Gut Microflora Influences Pathology in the Kawasaki Disease (KD) Vasculitis Mouse Model

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Intestinal Microbiota and Disease

Health

- Immune system
- Metabolism

Disease

- IBD (inflammatory Bowel disease)
- Allergy
- Arteriosclerosis
Changes in intestine of KD patients

A wide variety of bacteria was isolated from jejunal biopsies in the acute phase of KD as compared with those from control children. KD patients had a significantly lower incidence of Lactobacillus than disease control patients.

Macrophage/dendritic cells and activated CD4⁺ T cells were significantly increased in the lamina propria of KD patients in the acute phase.

Characteristic profile of intestinal microflora in Kawasaki disease

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KD patients had a significantly lower incidence of Lactobacillus than disease control patients
Coronary arteritis in mice following the systemic injection of group B Lactobacillus casei cell walls in aqueous suspension.

Coronary Arteritis

70-80% C57BL/6

Lactobacillus casei cell wall extract (LCWE)

Day 3
Mononuclear cells in adventitia

Day 14
Focal, asymmetric invasion of arterial wall, Lymphocytic

Day 28
Circumferential lesion with marked proliferation of intima/media

Day 56
Fibrous tissue, marked narrowing

Control

LCWE
LCWE-induced KD mouse model develops abdominal aorta aneurysms

Control

LCWE

Maximal aorta diameter (mm)

** P<0.01

Presented in The 32nd Kawasaki Disease Meeting (Kanto-area), Japan Red Cross Hospital in Tokyo, Dec 7th 2013
NOD2\(^{-/-}\) and Dectin-1\(^{-/-}\) mice are protected from LCWE-induced KD vasculitis

**Bacteria**

Peptidoglycan

**Fungi**

β-1,3-glucan

**NOD2**

**Dectin-1**

**Inflammatory cytokines**

<table>
<thead>
<tr>
<th>Heart vessels</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0/12</td>
</tr>
<tr>
<td>NOD2(^{-/-})</td>
<td>2/7</td>
</tr>
<tr>
<td>Dectin-1(^{-/-})</td>
<td>11/12</td>
</tr>
</tbody>
</table>
Germ-Free mice develop markedly decreased cardiovascular lesions in KD mouse model  

**SPF** (n=13) 
Germ Free (n=13) 

**Day7** 

Heart Abdominal Aorta 

SPF GF 

Heart vessels 

Inflammation score 

**P<0.01** 

**P<0.05** 

Incidence (%) 

10/13 
4/13 

(Coronal lesions)
Germ-Free mice develop markedly decreased cardiovascular lesions in KD mouse model (abdominal aorta lesions)
Depletion of commensal fungi and bacteria with fluconazole and antibiotic treatment

Anti-fungal drug (Fluconazole; Fluc) and/or Antibiotics cocktail (Abx) (Neomycin, Ampicillin, Vancomycin, Metronidazole)

Fungi amount

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fluc</th>
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</thead>
<tbody>
<tr>
<td>Fungal ITS1-2 DNA (Relative to total DNA)</td>
<td>1.00</td>
<td>0.50</td>
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</tbody>
</table>

* P<0.05

Bacteria amount

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Abx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial 16S rDNA (Relative to total DNA)</td>
<td>5.00</td>
<td>1.00</td>
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</table>

*** P<0.001
Fluconazole and/or antibiotics treatment decreased cardiovascular lesions in KD mouse model.
Fluconazole and/or antibiotics treatment decreased cardiovascular lesions in KD mouse model.
Intestinal permeability and disease development

Gut Microflora

Bacteria

Fungi

Products

Metabolites

Intestinal barrier dysfunction

Translocation of intestinal microflora

Inflammatory diseases

Host
LCWE injection increases intestinal permeability

LCWE (i.p.)

FITC-Dextran (p.o.)

Serum

* P<0.05

FITC-Dextran (ug/ml)

Control  8 hr  20 hr

Hours after LCWE injection
Conclusions

- LCWE-induced cardiovasculitis was decreased in germ free mice
- Depletion of gut commensal fungi and bacteria diminished KD vasculitis
- LCWE injection increased intestinal permeability

? Role of microbiome in KD pathogenesis, new diagnostic/therapeutic strategies
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