

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

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# BACKGROUND

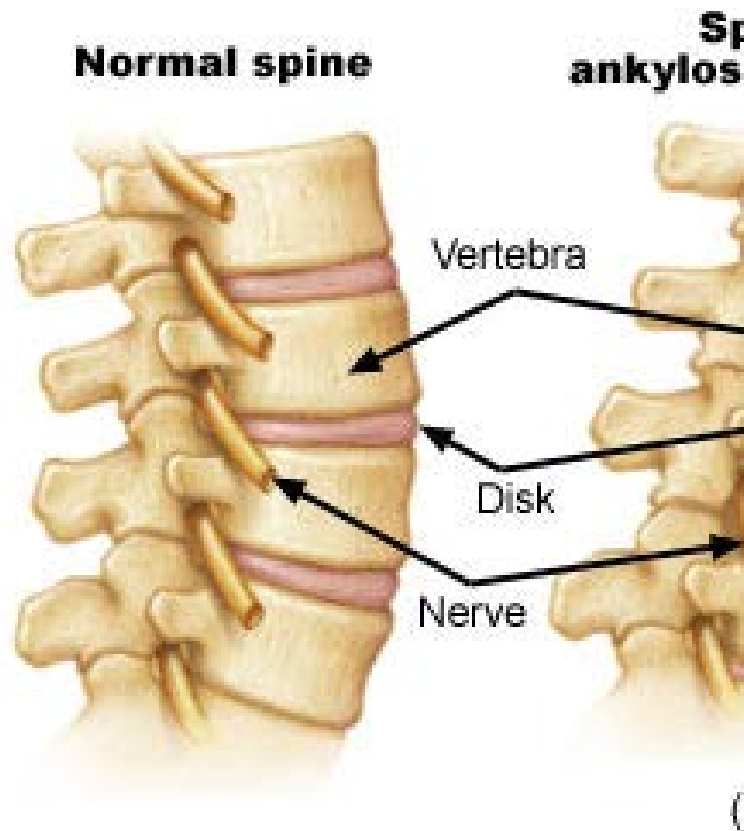


# ankylosing spondylitis (AS)

- =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

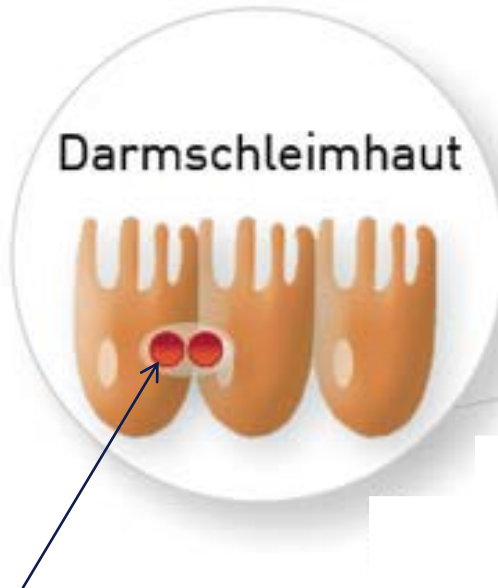
# ankylosing spondylitis (AS)

*ankylos* = stiffening, *spondylos* = vertebrae



# 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- binding 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



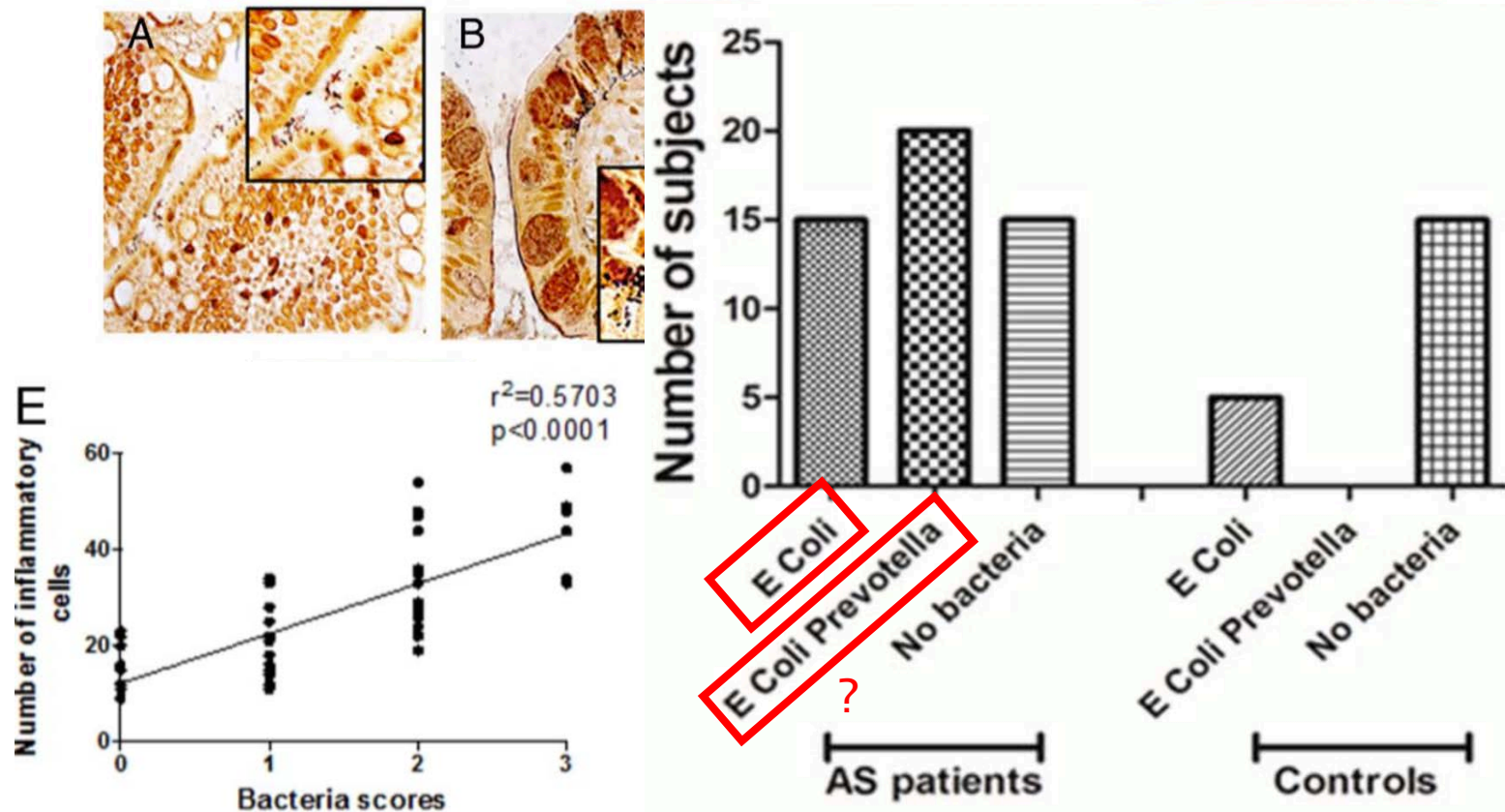
# Intestinal gut inflammation in AS

- 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

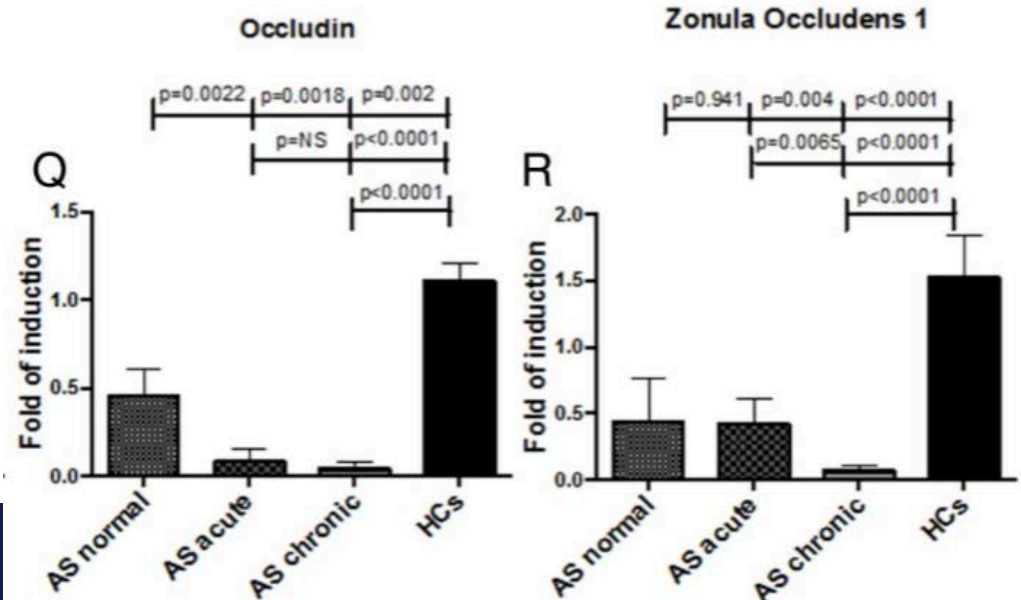
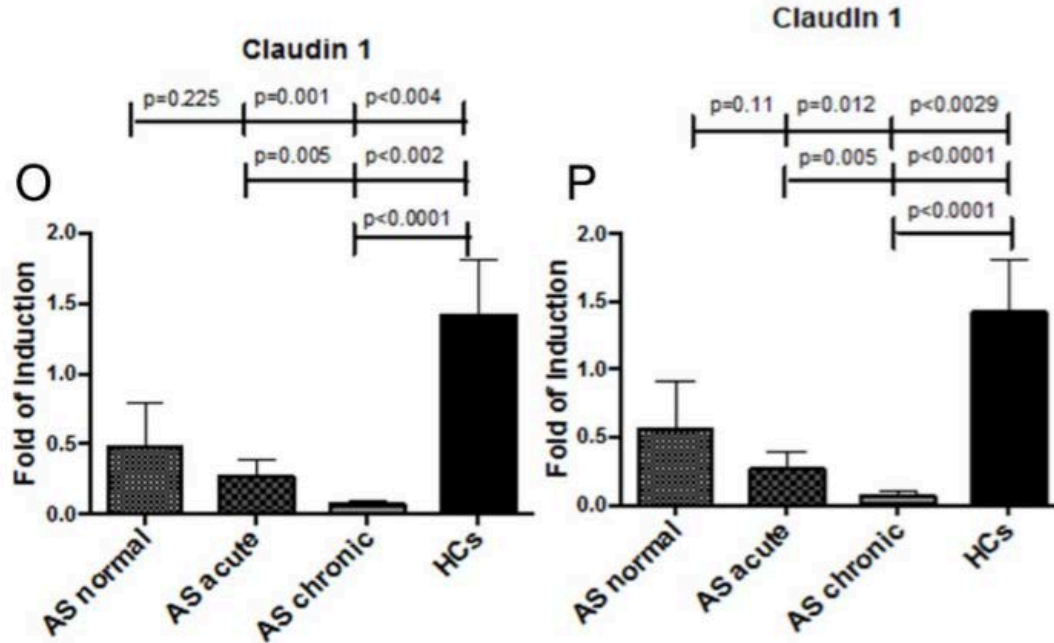


# Intestinal bacteria

- Adherent and invading bacteria present in AS (35/50), not in controls



# RT- PCR ileal samples

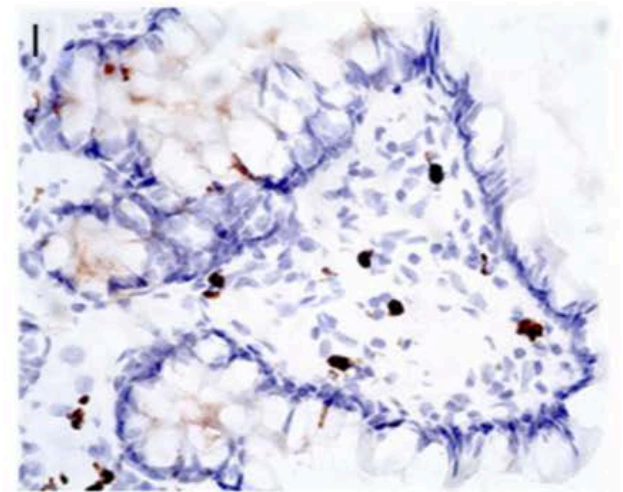
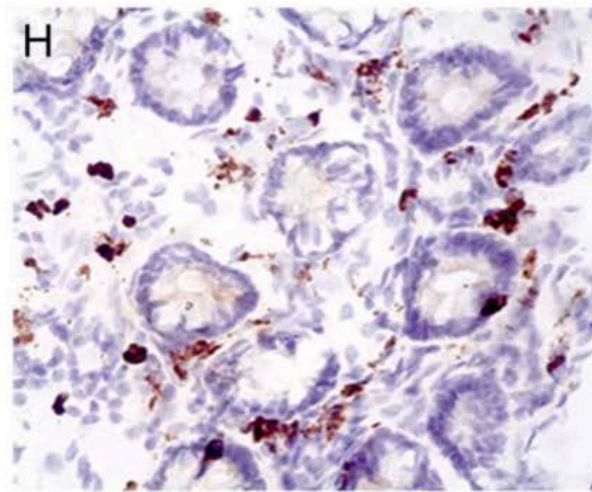
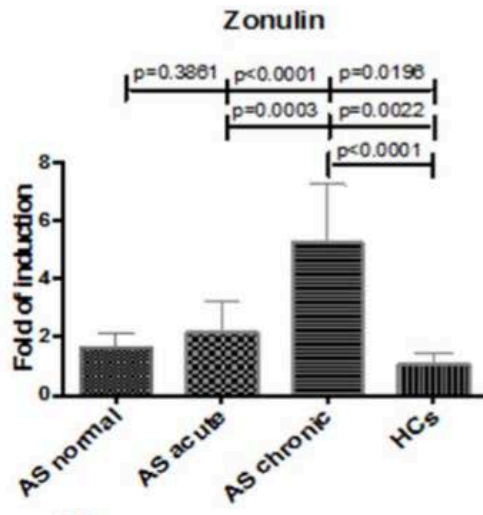


Downregulation of tight junction proteins in AS



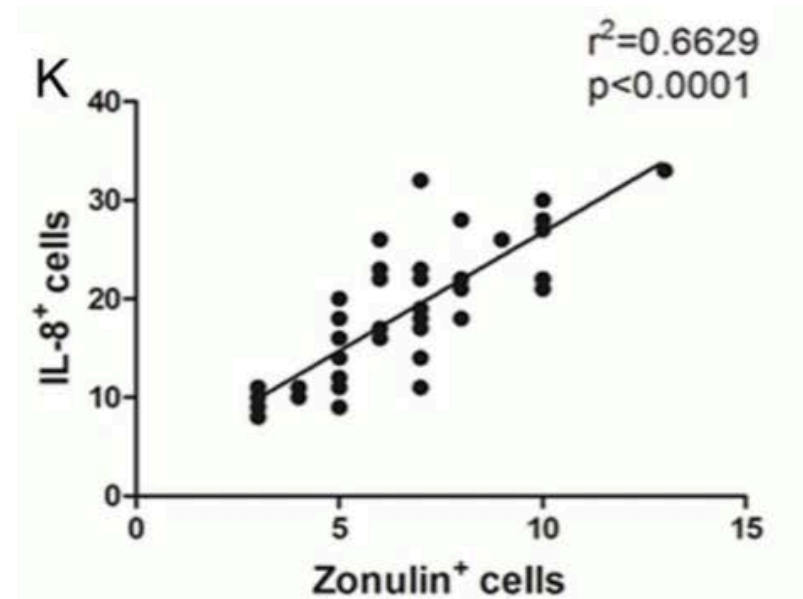
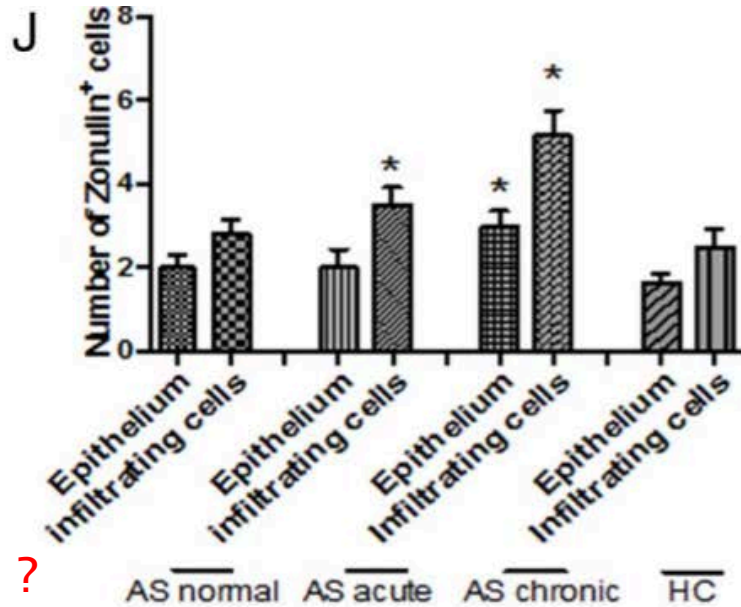
# Zonulin in ileal samples

G



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

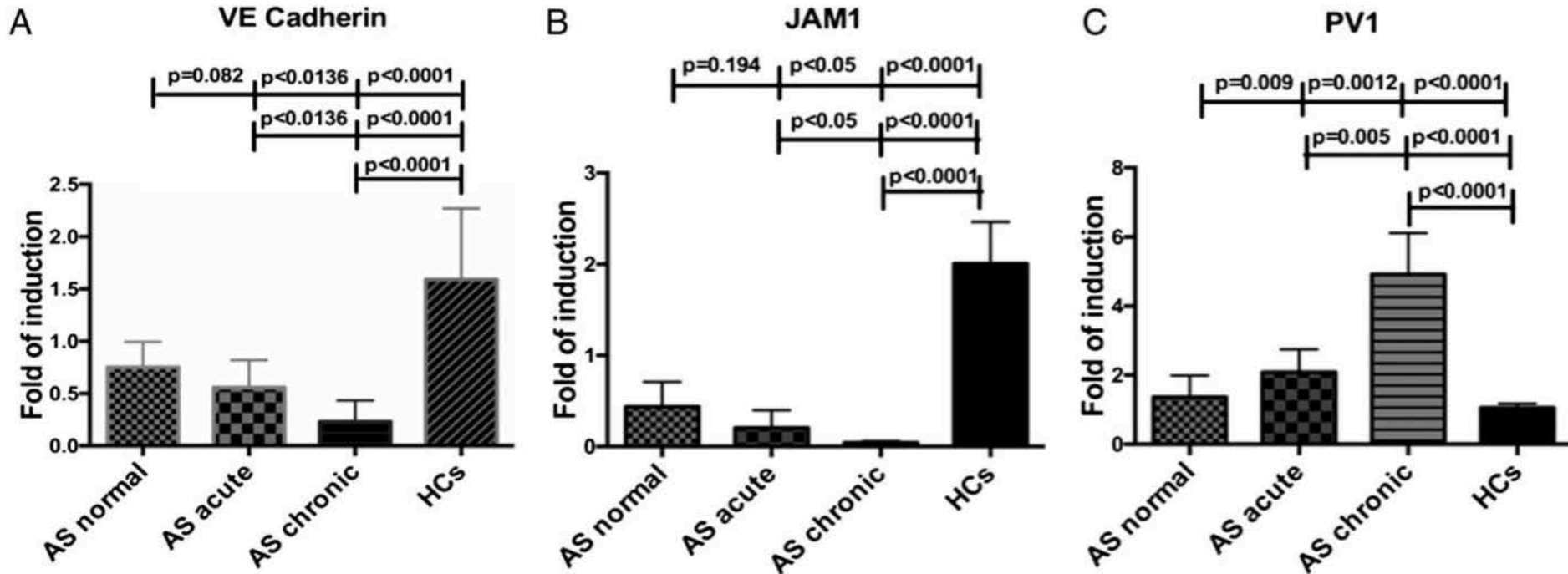
# Zonulin in ileal samples



(K) The number of zonulin positive cells was significantly and directly correlated with the number of IL-8 positive cells.

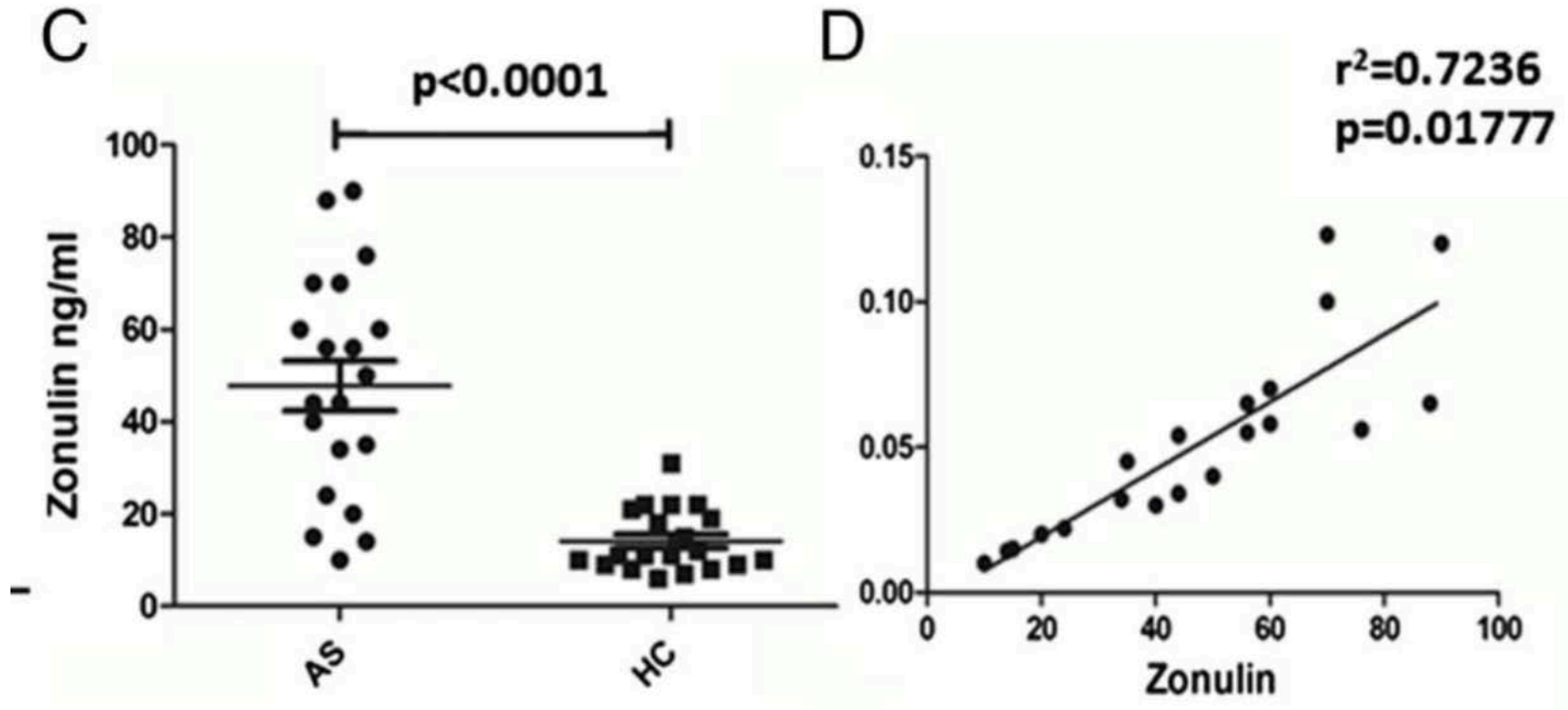
# Caco-2 cells incubated with AS- bacteria

# Gut vascular barrier (GVB) proteins in ileal samples



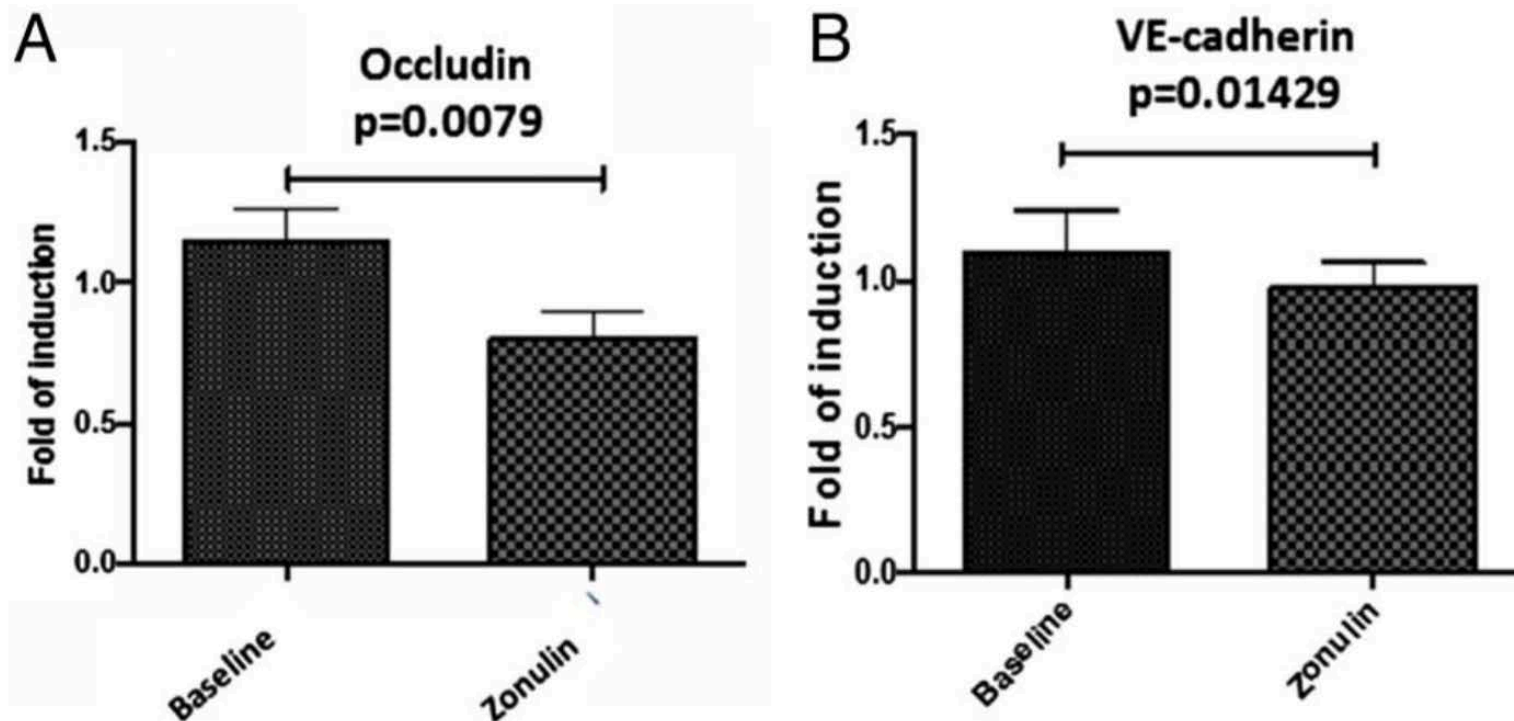
Reduced relative m-RNA levels of VE-cadherin (A), junctional adhesion molecule (JAM)-1 (B) and PV1 (C) were assessed by RT-PCR in AS

# serum zonulin



Serum levels of zonulin were evaluated in 20 patients with AS and 20 controls (C) and correlated with LA/MA ratio (D)

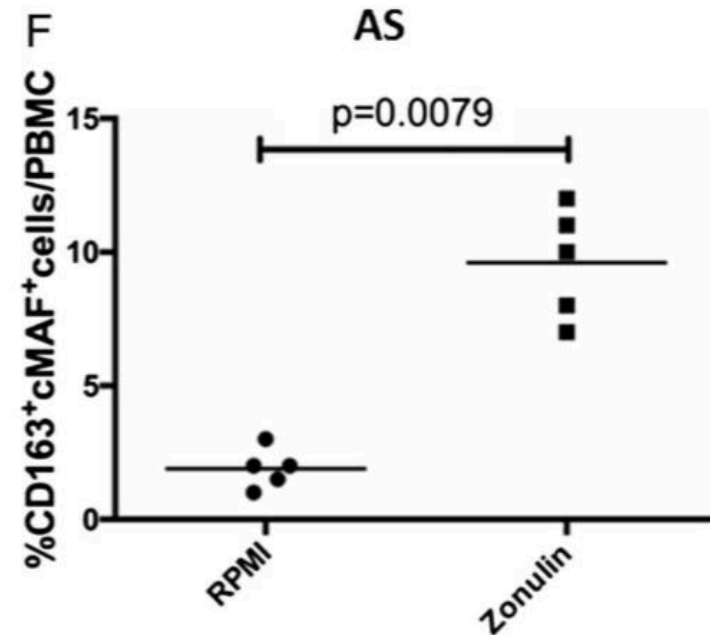
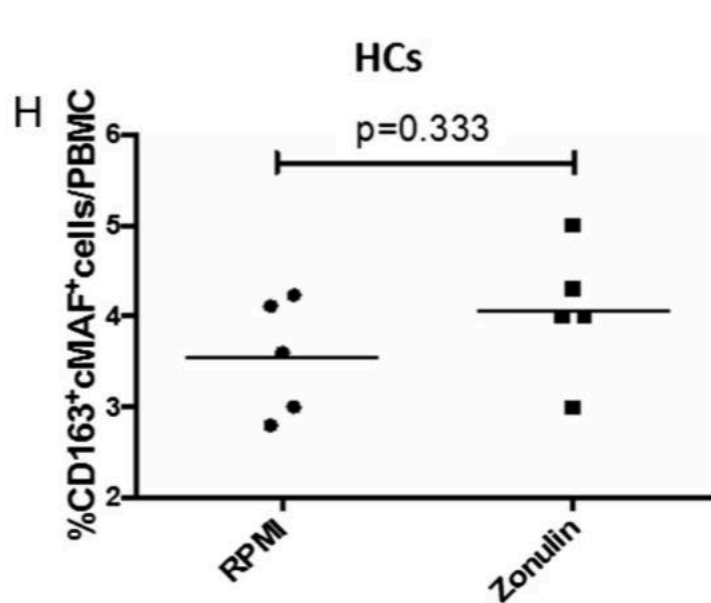
# In vitro effects of zonulin on human umbilical vein endothelial cells (HUVECs)



Downregulation of occludin (A) and VE-cadherin after zonulin treatment

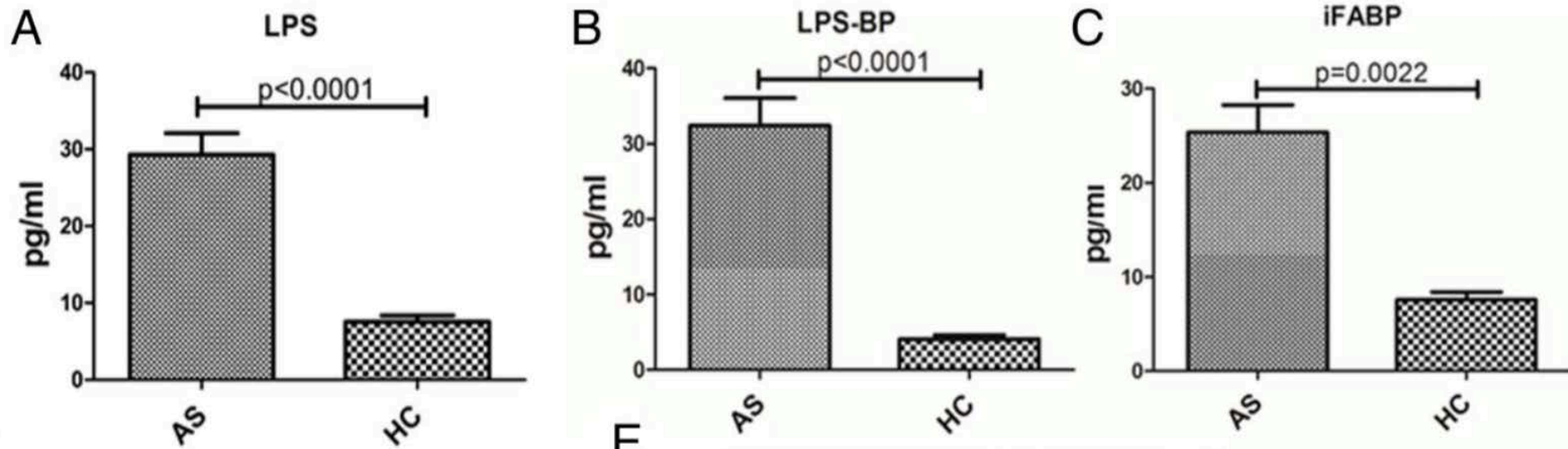
# In vitro effects of zonulin on peripheral monocytes

zonulin: CD163 binding motif



percentage of CD163+c-MAF+ cells increased after incubation with AS PBMCs  
=M2 polarised macrophages

# Serum LPS, LPS-BP, iFABP



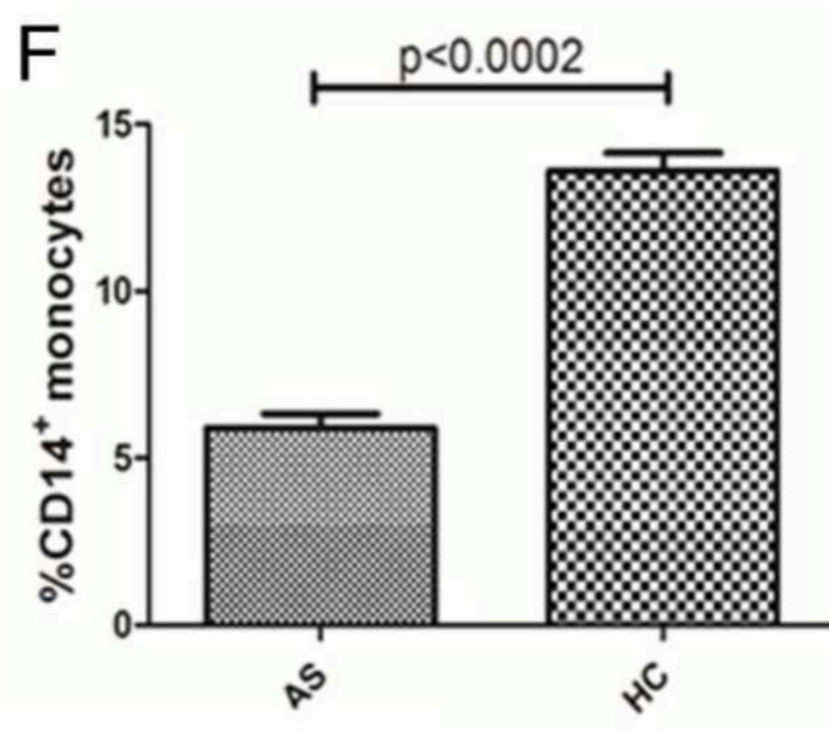
Elevated serum levels of lipopolysaccharide (LPS) (A), LPS-binding protein (BP) (B) and intestinal fatty acid-BP (iFABP) (C) in AS



# AS and CD14+

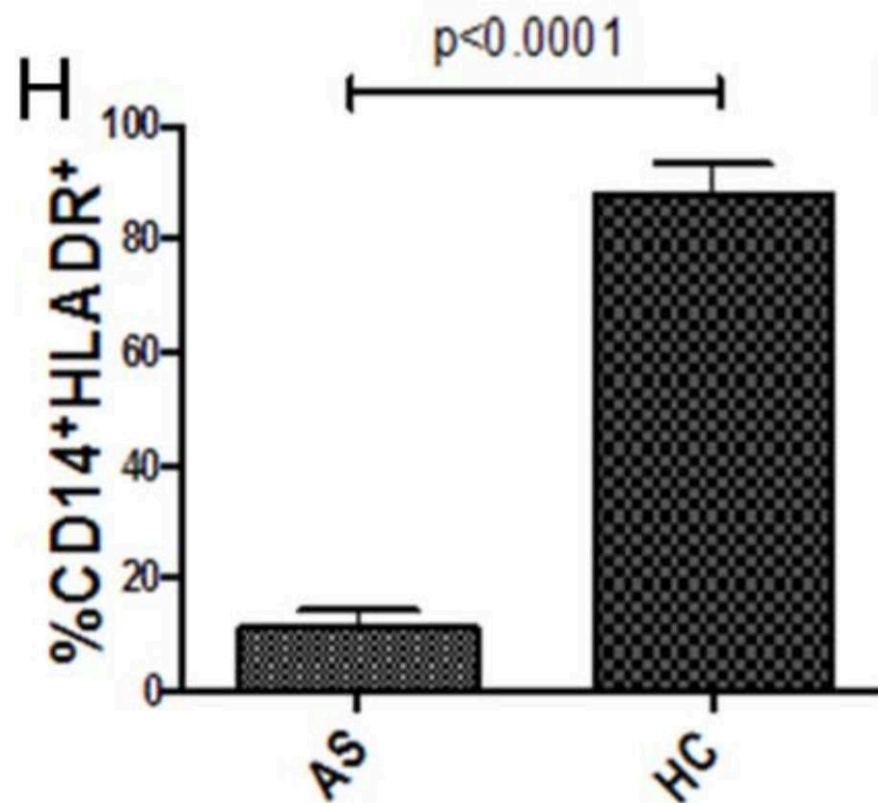
CD14= on monocytes/macrophages, involved in LPS binding

High LPS downregulates CD14



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

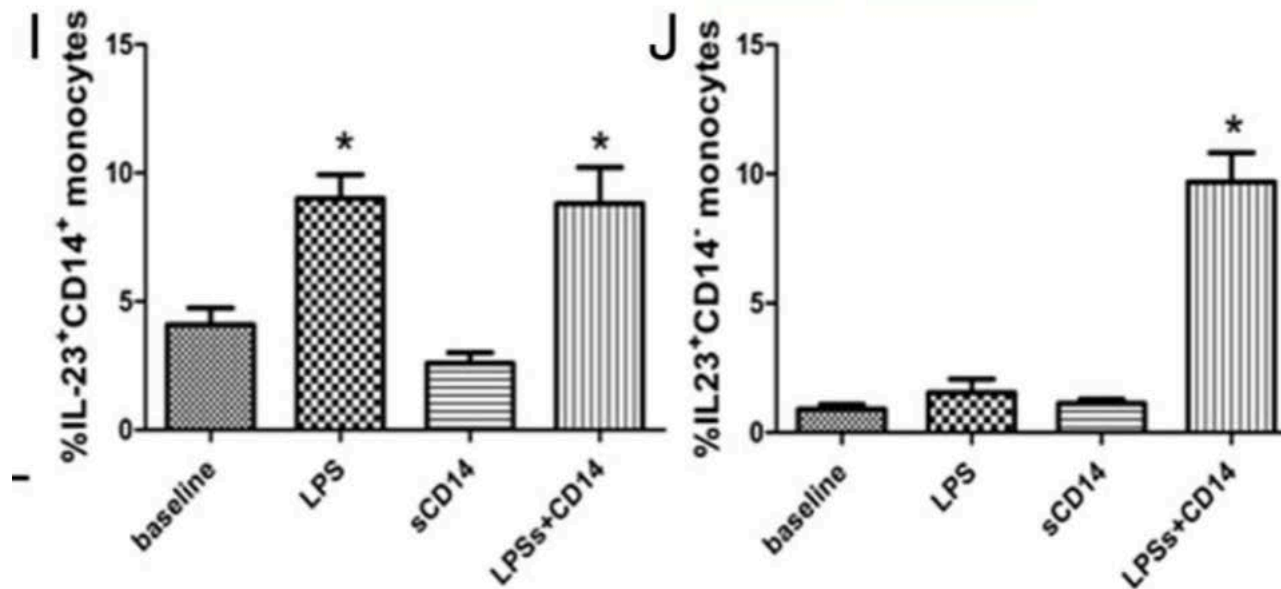
# AS and HLADR+



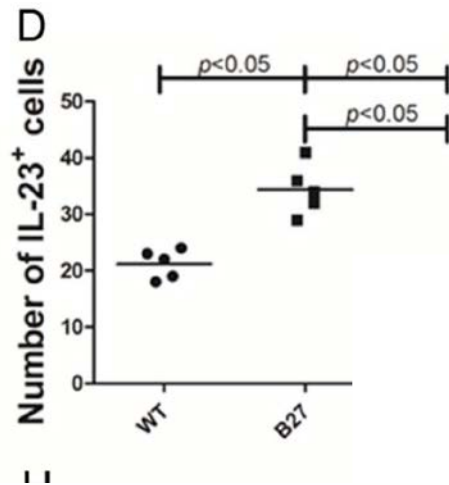
Percentages of CD14+ HLADR+ cells is reduced in peripheral blood mononuclear cells (PBMCs) from patients with AS.

# AS and CD14+

Effects of monocyte stimulation with LPS alone, sCD14 alone or sCD14+LPS on CD14+ and CD14- monocytes

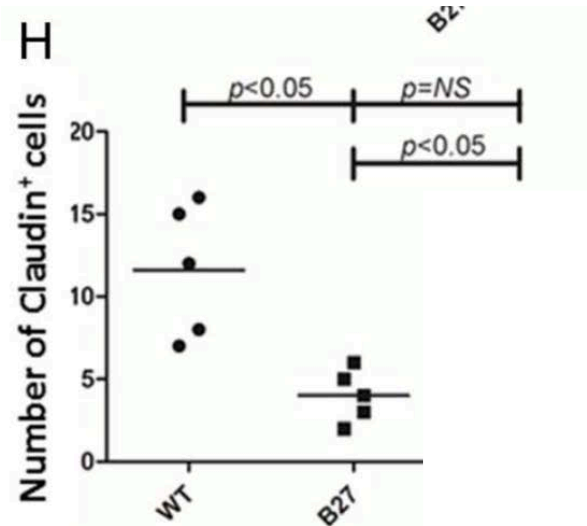


# HLA-B27 rats ileal samples

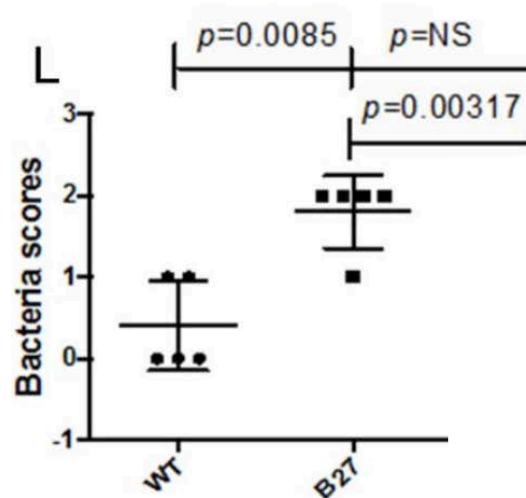


increased IL-23 expression

→ Antibiotic treatment



occludin  
downregulation



Presence of  
adherent bacteria

# 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??