Gut microbiota from high risk men who have sex with men drive immune activation in gnotobiotic mice and in vitro HIV infection

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Microbiome differences associated with MSM and HIV

- **HIV- MSW**: Bacteroides
- **HIV- MSM**: Prevotella, H. biformis, Desulfovibrio
  - Peripheral and colonic T cell activation
  - **??** HIV infection?
- **HIV+ MSM**: Prevotella, T. sanguinisis
  - Induces higher immune activation than MSM-associated microbiome in vitro
    - (Neff 2018 eBioscience)
    - (Armstrong 2018 BioRxiv)
Fecal transplant to gnotobiotic mice

MSW (HIV-)

MSM (HIV-)

MSM (HIV+)

21 days

gut tissue: immuno-phenotype

feces: 16S seq
MSM-associated composition differences are transferred.
Colonization fidelity consistent across groups

Donor-recipient unweighted unifrac distance

% Engraftment

Relative vector distance

% sequence variants transferred

HIV:  - - +

MSW  MSM  MSM

Colonization fidelity consistent across groups
Compositional changes following transfer to mice
Specific bacterial differences with MSM recapitulated in mice

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Recipients</th>
<th></th>
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<th></th>
<th>Donors</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HIV- MSW</td>
<td>HIV- MSM</td>
<td>HIV+ MSM</td>
<td></td>
<td>HIV- MSW</td>
<td>HIV- MSM</td>
<td>HIV+ MSM</td>
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<tr>
<td><strong>Increased with MSM</strong></td>
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<tr>
<td>Desulfovibrio desulfricans</td>
<td>0.00E+00</td>
<td>6.49E-03</td>
<td>5.63E-03</td>
<td></td>
<td>0.00E+00</td>
<td>2.42E-04</td>
<td>3.12E-04</td>
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<tr>
<td>Holdemanella biformis</td>
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<td>1.27E-03</td>
<td>1.86E-03</td>
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<td>6.29E-04</td>
<td>1.63E-02</td>
<td>1.41E-02</td>
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<tr>
<td>Howardella ureilytica</td>
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<td>1.61E-04</td>
<td>2.58E-04</td>
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<td>0.00E+00</td>
<td>1.38E-04</td>
<td>3.90E-05</td>
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<td><strong>Decreased with MSM</strong></td>
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<tr>
<td>Clostridium leptum</td>
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<td>1.45E-04</td>
<td>7.52E-04</td>
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<td>1.63E-04</td>
<td>2.38E-04</td>
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<tr>
<td>Bacteroides uniformis</td>
<td>1.39E-01</td>
<td>5.68E-02</td>
<td>4.31E-02</td>
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<tr>
<td>Flavonifractor plautii</td>
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<td>2.54E-04</td>
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<td>1.21E-04</td>
<td>6.63E-04</td>
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</tr>
</tbody>
</table>
Immune activation is higher in recipients of HIV- and HIV+ MSM
Immune activation in mouse recipients and donors correlate

Donor vs Recipient CD8 T cell activation

Donor CD4 T cell gut homing vs Recipient gut CD4 T cell activation

Mouse colon
%CD69+ CD4 T cells

Spearman r = 0.521
p = 0.048

Mouse ileum
%CD69+ CD8 T cells

Spearman r = 0.313
p = 0.0492

HIV-neg donor blood
%CD103+ CD4 T cells

Donor vs Recipient
CD8 T cell activation

HIV-neg donor blood
%CD38+ HLADR+ CD8 T cells
HIV- and HIV+ MSM microbiota stimulate in vitro HIV infection

In vitro HIV infection following FBC stimulation

Media HIV-
HIV-
HIV+

HIV-1 gag+

FBC:

* *

% HIV-1 gag+

Uninfected
Infected + Media

Infected + MSM (HIV-)
bacteria
Infected + MSM (HIV+)
bacteria

SSC-A

FSC-A

HIV-1 gag

SSC-A

HIV-1 gag

0 10 20 30 40 50

HIV-1 gag+

FBC: Media HIV- MSM HIV- MSM HIV+ MSM
### Consistent microbial correlates across recipients and donors

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Recipients</th>
<th>Donors</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ileum T cell activation</td>
<td>Colon T cell activation</td>
<td>Blood T cell activation</td>
</tr>
<tr>
<td><strong>Negatively correlated</strong></td>
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<tr>
<td>Akkermansia muciniphila</td>
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<td>-0.40</td>
<td>-0.36</td>
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<td>Oscillibacter ruminantium</td>
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<td>-0.42</td>
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<tr>
<td>Bacteroides uniformis</td>
<td>-0.27</td>
<td>-0.42</td>
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<tr>
<td>Pseudoflavonifractor capillosus</td>
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<td>-0.48</td>
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<tr>
<td><strong>Positively correlated</strong></td>
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<tr>
<td>Catenibacterium mitsuokai</td>
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<tr>
<td>Oscillibacter valericigenes</td>
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<tr>
<td>Howardella ureilytica</td>
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<tr>
<td>Desulfovibrio piger</td>
<td>0.25</td>
<td>0.40</td>
<td>0.44</td>
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<tr>
<td>Clostridium leptum</td>
<td>0.42</td>
<td>0.38</td>
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<tr>
<td>Holdemanella biformis (1)</td>
<td>0.31</td>
<td>0.44</td>
<td>0.61</td>
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<tr>
<td>Holdemanella biformis (2)</td>
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<td>0.33</td>
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<tr>
<td>Butyricimonas faecihominis</td>
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</tbody>
</table>
Addition of a single bacteria to MSW microbiome increases immune activation.
P. distasonis reduces immune activation by HIV+ microbiome

Colon CD4+ T cell activation

$p = 0.051$
Summary

• HIV- MSM donors have higher blood T cell activation and CD103+ T cell frequencies than HIV- MSW donors

• Fecal transplant results in engraftment of MSM-associated microbiome differences that maintain a distinct composition in mice

• These differences result in increased gut immune activation in mouse recipients (which correlated with donor immune activation), and in vitro HIV infection

• Addition of a single bacteria to the microbiome from HIV-negative MSW or HIV-positive MSM can respectively increase or decrease immune activation in mouse recipients