut, a German microbiologist who studied human gut flora
- Group of species formerly classified as Clostridium or Ruminococcus
- Gram-positive, non-motile, coccoid or oval-shaped, obligately anaerobic
- Generally sensitive to vancomycin and metronidazole
Unbiased approach: Do any bacterial subgroups impact on risk for gut GVHD?

- 64 adult patients transplanted from 9/09 to 10/12
- Evaluable for gut GVHD (survival until day 30, engrafted)
- 41% high-risk, 41% intermediate-risk, 17% low-risk
- 46% acute leukemia, 44% NHL
- 22% ablative, 42% reduced intensity, 36% nonablative
- 58% unmodified peripheral blood, 39% double cord blood
- Stool sample closest to BMT day 10, +/- 4 days
- Analyzed for abundance of bacterial subgroups, by 16S rRNA gene sequencing
Second cohort

- 51 adult patients transplanted from 8/11 to 8/13
- 34% high-risk, 30% intermediate-risk, 36% low-risk
- 56% acute leukemia, 34% NHL
- 18% ablative, 48% reduced intensity, 34% nonablative
- 60% unmodified peripheral blood, 36% double cord blood
- Evaluable for gut GVHD (survival until day 30, engrafted)
- Stool sample closest to BMT day 10, +/- 4 days
- Analyzed for abundance of bacterial subgroups, by 16S gene sequencing
Evaluating predictive power of Blautia abundance for lethal GVHD

- 64 patients from MSKCC
- Underwent allo BMT 9/09 to 10/12
- Analyzed by V1-V3 (454)
Evaluating predictive power of Blautia abundance for lethal GVHD

Identification cohort
- 51 patients from MSKCC
- Underwent allo BMT 8/11 to 8/13
- Analyzed by V4-V5

Validation cohort
- 64 patients from MSKCC
- Underwent allo BMT 9/09 to 10/12
- Analyzed by V1-V3

Cumulative incidence (%)

Years after BMT
- p=0.04
- median=0.05%

Years after BMT
- p=0.01
- median=0.05%
Blautia is associated with improved outcomes

Overall survival

p=0.00002

Two cohorts combined
Blautia is associated with improved outcomes

- Overall survival: p=0.0002
- Treatment-related mortality (non-GVHD): p=0.9
- Relapse-related mortality: p=0.03
- GVHD-related mortality: p=0.001

- Two cohorts combined
Blautia’s ability to predict GVHD-related mortality – when to look?
Blautia is most predictive for reduced lethal GVHD shortly after day 10

- Two cohorts combined
- Divided by Blautia abundance cutoff of 0.0001 (bottom tertile)
- p value cutoff of 0.0017 using Bonferroni correction for multiple comparisons (30)
Do known GVHD risk factors impact on Blautia abundance?

- MUD vs MRD:
- Patient race: W/B vs Asian:
- CMV neg/neg vs any positive:
- Donor gender: M vs F:
- Donor/recipient gender: F/M vs non-F/M:
- Performance status: <90 vs 90-100: p=

Acknowledgement: Sean Devlin
Do known GVHD risk factors impact on Blautia abundance?

- MUD vs MRD: $p=0.62$
- Patient race: W/B vs Asian: $p=0.69$
- CMV neg/neg vs any positive: $p=0.93$
- Donor gender: M vs F: $p=0.24$
- Donor/recipient gender: F/M vs non-F/M: $p=0.85$
- Performance status: <90 vs 90-100: $p=0.62$

Acknowledgement: Sean Devlin
Blautia is abundant on admission but rapidly declines.

- Two cohorts combined
- Blautia abundance trend
- 95% confidence intervals shown in gray
Determinants of Blautia abundance: exposure to anaerobic antibiotics and TPN use

- Conditioning intensity
  - Myeloablative
  - Reduced intensity
  - Nonmyeloablative

- Exposure to antibiotics with anaerobic activity
  - Abx
  - No abx

- TPN use
  - TPN <10 days
  - TPN ≥10 days

• Two cohorts combined
Blautia is reduced upon exposure to anaerobic antibiotics

- 7 patients with frequent stool monitoring
- 1-2 days prior to treatment and 2-3 days following treatment

p=0.02
Reduced caloric intake leads to loss of Blautia and Clostridiales

Blautia abundance (proportion)

Humans

Mice

p=0.007

p=0.0002

Before

After

50 samples from 5 patients collected early during transplant hospitalization (range day -8 to +11)

Acknowledgements: Tatanisha Peets and Melissa Lumish
Effects of GVHD on Blautia and Clostridiales
Effects of GVHD on Blautia and Clostridiales

Humans

\[ p = 0.02 \]
Effects of GVHD on Blautia and Clostridiales

**Humans**

Before After

Abundance (%) $p=0.02$

**Mice**

Untreated BMT GVHD

Abundance (%) $p<0.001$
Bacterial re-introduction after microbiota injury reduces experimental GVHD

- Two experiments
- B10.BR into B6, BM + T
- Mice treated pre-BMT with cocktail of abx and indicated bacteria by gavage twice
- Acknowledgement: Silvia Caballero

- Single experiment
- B10.BR into B6, BM + T
- Mice treated pre-BMT with cocktail of abx and indicated bacteria by gavage twice
- Acknowledgement: Kenya Honda
Possible anti-inflammatory mechanism of Blautia
Possible anti-inflammatory mechanism of Blautia

- Clostridial strains support colonic Tregs and produce short-chain fatty acids (Honda, 2011, 2013)
- Administration of acetate, propionate or butyrate increase colonic Tregs (Garrett et al, 2013)
- Administration of butyrate induces Foxp3 via HDAC inhibitor effects on CD4T cells and dendritic cells (Rudensky et al, 2013, Ohno et al, 2013)
Association of Blautia abundance with short-chain fatty acid concentrations

- Acetate concentration
  - p=0.04

- Butyrate concentration
  - p=0.02

Blautia abundance
Blautia introduction after antibiotics restores short-chain fatty acid levels

Short-chain fatty acid content (stool)

Absorbance (400 nm)

Retention time (min)

- butyrate
- propionate

Acknowledgements: Nick Arpaia and Paul DeRoos
Prebiotics
• Encouraging eating
• Gastric nutritional supplementation
• Flora-targeted nutritional supplements

Probiotics
• Re-introducing endogenous flora (autologous fecal microbiota transplant)
• Re-introducing selected bacteria with beneficial potential

Antibiotics
• Selecting antibiotics that spare bacteria with beneficial potential

Postbiotics
• Identifying and introducing bacterial metabolites that mediate the anti-inflammatory effects
Strategies to prevent GVHD: prebiotics and postbiotics

- Three experiments
  - B6 into 129S1
  - Mice treated with sodium acetate drinking water beginning 1 week pre-BMT

- Two experiments
  - B10.BR into B6, BM + T
  - Mice treated with xylose drinking water beginning 1 week pre-BMT
Strategies to prevent GVHD: prebiotics and postbiotics

- Two experiments
- B10.BR into B6, BM + T
- Mice treated with xylose drinking water beginning 1 week pre-BMT

- Three experiments
- B6 into 129S1
- Mice treated with sodium acetate drinking water beginning 1 week pre-BMT
Antibiotic strategies to prevent GVHD: selecting those that spare Clostridiales

**Aztreonam**

**Cefepime**

**Imipenem**

**Metronidazole**

Antibiotic Treatment; BID SC x2 daily, 3 days at 100 mg/kg

* Mice treated with TAZ/PIPC had no amplifiable bacterial DNA

SC ↓↓↓↓↓ Harvest

1 2 3 4
Antibiotic strategies to prevent GVHD: selecting those that spare Clostridiales

Antibiotic Treatment; BID SC x2 daily, 3 days at 100 mg/kg

* Mice treated with TAZ/PIPC had no amplifiable bacterial DNA
Antibiotic strategies to prevent GVHD: selecting those that spare Clostridiales

Antibiotic Treatment; BID SC x2 daily, 3 days at 100 mg/kg

* Mice treated with TAZ/PIPC had no amplifiable bacterial DNA
Clostridiales eliminating antibiotic Imipenem worsens GVHD survival

C57BL/6 -> 129
1000 cGy, $1 \times 10^6$ T cells
100 mg/kg s.c.
Day 10~24, 3 times/week
$n = 10$
Lethal GVHD and anaerobic antibiotics

- 931 patients from MSKCC
- Underwent allo BMT 1991 to 2013
- Ex vivo or in vivo TCD excluded
- No oral antibiotic prophylaxis
- Stratified by exposure to antibiotics with anaerobic coverage during transplant hospitalization
Lethal GVHD and anaerobic antibiotics

- 931 patients from MSKCC
- Underwent allo BMT 1991 to 2013
- Ex vivo or in vivo TCD excluded
- No anaerobic antibiotic prophylaxis
- Stratified by exposure to antibiotics with or without anaerobic coverage during transplant hospitalization
Lethal GVHD and anaerobic antibiotics

• To address a “sick bias”, specifically looked at subset of patients, all treated for neutropenic fever
• Stratified by empiric antibiotics with or without anaerobic coverage during transplant hospitalization
Lethal GVHD and anaerobic antibiotics

- To address a “sick bias”, specifically looked at subset of patients, all treated for neutropenic fever
- Stratified by empiric antibiotics with or without anaerobic coverage during transplant hospitalization

![Graph showing cumulative incidence of GVHD-related mortality](image)

- Allo BMT patients treated for neutropenic fever
- Antibiotics with anaerobic coverage (n=231)
- Antibiotics without anaerobic coverage (n=60)

p=0.04
Summary

• GVHD is associated with:
  – Increase in Lactobacillales in gut flora
  – Loss of microbial diversity
  – Loss of Blautia

• Potential therapies to decrease gut GVHD
  – Prebiotics that support Clostridiales
  – Probiotic therapy with Blautia or pre-BMT flora
  – Short chain fatty acids ("postbiotics")
  – Antibiotics which spare Clostridiales
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