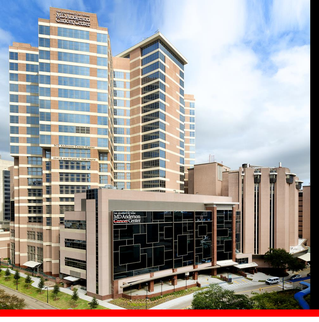

Generation of in vivo mouse model to recapitulate arthritis after ICI therapy

Nigel Blackwood[1, 2], Roza Nurieva[2], Sang Taek Kim[3]

[1]Tulane School of Medicine, New Orleans, LA;
[2]Department of Immunology and [3]General Internal Medicine, The University of Texas MD Anderson Cancer Center[2], Houston, TX

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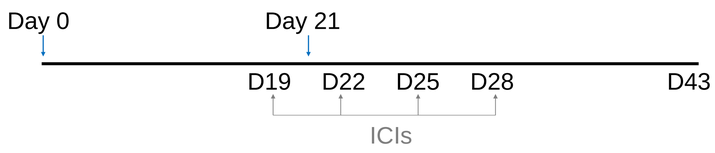
Introduction

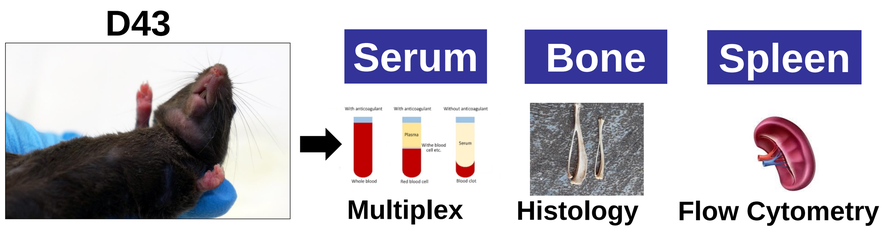
- Immune checkpoint inhibitor (ICI) therapy has revolutionized cancer therapy; however, ICIs cause life/organ-threatening inflammatory phenomenon, termed immune-related adverse events (irAEs). Inflammatory arthritis is the most common rheumatic irAEs, occurring 3-5% of patients receiving ICI therapy.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

Methods



Group	D0	D21	ICIs	Significance
A	CII/CFA	IFA	None	Neg Cont
B	CII/CFA	IFA	aCTLA-4	Group of interest
C	CII/CFA	IFA	aPD-1	Group of interest
D	CII/CFA	IFA	aCTLA-4 + aPD-1	Group of interest
I	CII/CFA	CII/CFA	aCTLA-4 + aPD-1	Pos Cont

Table 1. We immunized chicken collagen (CII) emulsified with complete Freud media (CFA) in 8-10 weeks male C57/B6 mice on Day 0. Mice were re-challenged on Day 21 with incomplete Freud media (IFA). PBS, anti-CTLA-4, and/or anti-PD-1 antibodies were injected on Day 19, 22, 25, and 28. CIA mice (CII-CFA on Day 0 and Day 21) receiving combined ICIs were used as positive controls. The incidence and severity of arthritis were measured until they were sacrificed on Day 43. At sacrifice, we harvested spleen, bones, and serum.



	T Cells	Myeloid	ICS	NF
BV421		SiglecF	IL-17	IL-10
AmCyan	Dead	Dead	Dead	Dead
BV786		Ly6G	IFNg	IFNg
PerCP	CD8	MHCII	GM-CSF	CTLA-4
FITC-A	CXCR3	CD19	CD8	FoxP3
PE-A	CXCR5	CD3	CX3CR1	CD3
Alexa Fluor 700-A	CD4	F4/80	CD4	
PE-Cy7-A		CD11B	TNFa	
PE-CF594A				CD8
APC-A	CCR6	Ly6C	IL-4	IL-17
BUV396				
APC Cy7	CD3	CD11C	TCRgd	CD4

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

Results

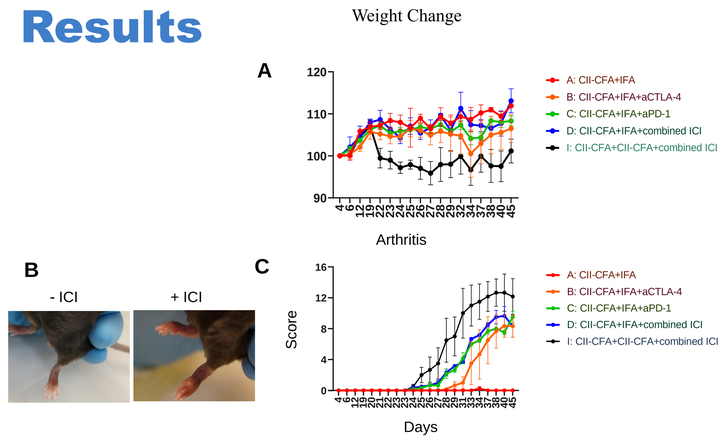


Fig 1. (A) Weight Change over time (B) Representative pictures of CII-CFA+CII-IFA mice receiving PBS (no ICI) or ICI. (C) Arthritis score over time based on ICI regimen.

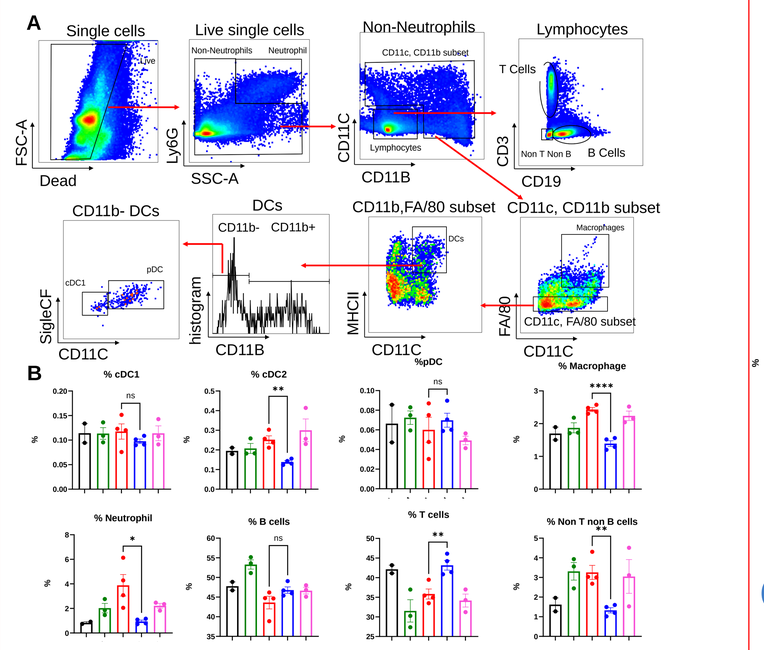


Fig 2. Delineation of major immune cell subsets of spleen. (A) Flow cytometry to show the gating strategy. (B) Quantitative analysis.

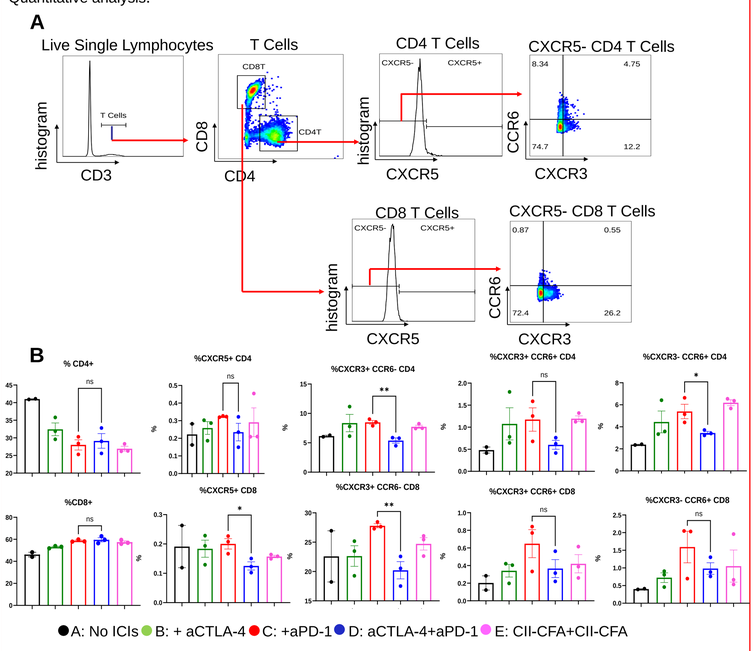
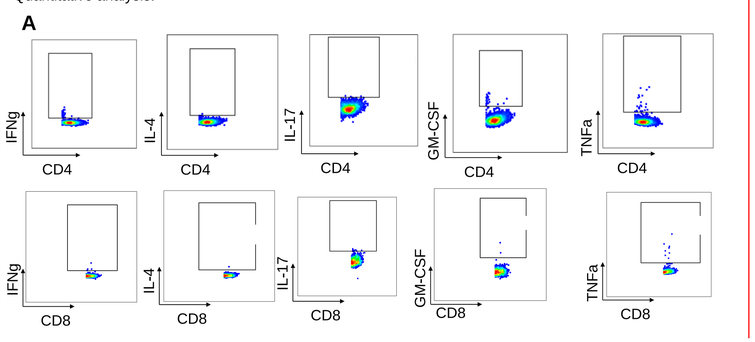


Fig 3. Delineation of major T cell subsets of spleen. (A) Flow cytometry to show the gating strategy. (B) Quantitative analysis.



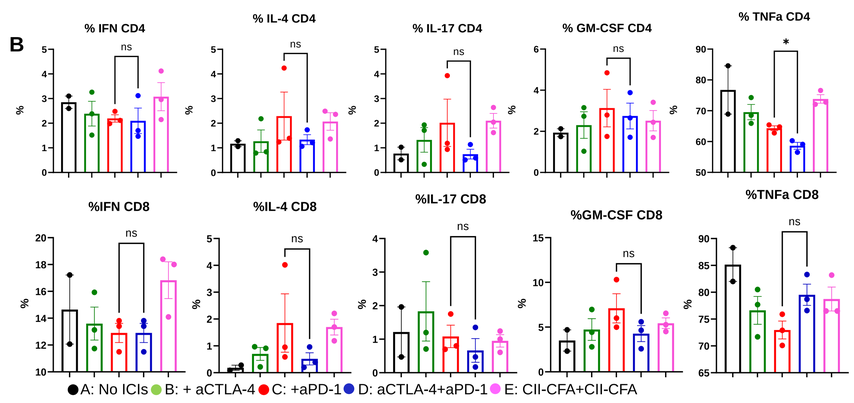


Fig 4. Comparison of intracellular cytokine expression levels. (A) Flow cytometry to show the gating strategy. (B) Quantitative analysis.

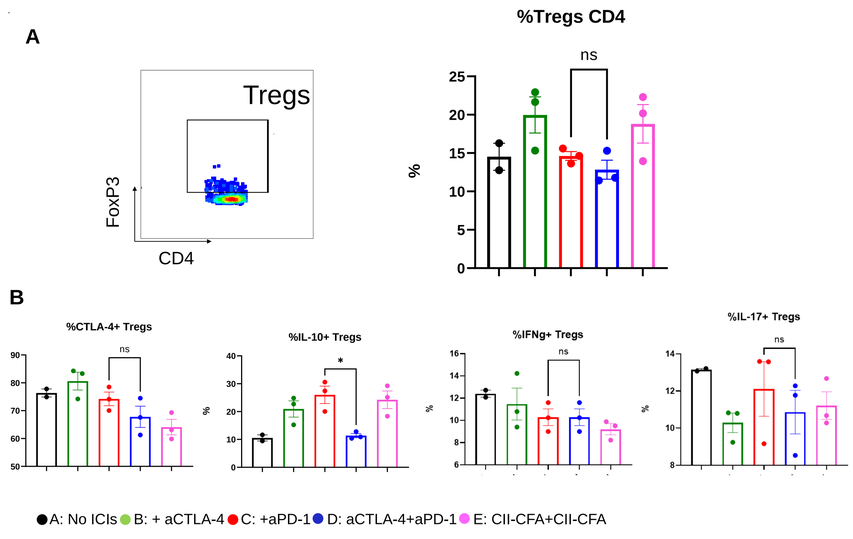


Fig 5. Comparison of Treg subpopulation. (A) Flow cytometry to show the gating strategy and quantitative analysis of all Tregs. (B) Quantitative analysis on anti-inflammatory Tregs .

Conclusion

- ICI induced arthritis in mice receiving CII/CFA and IFA
- CTLA4 monotherapy induced arthritis at later time and was associated with transient weight loss (day 34)

Cell populations expanded in aPD1 arthritis (%)	Cell populations expanded in combined ICI arthritis (%)
cDC2	T Cells
Macrophages	
Neutrophils	
Non T Non B Cells	
CXCR3+ CCR6- CD4+ T Cells	
CXCR3-CCR6+ CD4+ T Cells	
CXCR5+ CD8+ T Cells	
CXCR3+ CCR6- CD8+ T Cells	
TNFa+ CD4+ T Cells	
Tc1 and Tc1.17 CD8+ T Cells	

Future Directions

1. Since CTLA-4 monotherapy group developed arthritis rapidly on D32 after weight loss, we should sacrifice mice before D32
2. *Pending analysis of serum multiplex and bone histology*

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