Generation of in vivo mouse model to recapitulate arthritis after ICI therapy

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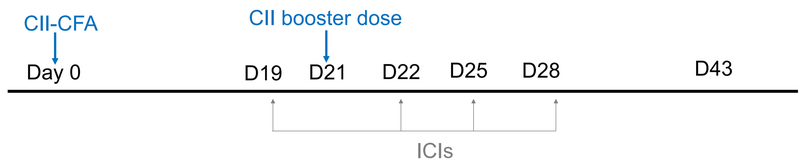
Introduction

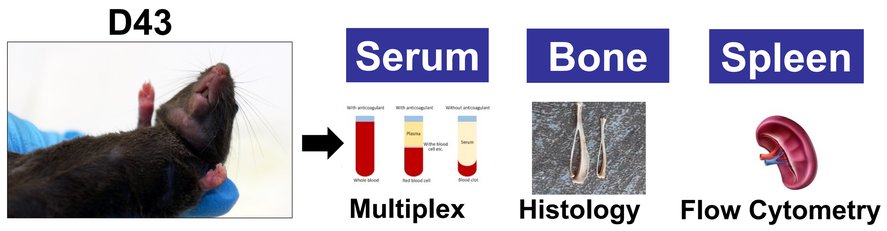
- Immune checkpoint inhibitor (ICI) therapy has revolutionized the cancer therapy; however, ICIs caused life/organ-threatening inflammatory phenomenon, termed immune-related adverse events (irAEs). Inflammatory arthritis is the most common rheumatic irAEs, occurring 3-5% of patients receiving ICI therapy.
- CTLA-4 monotherapy barely induces arthritis-irAE, while PD-1 monotherapy and combination therapy does.
- Mechanism of irAEs is unknown, mainly due to lack of pre-clinical mouse models.
- Hence, we would like to generate pre-clinical murine model recapitulating patients with arthritis induced by immune checkpoint inhibitors (arthritis-irAE)

Methods



Group	D0	D21	ICIs	Significance
A	CII/CFA	CII-IFA	None	Negative Cont
B	CII/CFA	CII-IFA	aCTLA-4	Group of Interest
C	CII/CFA	CII-IFA	aPD-1	Group of Interest
D	CII/CFA	CII-IFA	aCTLA-4 + aPD-1	Group of Interest
E	CII/CFA	CII/CFA	None	Positive Cont

Table 1. We immunized chicken collagen (CII) emulsified in complete Freud adjuvant (CFA) to 8-10 weeks male C57/B6 mice on Day 0. Mice were re-challenged on Day 21 with CII emulsified in incomplete Freud adjuvant (IFA) on Day 21. 100-200 ug of PBS, anti-CTLA-4, and/or anti-PD-1 antibodies were intraperitoneally implemented on Day 19, 22, 25, and 28. Conventional CIA mice (CII-CFA on Day 0 and Day 21) were used as a positive control. The incidence and severity of arthritis were measured until they were sacrificed on Day 43. At end point of study, we harvested spleen, bones, and serum.



	T Cells	Myeloid	ICS	NF
BV421	CXCR5	SiglecF	IL-17	IL-10
AmCyan	Dead	Dead	Dead	Dead
BV785		Ly6G	IFNg	IFNg
PerCP	CD8	MHCII	GM-CSF	CTLA-4
FITC-A	CXCR3	CD19	CD8	FoxP3
Phycoerythrin (PE)		CD3	CX3CR1	CD3
PE-DAZ				CD8
PE-Cy7		CD11B	TNFa	
PE-CF594A	CD3			
Allophycocyanin (APC)	CCR6	Ly6C	IL-4	IL-17
APC 700	CD4	FA/80	CD4	
APC Cy7		CD11C	TCRgd	CD4

Table 2. Markers and dyes used in flow cytometry. Four panels (T cells, myeloid cells, intracellular cytokine for T cells and gd T cells, nuclear factor in Tregs) are included.

Results

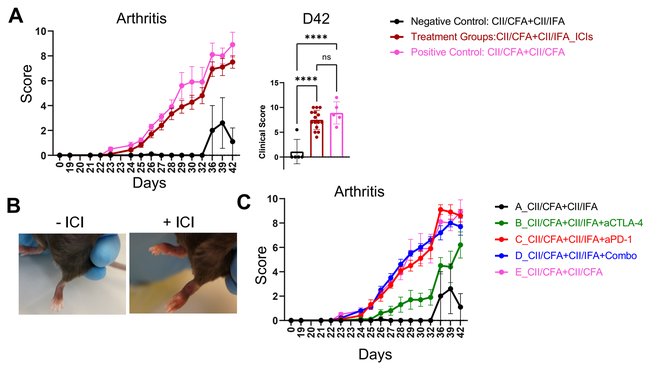


Fig 1. (A) Arthritis score at different time points (left panel). One-way ANOVA test. * P< 0.05, ****P<0.0001. (B) Representative pictures of CII-CFA+CII-IFA mice receiving PBS (no ICI) or ICI. (C) Arthritis score over time based on ICI regimen.

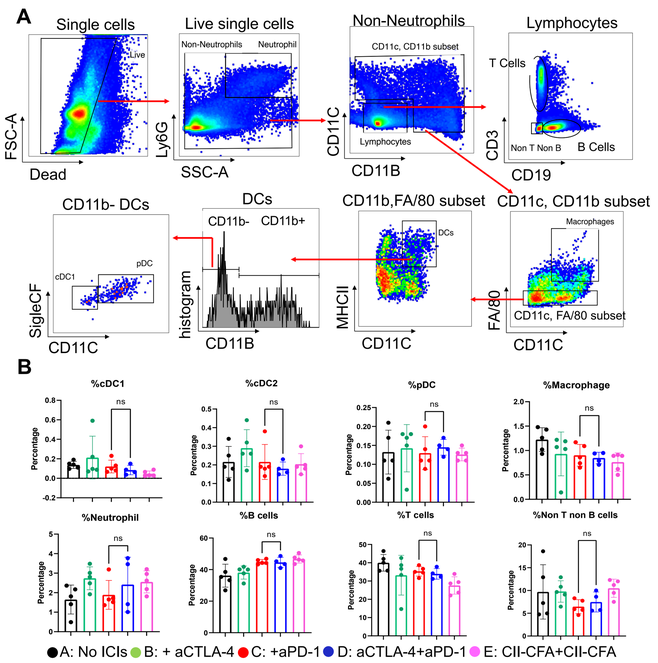


Fig 2. Delineation of major immune cell subsets in spleen. (A) Gating strategy for Flow Cytometry analysis. (B) Quantitative analysis.

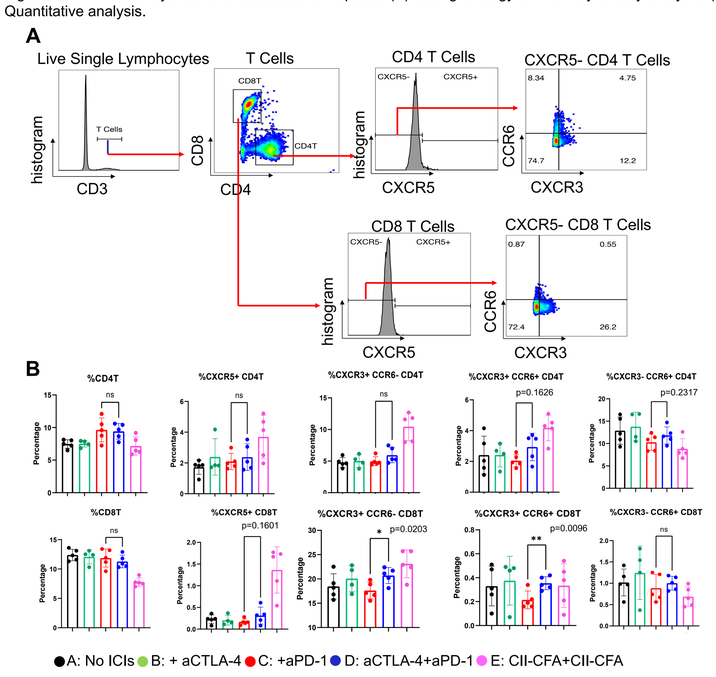
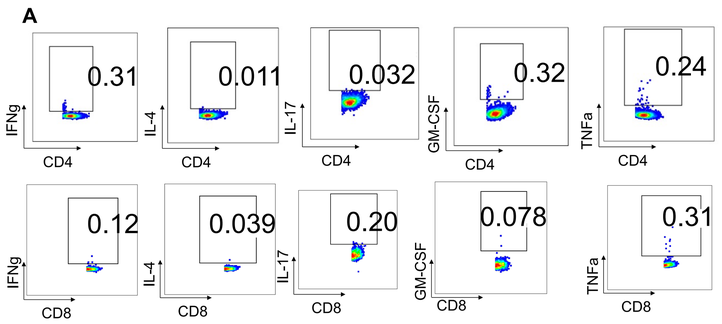


Fig 3. Delineation of major T cell subsets in spleen. (A) Gating strategy for Flow Cytometry analysis. (B) Quantitative analysis.



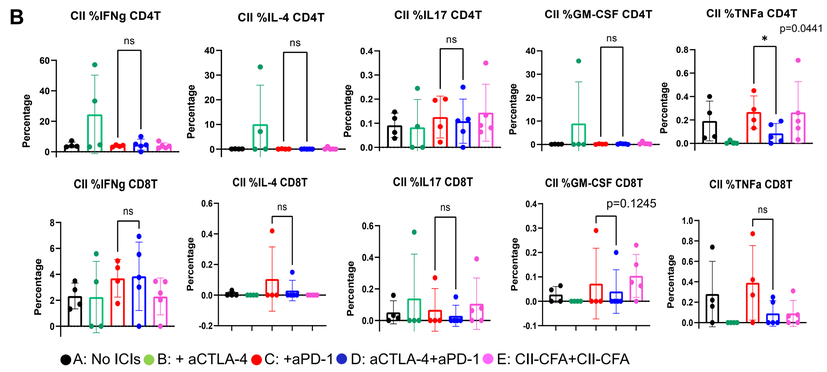


Fig 4. Comparison of cytokine expression by T cells. (A) Gating strategy for Flow Cytometry analysis. (B) Quantitative analysis.

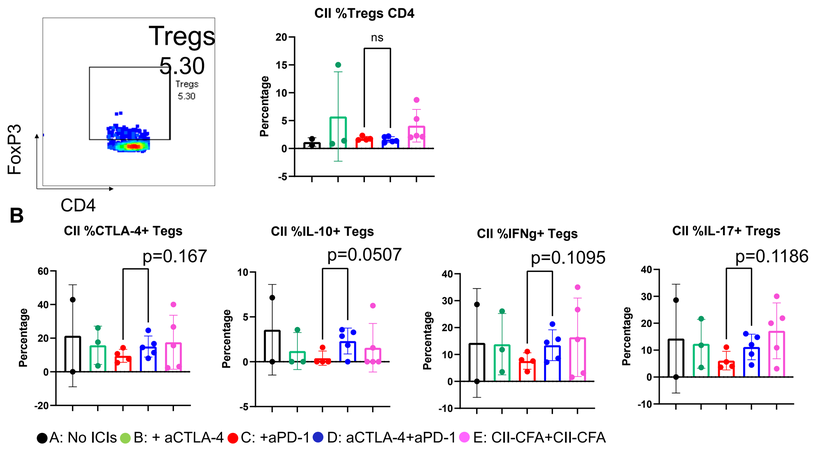


Fig 5. Comparison of Treg cell subpopulations. (A-B) Gating strategy (A) and quantitative analysis of Tregs (A-B).

Conclusion

- Compared with PD-1 inhibitor arthritis group, Th17, Th1.17, CXCR5+ CD8 T cells, Tc1, Tc1.17 were expanded in the combined ICI arthritis group.
- In contrast, TNFa+ CD4 T cells, GM-CSF+ CD8 T cells, both pro-inflammatory and anti-inflammatory Tregs were expanded in PD-1 inhibitor arthritis group.
- Together, like humans, our data suggested that immune profiles underpinning arthritis differ by ICI regimen in our in vivo system.
- We successfully generated in vivo murine model recapitulating the human arthritis-irAE settings. Our model will serve as a powerful tool for us to understand mechanisms underlying arthritis-irAE as well as formulate appropriate therapeutic strategies for arthritis-irAE.

Future Directions

1. Since CTLA-4 monotherapy group developed arthritis rapidly on D32 after first CII immunization, we need to analyze mice before D32
2. Experiment needs to be repeated in order to detect pro-inflammatory and anti-inflammatory Tregs and other intracellular cytokine changes between groups of interest.

Acknowledge

- I am grateful for Dr. Kim's extraordinary mentorship. I am also thankful to Dr. Nurieva and all Dr. Nurieva's lab members for help and support in improving experimental skills.
- This work was supported by the University of Texas MD Anderson Cancer Center Division of Internal Medicine Developmental Funds (STK.), National Institutes of Health (NIH) R01 grants (RN: R01HL141966 and R01HL143520), Cancer Prevention and Research Institute of Texas grant (RN: RP190326), Wilkes Melanoma Foundation grant (STK, RN, PW, and AD), and the CPRIT Research Training Program at MD Anderson Cancer Center (KK and SW).
- I am thankful to the Program Manager, Ms. Brittney Edwards, and Program Directors - Drs. Khandan Keyomarsi, Stephanie Watowich, and Kara Lewis - for CPRIT Research Training Grant for guidance and support.

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