CFA injection changed the fecal, but not ileal microbiota with altered Ig isotype responses

Sundar Khadka, Seiichi Omura , Fumitaka Sato, Ijaz Ahmad , Ikuo Tsunoda

近畿大学 KINDAI UNIVERSITY

Department of Microbiology, Kindai University Faculty of Medicine, Osaka, Japan

PDF Poster

Sundar Khadka, MS
PhD (MEXT) student
cls.sundar@gmail.com

Abstract

Animal models of autoimmune diseases, including encephalomyelitis and myocarditis, are induced by sensitization of autoantigens emulsified in complete Freund's adjuvant (CFA) that contains heat-killed *Mycobacterium tuberculosis*. Although the gut microbiota has been shown to affect the susceptibility to autoimmune models, only a few microbiota studies in autoimmune models have set up the control animals receiving CFA alone to examine the compositions of gut microbiota. We aimed to determine whether CFA injection alone in mice could affect the fecal and ileal microbiota using 16S rRNA sequencing. We found that CFA injection significantly decreased alpha diversity in the fecal, but not ileal, microbiome. Principal component analysis of the microbiome separated between the naïve control and CFA injected in feces, but not in the ileum; principal component (PC) values moderately correlated with the serum total IgG1 levels. In feces, the factor loading showed that the relative abundance of the genera *Lachnospiraceae NK4A136* group and *Alistipes* positively and negatively contributed to PC values, respectively. We also found changes in the relative abundance of individual bacteria in the CFA injected group: 16 bacterial genera in feces and eight genera in the ileum. Among them, the relative abundance of the genus *Facklamia* strongly correlated with the serum anti-PPD (purified protein derivative) antibody and total IgG2c titers; that of the genus *Atoptostipes* strongly correlated with the serum total IgG1 and IgA titers. Since CFA injection alone could alter the gut microbiota, "dysbiosis" reported in autoimmune models might be partly due to the effects of CFA.

Background

Complete Freund's adjuvant (CFA)

- CFA = incomplete Freund's adjuvant (IFA) + *Mycobacterium tuberculosis*
- IFA = paraffin oil and mannide monooleate
- Used as an immunopotentiator
- Mechanism of action: depot formation, cytokines/chemokines induction, antigen uptake and presentation enhancement
- Emulsified with autoantigen solution and used for induction of animal models of autoimmune diseases

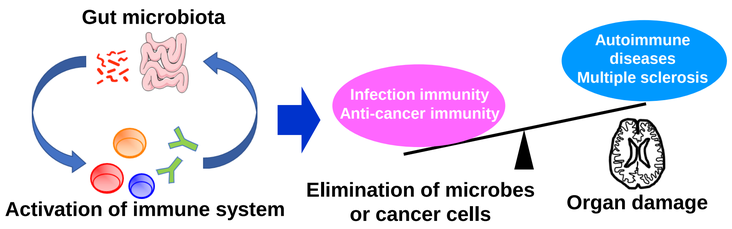
Autoantigen/CFA-induced autoimmune models

Human diseases	Animal models	Induction methods (Autoantigen +CFA) / Animal	Microbiota study (CFA alone)
Guillain-Barré syndrome (GBS)	Experimental autoimmune neuritis (EAN)	Myelin P2 protein + CFA / Lewis rats	No
Multiple sclerosis (MS)	Experimental autoimmune encephalomyelitis (EAE)	Myelin peptide + CFA / C57BL/6 mice	Yes
Myasthenia gravis	Experimental autoimmune myasthenia gravis (EAMG)	Acetylcholine receptor + CFA / Lewis rats	No
Myocarditis	Experimental autoimmune myocarditis (EAM)	Cardiac myosin peptide + CFA / BALB/c mice	No
Orchitis	Experimental autoimmune orchitis (EAO)	Testicular homogenate + CFA / A/J mice	No
Rheumatoid arthritis (RA)	Collagen-induced arthritis (CIA)	Collagen type II + CFA / SD rats, ICR mice	No
Thyroiditis	Experimental autoimmune thyroiditis (EAT)	Thyroglobulin + CFA / CBA/J mice	No
Uveoretinitis	Experimental autoimmune uveoretinitis (EAU)	Retinal soluble antigen + CFA / B10.RIII mice	No

Autoantigen + CFA : used for autoimmune model induction

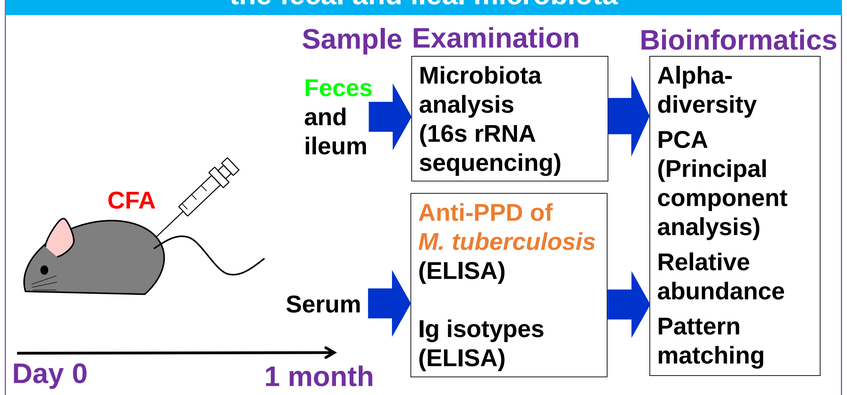
Gut microbiota

- Fecal sample has been widely used for microbiota analysis.
- Microbiota in the ileum affects various immune responses, including Th17 differentiation, IL-10 production.
- Communications between gut microbiota and immune system activate systemic immune responses that eliminate microbes and cancer.
- Dysbiosis, an altered state of microbiota, could induce uncontrolled excessive immune responses, leading to immune-mediated tissue damage, not only in the intestine but also in other organs, including the central nervous system (CNS)



Aim and Methods

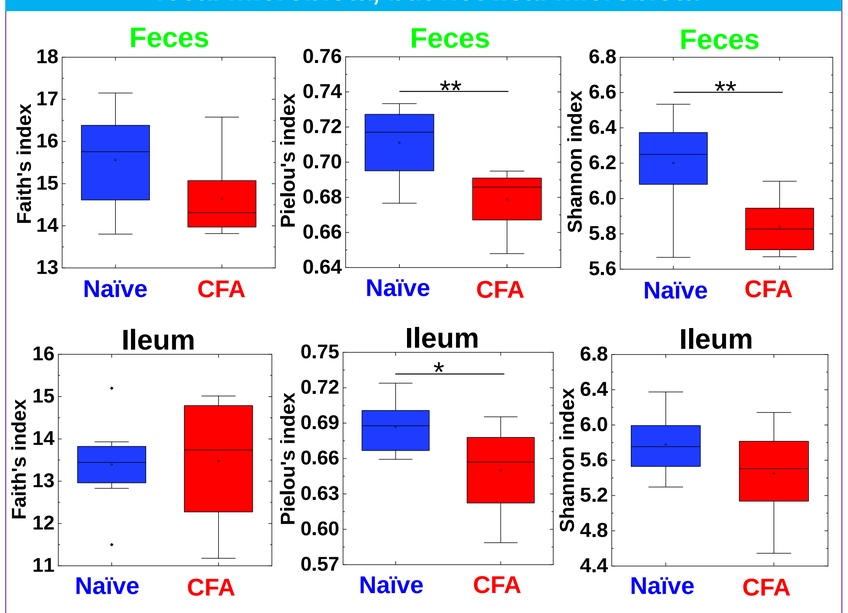
Aim: To determine whether CFA injection alone could affect the fecal and ileal microbiota



- C57BL/6 mice were injected subcutaneously with CFA composed of IFA and *Mycobacterium tuberculosis*.
- 'Naïve' mice were used as the controls

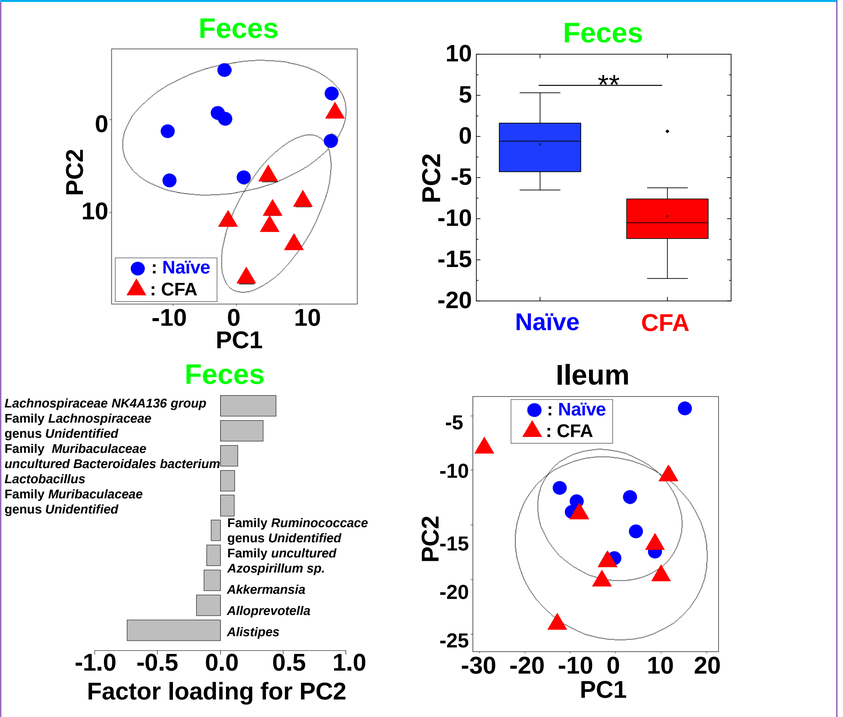
Results

CFA injection decreases alpha-diversities of fecal microbiota, but not ileal microbiota



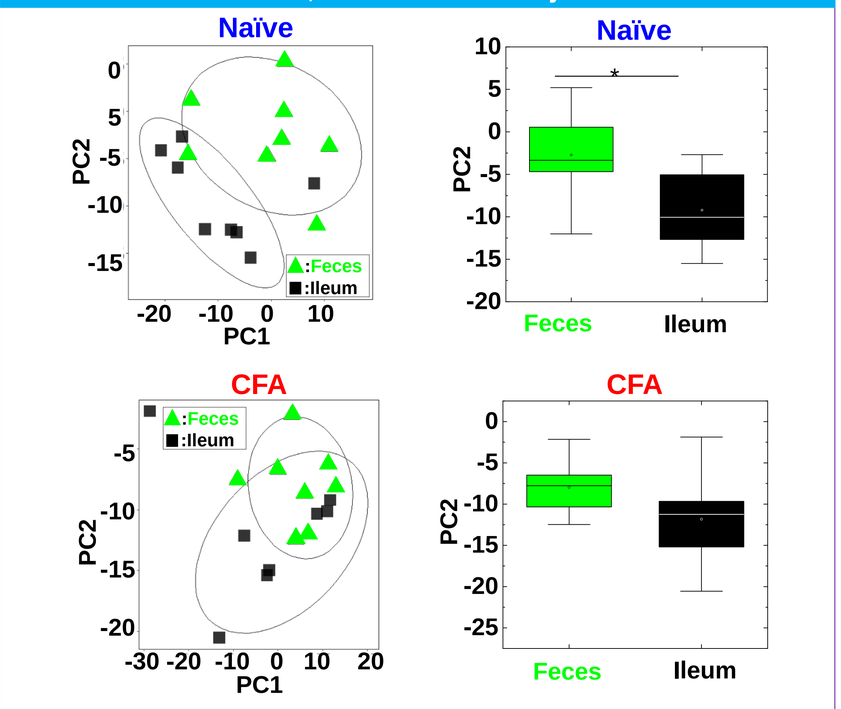
Using QIIME 2, we compared the number of genera, evenness, and combination of them by Faith's phylogenetic diversity index, Pielou's evenness index, and Shannon index respectively, between the naïve and CFA-injected groups. * $P < 0.05$, ** $P < 0.01$

CFA injection alters fecal microbiota, but not ileal microbiota



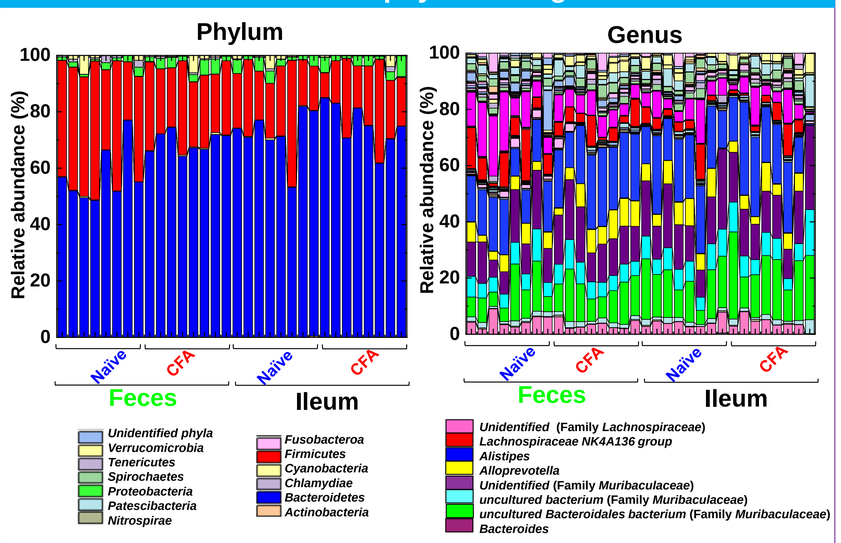
PCA was conducted using fecal and ileal microbiome from the naïve and the CFA-injected groups. PCA separated microbiome only in feces, but not in the ileum. In feces, the PC2 values were statistically different between the two groups. ** $P < 0.01$

Gut microbiota differs between feces and the ileum in naïve mice, but not in CFA-injected mice



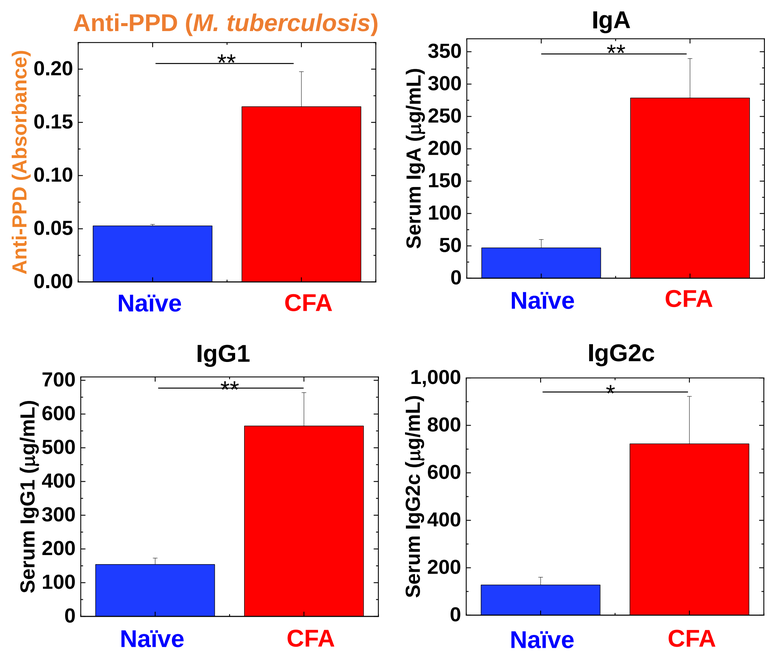
PCA was conducted using microbiome data in the naïve and CFA-injected groups. PCA separated the ileum from feces in the naïve group but not in the CFA-injected group. * $P < 0.05$

CFA injection alters the relative abundance of individual bacteria at both the phylum and genus levels



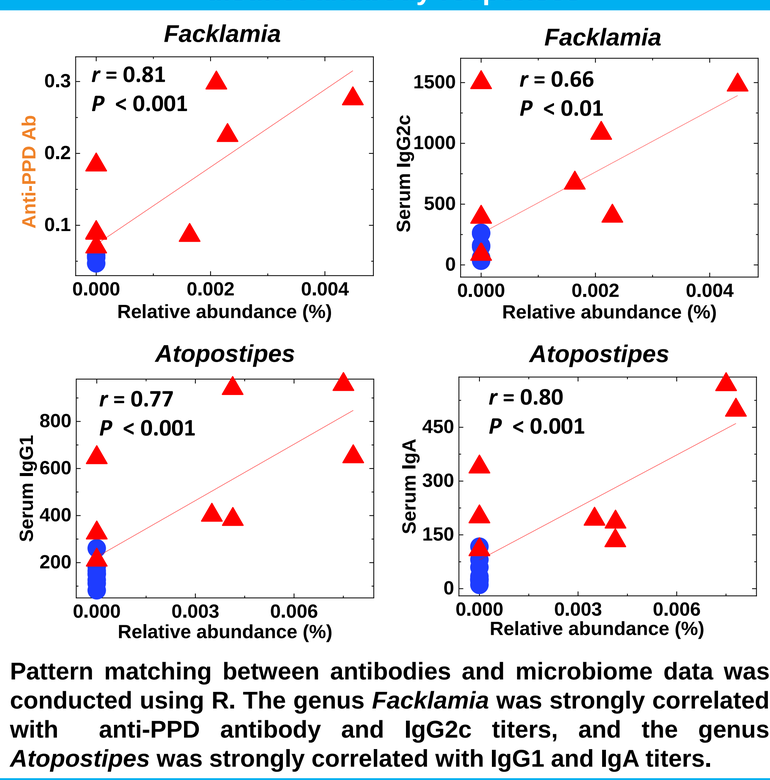
Using 16S rRNA sequencing, we analyzed the relative abundance of individual bacteria in fecal and ileal samples. We found changes in the relative abundance of individual bacteria in the CFA-injected group: 16 bacterial genera in feces and eight genera in the ileum.

CFA injection induces higher anti-mycobacterium and immunoglobulin isotype responses



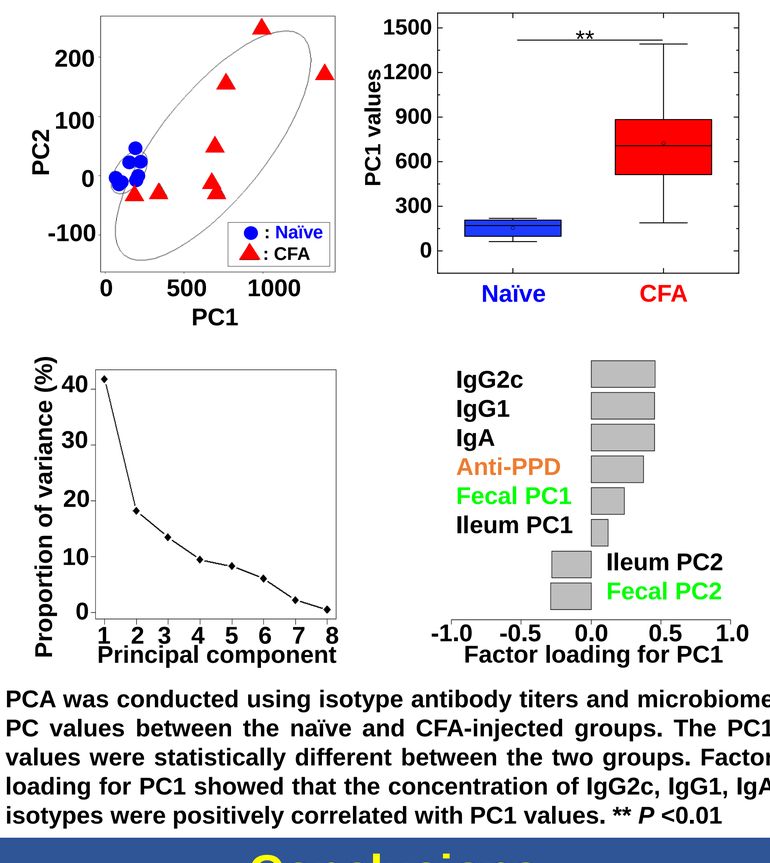
Serum anti-PPD (purified protein derivative) antibody, IgA , IgG1, and IgG2c were quantified by ELISAs. * $P < 0.05$, ** $P < 0.01$

Individual bacterial abundance associates with distinct antibody responses



Pattern matching between antibodies and microbiome data was conducted using R. The genus *Facklamia* was strongly correlated with anti-PPD antibody and IgG2c titers, and the genus *Atopostipes* was strongly correlated with IgG1 and IgA titers.

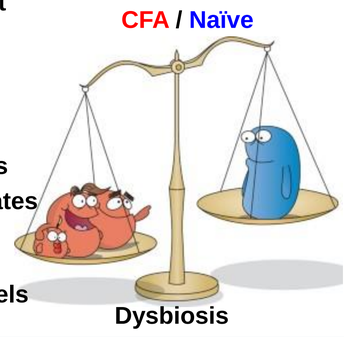
Immunoglobulins associate with CFA injection



PCA was conducted using isotype antibody titers and microbiome PC values between the naïve and CFA-injected groups. The PC1 values were statistically different between the two groups. Factor loading for PC1 showed that the concentration of IgG2c, IgG1, IgA isotypes were positively correlated with PC1 values. ** $P < 0.01$

Conclusions

- CFA injection alone alters fecal, but not ileal, microbiota, compared with naïve group
- CFA injection induces anti-mycobacterium Ab and enhances Ig isotype (IgA, IgG1, IgG2c) responses
- Individual bacterial abundance associates with distinct antibody responses
- Microbiota changes reported in CFA-induced autoimmune animal models could be partly due to CFA



References

- Khadka S. et al. (2021), Curcumin β-D-glucuronide modulates an autoimmune model of multiple sclerosis with altered gut microbiota in the ileum and feces. *Front Cell Infect Microbiol*., 11:772962.
- Johanson DM. et al. (2020), Experimental autoimmune encephalomyelitis is associated with changes of the microbiota composition in the gastrointestinal tract. *Sci Rep*.,10:15183.
- Freund J. (1956), The mode of action of immunologic adjuvants. *Bibl Tuberc*., 10:130-48.

COI and Acknowledgements

- The authors have no financial relationships to disclose
- Monbukagakusho (MEXT 文部科学省) Scholarship, Japan
- Sundar Khadka is a PhD MEXT (2018) student from Nepal