Linking Gut Dysbiosis and Peripheral Arthritis in Spondyloarthropathy through Increased Villous Permeability that Allows Vascular Bacteria Dissemination

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Background

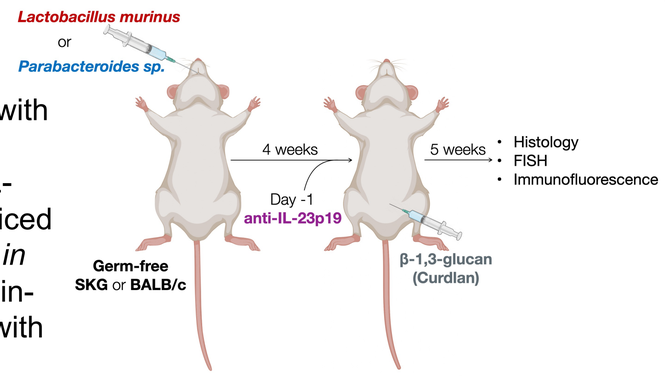
- Spondyloarthropathies (SpA) are progressive, chronic inflammatory diseases characterised by arthritis, ileitis and psoriasis, affecting 1-3% of the population, strongly associated with HLA-B27 genetic disposition.
- Up to 70% of SpA patients have extra-articular gut symptoms, suggesting that gut inflammation is involved in the disease pathogenesis.
- The SKG mouse model possess a spontaneous W163C mutation in the ZAP70 protein, important for T cell functions, and is a model for human SpA when treated with β-1,3-glucan (curdlan) with the cytokine IL-23 playing a key role.
- Dysbiotic gut microbiome has increased Gram-negative bacteria over protective commensals; however, it is currently unclear if dysbiosis of gut pathobionts drives joint inflammation.

Hypotheses

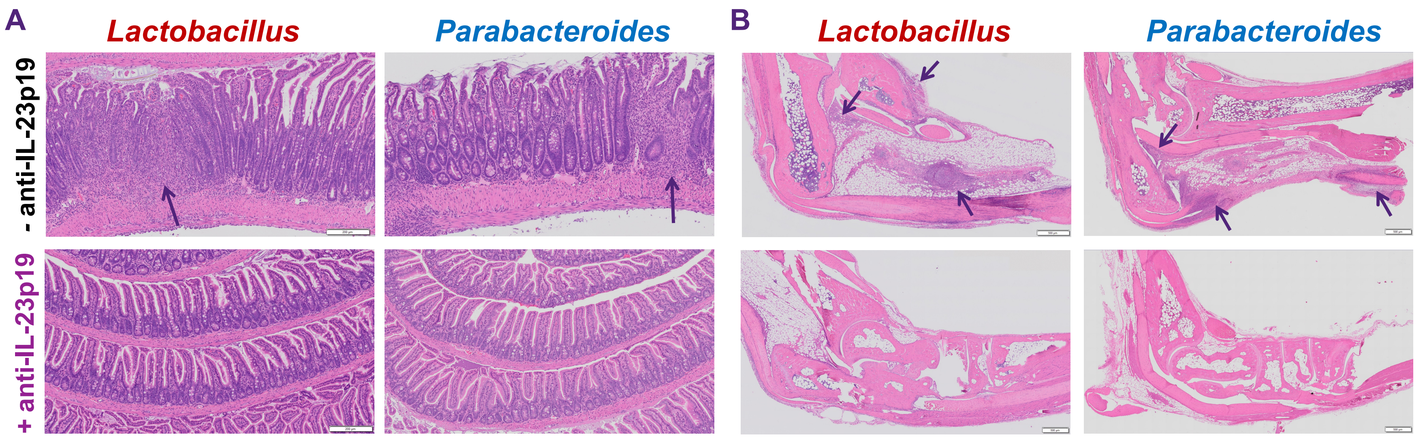
1. Colonisation of germ-free SKG mice with a single pathobiont drives arthritis after a microbial (curdlan) inflammatory trigger.
2. Curdlan-induced increased gut permeability allows pathobionts to travel from the gut to the peripheral joints via blood vessels.

Methods

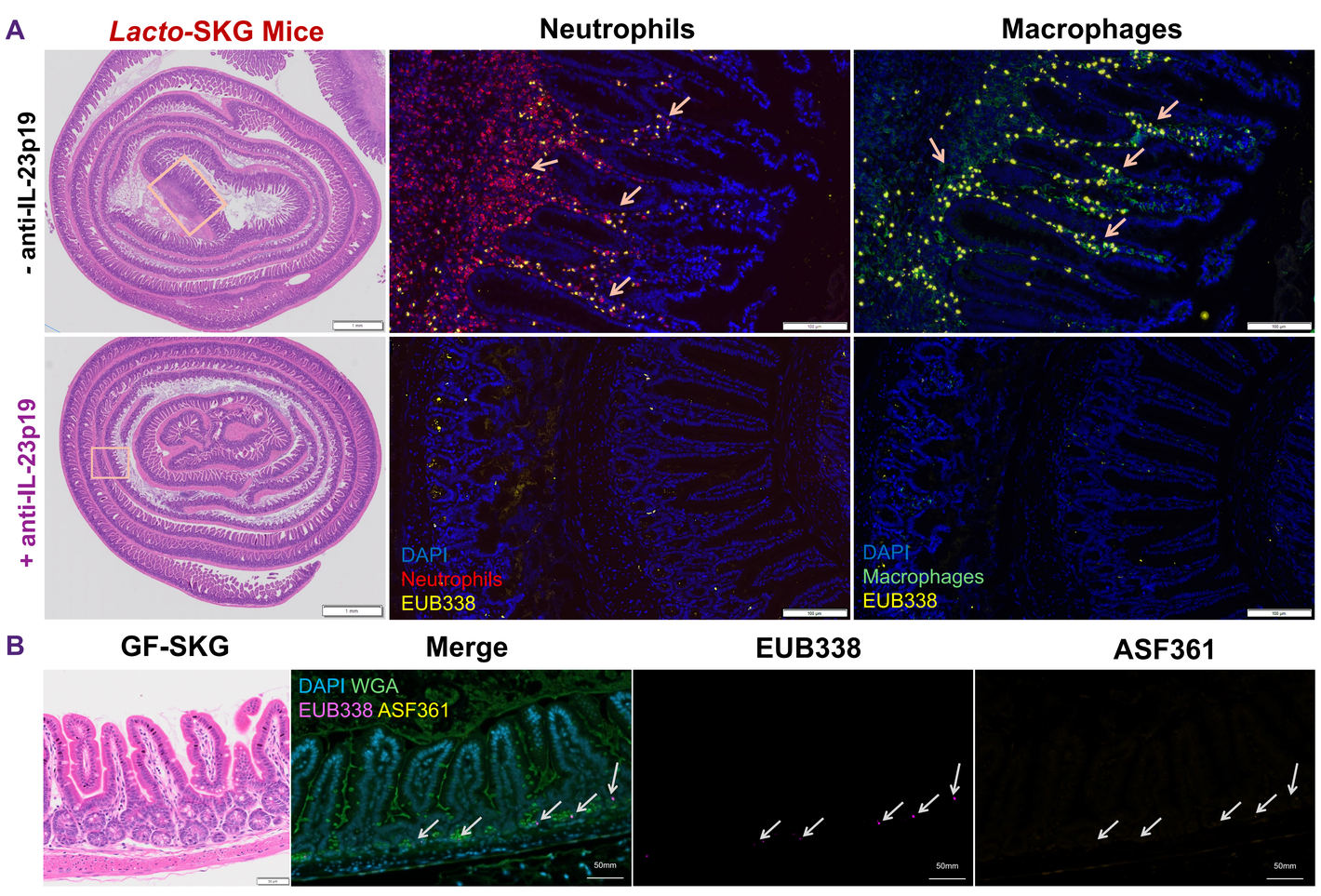
4 weeks prior to 3mg intraperitoneal curdlan injection, germ-free SKG and BALB/c mice were monocolonised with Gram-negative *Lactobacillus murinus* (Lacto) or *Parabacteroides sp.* (Para). Some mice received anti-IL-23p19 treatment 1 day before curdlan. Mice were sacrificed at week 5 for organ collection, histological analysis and *in vitro* imaging. Bacteria were detected using fluorescent in-situ hybridisation (FISH), tissue sections were labelled with antibodies and imaged with confocal microscopy.



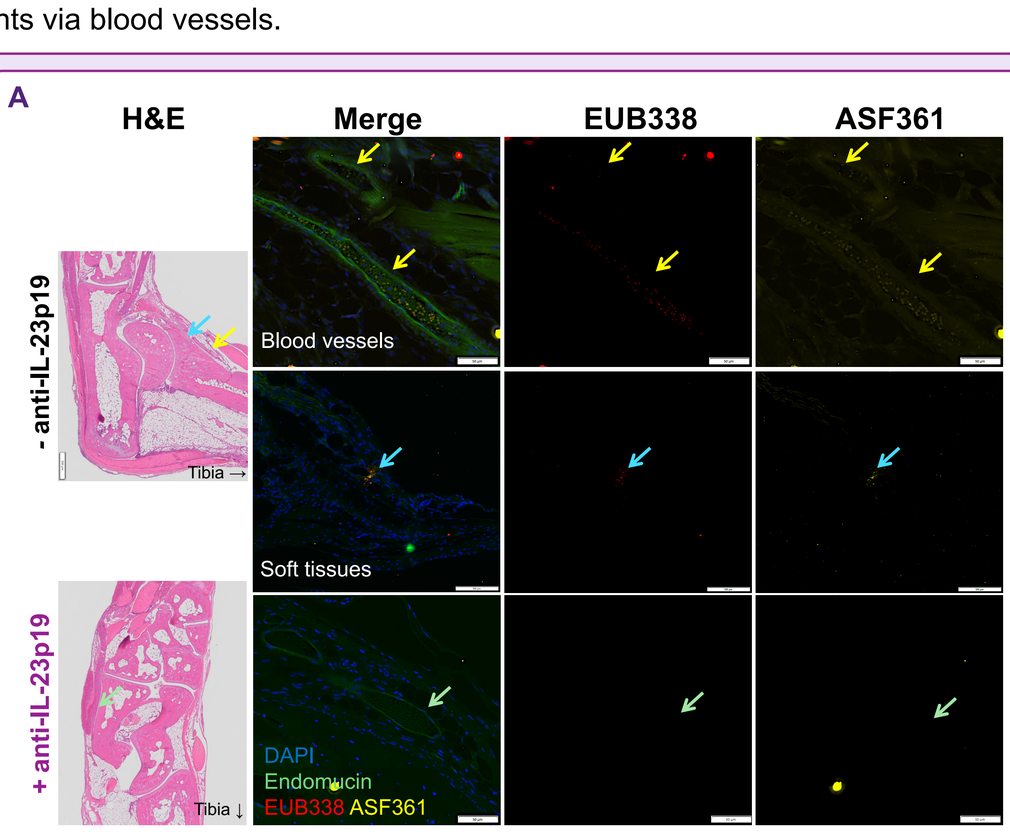
Results

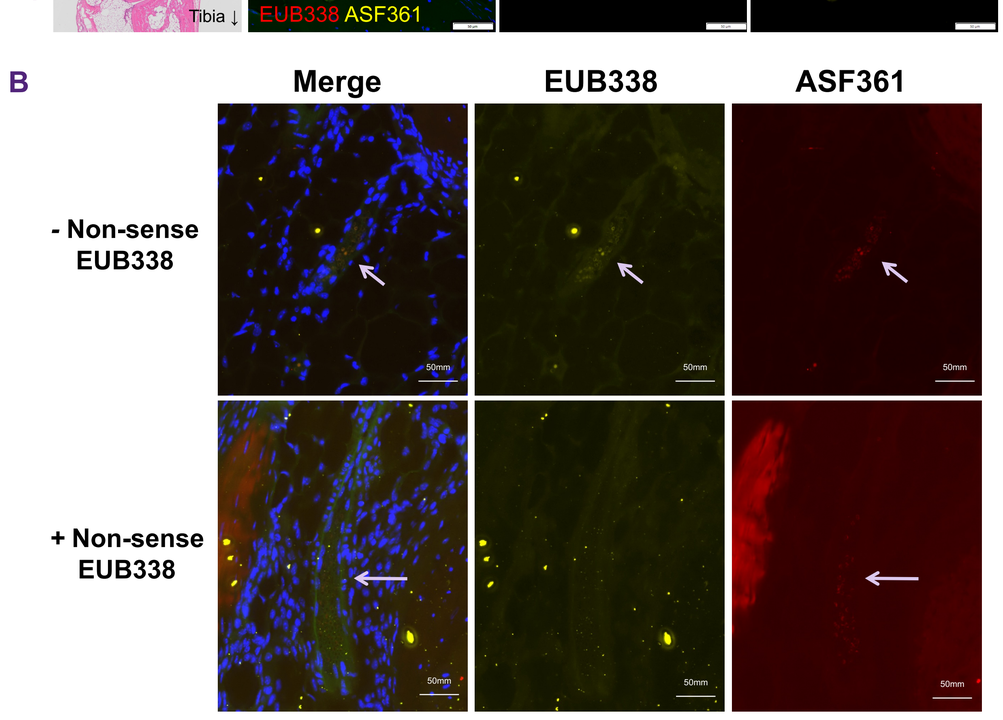


▲ **Figure 1 | *Lactobacillus murinus* or *Parabacteroides sp.* monocolonised germ free SKG mice develop IL-23 dependent gut and joint inflammation following β-1,3-glucan treatment.** Representative H&E staining of ileum **(A)** and rear ankle **(B)**, showing areas of inflammatory infiltration (arrows). n=4-7 across 2 experiments.



▲ **Figure 2 | *Lactobacillus murinus* translocates from lumen to villi, which are captured by neutrophils and macrophages.** **(A)** Ileum sections from *Lactobacillus*-colonised germ-free SKG mice were assessed for the colocalisation of *Lactobacillus*, neutrophils and macrophages, using universal bacterial FISH EUB 338 probe and IF antibodies (right), along with representative H&E images (left), in mice with (bottom) and without (top) anti-IL-23p19 treatment. Arrows point to areas of colocalisation of bacteria, neutrophils and macrophages; **(B)** Ileum sections from germ-free SKG mice stained with universal bacteria EUB338 and ASF361 FISH probes shows non-specific binding of EUB338 (arrows) but not ASF361 probes (arrows) in the Paneth cells, along with an representative H&E image (left).





▲ **Figure 3 | *Lactobacillus* translocation to the ankle which triggers arthritis is prevented by anti-IL-23p19 treatment.** **(A)** Representative H&E (left) and fluorescent images (right) of ankle sections from *Lactobacillus*-monocolonised germ-free SKG mice at 5 weeks post-curdlan labelled with EUB338 and ASF361 FISH probes, without (top, middle) or with (bottom) anti-IL-23p19 treatment. Arrows point to bacteria from sections without anti-IL-23p19 treatment (top, middle) and lack of bacteria in the blood vessel when treated with anti-IL-23p19 (bottom). **(B)** Fluorescent images from ankle at 5 weeks post-curdlan labelled with EUB338, non-sense EUB338 and ASF361 FISH probes, shows staining specificity. Arrows point to positive bacteria signals and scale bars are 50 microns in length.

Conclusions

Lactobacillus or *Parabacteroides* monocolonisation of germ-free SKG mice and β-1,3-glucan treatment are sufficient to induce ileitis and arthritis:

- Dysfunctional ileal barrier allows gut-derived bacteria to perforate, subsequently captured by neutrophils and macrophages.
- In the inflamed peripheral joints, these bacteria are found in the blood vessels and enthesis, suggesting vascular bacteria translocation.
- Blocking IL-23 mitigates ileitis, arthritis and bacteria translocation.

Overall, this proof-of-concept study links gut dysbiosis to its outcomes in the peripheral joints, proposing a potential mechanism by which dysbiosis induces arthritis in SpA.

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