SINGHEALTH · DUKE-NUS SCIENTIFIC CONGRESS
17 - 18 SEPTEMBER 2021

REVOLUTIONISING MEDICINE - THE NEW FRONTIER

The host and bacterial determinants of immunomodulation by a commensal Clostridium

Chin Yee Tan[1,2,4] and Neeraj K Surana[1,2,3]

[1]Department of Pediatrics, [2]Department of Molecular Genetics and Microbiology, [3]Department of Immunology, Duke University, Durham, North Carolina, USA; [4]Duke-NUS Medical School

1. *Clostridium immunis* protects against colitis-mediated death

- The microbiota is intricately **linked to human health**.
- Specific **members of the intestinal microbiota regulate the immune system**, and therefore have the potential to **treat inflammatory disease**.
- ***Clostridium immunis*, a newly discovered** commensal bacterial species, **protects mice against colitis[1].** Current efforts are focused on developing it as a **therapeutic for inflammatory bowel disease (IBD).** However, its **mechanism of action is unknown**

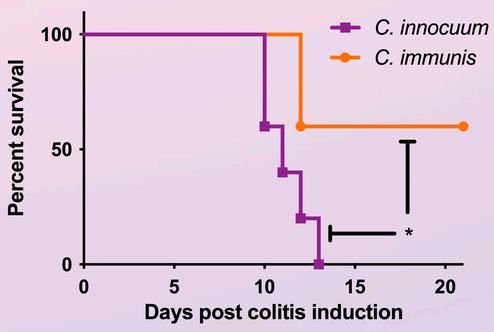

Figure 1. *C. immunis* protects against colitis-mediated death

Mice were treated orally with *C. immunis* or *C. innocuum* (as a control), 7 days before challenge with dextran sodium sulphate induced colitis. *, p < 0.05

2. *C. immunis* alters disease outcome by negatively regulating ILC3s

2a. *C. immunis* specifically downregulates group 3 innate lymphoid cells (ILC3s)

- To understand how *C. immunis* affects the host, we analyzed the immune system of mice administered *C. immunis.*
- *C. immunis* **specifically decreased the number** of **colonic ILC3s and ILC3-produced cytokine GM-CSF**. IL-6, a proinflammatory cytokine produced by other cell types, was unchanged.

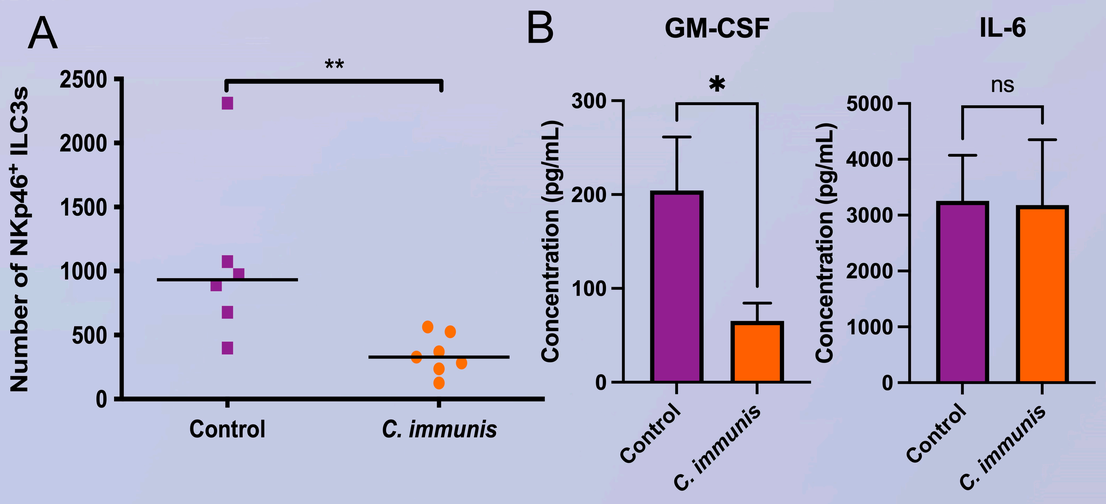

Figure 2. *C. immunis* specifically decreases colonic ILC3 numbers and ILC3-associated cytokines
(A) Mice were treated orally with *C. immunis*, and 7 days later, colonic immune cells were isolated and analyzed with flow cytometry. (B) Colonic explants from mice were incubated with *C. immunis* and grown under cell culture conditions for 24 hours. Culture supernatant was analyzed with a multiplex cytokine assay. *, p < 0.05; **, p < 0.01; ns, not significant

- ILC3s are **rare but critical tissue-resident innate immune cells** that have been **implicated in autoimmune and metabolic diseases**.
- To interrogate the role of *C. immunis,* ILC3s, and disease, we used animal disease models in which ILC3s have been implicated:
  - Infection with the enteric pathogen ***Citrobacter rodentium***
  - **Experimental autoimmune encephalomyelitis**, a model of multiple sclerosis.

2b. *C. immunis* modulates disease outcomes in an ILC3-dependent manner

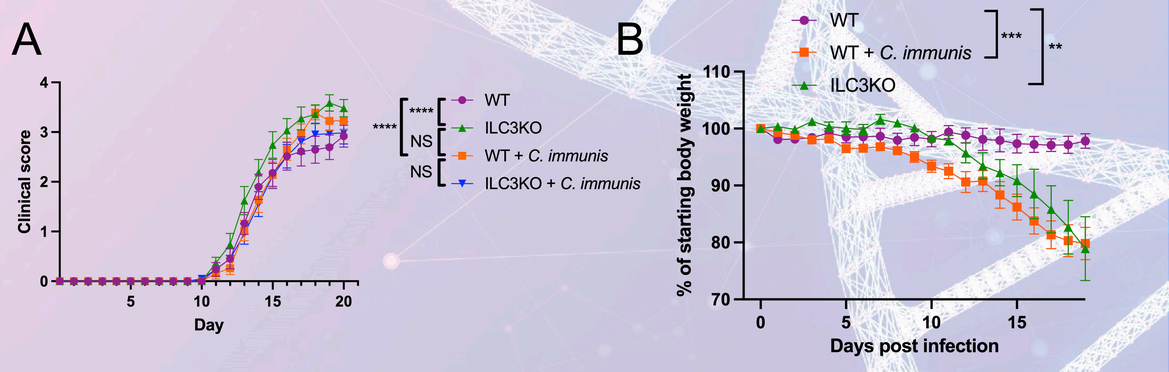

Figure 3. *C. immunis* modulates disease outcomes in an ILC3-dependent manner
Wildtype (WT) or ILC3-deficient (ILC3KO) mice were treated orally with *C. immunis* 3 times a week and challenged with either (A) experimental autoimmune encephalomyelitis or (B) *Citrobacter rodentium* infection, and clinical severity or weight were quantified respectively **, p < 0.01; ***, p < 0.001; ****, p < 0.0001

3. *C. immunis* is relevant to human health

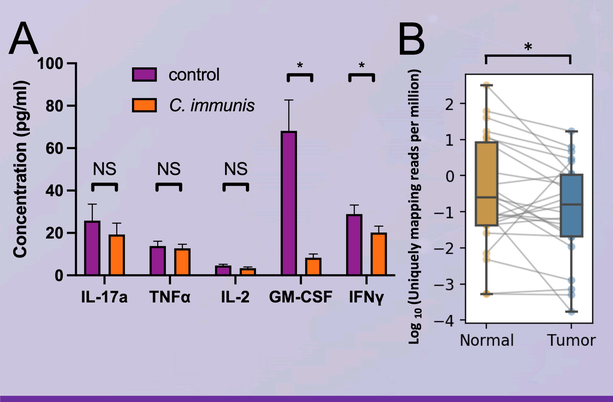

Figure 4A. *C. immunis* modulates ILC3-related cytokines in human IBD. Biopsy samples from IBD patients were cultured with *C. immunis* and cytokines were measured as in Fig 2B.
Figure 4B. *C. immunis* is enriched in normal compared to tumor tissue
Sequencing reads from tumor or adjacent normal tissue were mapped to the *C. immunis* genome. *, p < 0.05

4. Eight candidate *C. immunis* genes are associated with immunomodulation

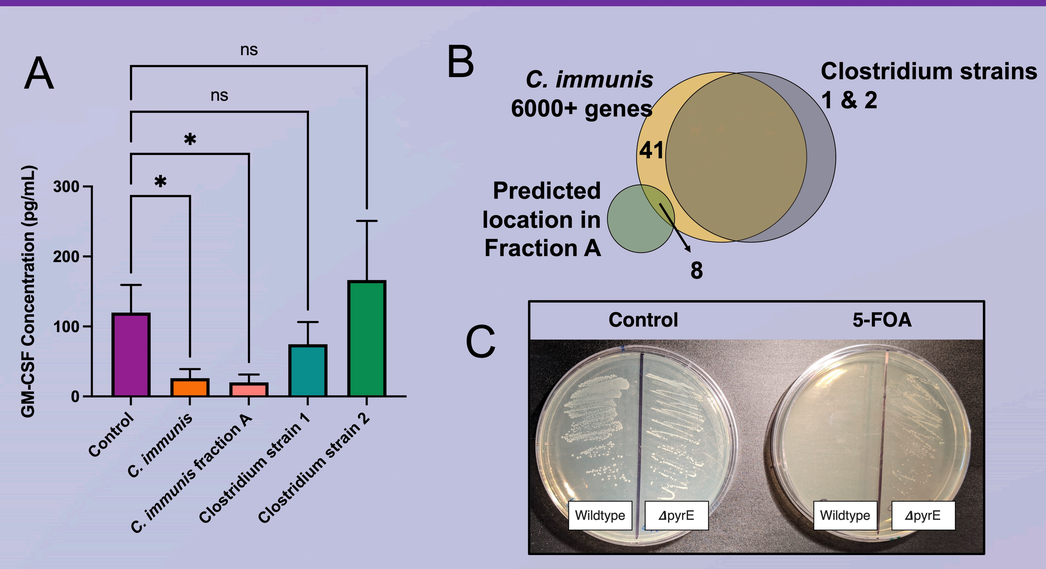

Figure 4. Comparative genomics and biochemical analysis reveal 8 candidate genes that are associated with GM-CSF modulation.
(A) Colonic explants from mice were incubated with biochemical fractions from *C. immunis*, or closely related Clostridium strains, and GM-CSF was measured as in Figure 2B. (B) Exclusion of *C. immunis* genes in the two Clostridium strains that are unable to downregulate GM-CSF, combined with inclusion of genes with products predicted to be in Fraction A, yields 8 candidate genes associated with GM-CSF modulation. (C) CRISPR-mediated deletion of *C. immunis pyrE* gene results in successful growth on 5-FOA, a precursor bactericidal molecule that is activated by functional *pyrE.* *, p < 0.05; ns, not significant

Conclusions

- *Clostridium immunis* is a **newly discovered commensal bacterial species** that may be used to **treat diseases driven by pathogenic ILC3s, such as IBD.**
- Ongoing efforts are to test and **isolate the biochemical products from *C. immunis*** for use as second-generation microbiome-derived therapeutics

SingHealth MedSG200 Defining Tomorrow's Medicine

ORGANISER
SingHealth DukeNUS ACADEMIC MEDICAL CENTRE

SECRETARIAT
SingHealth Academy