The role of gut microflora and autoreactive CD4+ T cells in the development of spondyloarthritis and inflammatory bowel disease in β-glucan-treated SKG mice

Merja Ruutu, Linda Rehaume, Jared Velasco, Daniel Aguirre, Helen Benham, Mark Morrison, Michael McGuckin and Ranjeny Thomas

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SpA – relationship of arthritis with IBD

- SpA/IBD: Either arthritis or IBD may present first. Can vary family members. Can be subclinical
- Gut flora critically involved in IBD pathogenesis: differences in flora in Crohn’s associated with mucous/epithelial integrity
- Microbial trigger or interaction at the genetic interface of SpA e.g. NOD2, CARD9

SKG mouse model

- Point mutation in T cell receptor ZAP70 reduces strength of signalling and function, consequent thymic selection defect
  - Relative lymphopenia, increased peripheral autoreactive T cells
  - IL-6 required for disease, CD4+ Th17 transfer arthritis: Hirota JEM 2007
- Environmental fungal infection of lung triggers disease in clean conventional conditions
  - C.albicans: dectin-1, dectin-2, FcR, mincle, MMR, TLR2
  - Zymosan (containing β-glucans, mannan, chitin and protein): dectin-1, MMR, mincle, TLR2

Infection, IL-23 signaling pathway and SpA

- Common genes associated with AS, psoriasis and IBD: Card9 IL12B, IL23R, Stat3
- IL-23R protective allele reduced signal strength
  - IL-23 signaling role in disease

Development of Spondyloarthropathy in SKG mice treated with i.p.curdian

Crohn's-like ileitis, vasculitis, granulomas, goblet cell hyperplasia, increased mucous secretion, “Skip lesions”
SpA with IBD in response to dectin-1 signaling in autoimmune-prone SKG mice

How are gut and joints related in the same disease?

Hypotheses

1. Autoimmune cross-reactivity towards joint and gut antigens

2. Coincident or sequential development of joint and gut inflammation driven by common innate mechanisms, potentially triggered by infection

1. Autoimmune cross-reactivity towards joint and gut antigens?

Timing

Arthritis and spondylitis starts within 1 week of curdlan, develops in 100% of mice ileitis evident histologically in 70% of mice 8-14 weeks after curdlan

Evidence of autoimmunity

T cell transfer

Autoantibodies

SKG autoreactive CD4 T cells are necessary and sufficient for arthritis and spondylitis but not ileitis

CD4 T cells autoreactive towards joint/disc.

Relevant autoantigens?

Fibrocartilage: type II collagen, Proteoglycan (aggrecan, versican)

Summary 1

- CD4+ SKG T cell dependent arthritis and spondylitis
  - Arthritis, spondylitis rapid associated with SKG CD4 T cell-dependent cartilage-specific AB
  - Ileitis delayed by 2 months, CD4-independent: unlikely cross-reactive autoantigen

- Regulation is critical to disease control in the face of SKG CD4+ T cells
- CD4+ SKG T cells from untreated mice transfer to SCID
- CD4+ SKG T cells transfer colitis, which does not occur in the host
  - Tanaka et al. J Immunol 2010:
    - Colitis upon CD4+ SKG T cell transfer. More frequent if Treg depleted
  - Skg/ZAP70 with more severe signaling defect: spontaneous disease in SPF
  - i.e. reduced regulation promotes autoimmune disease without trigger
2. Coincident or sequential development of joint and gut inflammation driven by common innate mechanisms, potentially triggered by infection.

**Role of microflora**

Re-derive animals to germ free animal house: WEHI

Rederived to germ free, remained germ free. 14w after curdlan, 2/10 males and 0/10 females developed mild arthritis. 0/20 ileitis or colitis.

**Role that microflora might play in initiation of SpA**

1. Gut microflora required to supply cross-reactive peptides for joint autoimmunity e.g. HSP-70
2. Gut microflora required as adjuvant for priming autoimmunity:
   1. Are joint-specific autoantibodies primed in GF?
   2. Is there a pro-inflammatory effect of curdlan?

**Evidence for adjuvant effect on inflammation: reduced initial neutrophil and macrophage infiltration of curdlan-treated GF mice**

**Evidence for adjuvant effect on inflammation: reduced initial cytokine response of curdlan-treated GF mice**

**Increased titres of CII and PG autoantibodies only in arthritic GF SKG mice**

**Commensal microflora required for arthritis, ileitis, skin inflammation after curdlan in SKG mice**

**Although gut microflora may supply cross-reactive peptides, this is not required for development of cartilage autoimmunity and arthritis/spondylitis**
Evidence for adjuvant effect on inflammation: increased intracellular reactive oxygen species in peritoneal cavity of GF mice

Summary 2

- Gut/skin microflora = common innate mechanism required for development of arthritis and IBD
  - β-glucan usually insufficient without bacterial adjuvant, and commensal microflora sufficient to supply this (stochastic, ROS may regulate)
  - Adjuvant effect required for:
    - Pro-inflammatory response to curdlan: macrophage and neutrophil recruitment and pro-inflammatory cytokines
    - Priming of autoantibodies/autoimmune arthritis

Collaboration between β-glucan and microflora to trigger arthritis and IBD

Collaboration between β-glucan and microflora to trigger disease in SKG mice

UQ Diamantina Institute
Merja Ruutu, Linda Rehaume, Jared Velasco, Helen Benham
Metagenomics, CSIRO
Daniel Aguirre, Mark Morrison,
Mater Medical Research Institute
Michael McGuckin
Princess Alexandra Hospital Pathology
Geoff Strutton