Dysbiosis and zonulin upregulation alter gut epithelial and vascular barriers in patients with ankylosing spondylitis

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BACKGROUND
ankylosing spondylitis (AS)

- Bechterew's disease
- Chronic inflammatory disease
- Prevalence: 0.1-2%
- Men > women
- Seronegative spondylarthritis
- Mainly affects spine, sacroiliacal joints
- Peripheral joints, eyes, bowel involvement
- Genetic (HLA-B27) and environmental factors
ankylosing spondylitis (AS)

ankylos = stiffening, spondylos = vertebra, -itis = inflammation
Zonulin

Dysbiosis

Tight junction proteins
- claudin 1
- claudin 4
- occludin
- zonula occludens 1
AS and the gut

• Dysbiosis
• Subclinical gut inflammation
• Cause or consequence?
Study aim

• tissue localization of bacteria in the gut of patients with AS
• gut-epithelial barrier and gut vascular barrier (GVB) integrity.
• Role of zonulin
  • modulating intestinal permeability
  • Monocyte activation
• Associations with systemic inflammation?
METHODS
patients

- Ileal biopsies
  - 50 patients with AS
  - 20 healthy controls
- Interleukin (IL)-8 in the tissue
- Histologically divided:
  - normal
  - Acute inflammation
  - Chronic inflammation
- RT-PCR
- Lactulose/ mannitol ratio test for gut permeability
bacteria

• Ileal biopsies from AS patients and controls
• Cultures for aerobic and facultative anaerobic bacteria

• Bacteria isolated from 5 AS patients
• Incubated with Caco-2 epithelial cells
• Modulation of zonulin mRNA assessed by RT-PCR
Sera

- Levels of lipopolysaccharide (LPS), LPS-binging protein (BP), intestinal fatty acid-BP (iFACBP) and zonulin proteins
- Analysed in sera of all AS patients and controls
- In vitro effects of recombinant human zonulin
  - on human umbilical vein endothelial cells (HUVECs)
  - On peripheral monocytes
Human leukocyte antigen (HLA)-B27 TG rats

• 5 HLA-B27 TG rats, 5 WT
• Ileal samples
RESULTS
Intestinal gut inflammation in AS

• IL-8 overexpressed in AS with chronic inflammation

• 50 AS patients
  • N=20 no gut inflammation
  • N=11 acute gut inflammation
  • N=19 chronic gut inflammation
Intestinal bacteria

- Adherent and invading bacteria present in AS (35/50), not in controls.
RT-PCR ileal samples

Downregulation of tight junction proteins in AS
Zonulin in ileal samples

(G) Elevated Zonulin levels in AS chronic (RT-PCR); (H) AS, (I) control
Zonulin in ileal samples

(K) The number of zonulin positive cells was significantly and directly correlated with the number of IL-8 positive cells.
Caco-2 cells incubated with AS- bacteria
Gut vascular barrier (GVB) proteins in ileal samples

Reduced relative m-RNA levels of VE-cadherin (A), junctional adhesion molecule (JAM)-1 (B) and PV1 (C) were assessed by RT-PCR in AS
Serum levels of zonulin were evaluated in 20 patients with AS and 20 controls (C) and correlated with LA/MA ratio (D).
In vitro effects of zonulin on human umbilical vein endothelial cells (HUVECs)

Downregulation of occludin (A) and VE-cadherin after zonulin treatment
In vitro effects of zonulin on peripheral monocytes

zonulin: CD163 binding motif

percentage of CD163+c-MAF+ cells increased after incubation with AS PBMCs = M2 polarised macrophages
Elevated serum levels of lipopolysaccharide (LPS) (A), LPS-binding protein (BP) (B) and intestinal fatty acid-BP (iFABP) (C) in AS
AS and CD14+

CD14= on monocytes/macrophages, involved in LPS binding

High LPS downregulates CD14

Percentages of CD14+ cells is reduced in peripheral blood mononuclear cells (PBMCs) from patients with AS.
Percentages of CD14+ HLADR+ cells is reduced in peripheral blood mononuclear cells (PBMCs) from patients with AS.
AS and CD14+

Effects of monocyte stimulation with LPS alone, sCD14 alone or sCD14+LPS on CD14+ and CD14- monocytes
HLA-B27 rats ileal samples

- Increased IL-23 expression
- Occludin downregulation
- Presence of adherent bacteria

→ Antibiotic treatment
Discussion

• Adherent and invading bacteria in ileum of AS patients associated with alteration of epithelial barrier and the GVB

• Zonulin- dependent leaky epithelium and endothelium in AS ileum

• → translocation of zonulin and bacterial products into the bloodstream

• → inducing modulation of innate immune system in AS

• E. coli, Prevotella spp.
My opinion

Pros:
• Assessed several aspects- descriptive, functional
• Human samples

Cons:
• Tipping errors, incorrect legends
• Bacterial score?

• Link bacteria- zonulin?
• CD14+HLADR+/ LPS??