Intestinal toxicity to CTLA-4 blockade driven by IL-6 and myeloid infiltration

Yihan Zhou1, Lusia B. Maddi, Bharath Palati, Daniel M. Zambuk, Hala El-Sayed, Thomas Czapiga, Austin Glowak2, Elizabeth M. Park1, Radhika Saboock2, Taylor T. Christou3, Laura M. Kahn2, Allison M. Dyevich1, Joseph R. Pecina1, Matthew C. Wong1, Athalya K. Mishra, Samuel H. Gais1, Alejandro P. Cogoli2, Daniel H. Johnson3, Sarah B. Johnson1, Khaliha Wani1, Debona A. Ledesma1, Courtney W. Hudgens1, Jingjing Wang1. 1Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX. 2Department of Geriatric Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX. 3Department of Dermatology, The University of Texas MD Anderson Cancer Center, Houston, TX.

Abstract

Immunotherapies such as anti-CTLA-4 (αCTLA-4) immune checkpoint blockade (ICB) have revolutionized cancer treatment, yet quality of life and continuation of therapy can be constrained by off-target tissue damage or immune-related adverse events (irAEs). At present, there is limited understanding of irAE mechanisms, hampering development of approaches to mitigate their damage. We addressed this problem by generating animal models of intestinal irAE. Our results show that disruption of homeostatic immunity by genetic predisposition to intestinal inflammation or acute gastrointestinal infection sensitizes mice to αCTLA-4-mediated intestinal toxicity. Inflammation-prone mice treated with αCTLA-4 showed neutrophil accumulation, systemic interleukin-6 (IL-6) release, and dysbiosis. Significantly, IL-6 blockade combined with antibiotic treatment improved αCTLA-4 therapeutic efficacy and reduced intestinal irAEs. Immune signatures were validated in biopsies from patients who developed colitis during ICB, supporting the utility of our models. This study provides new pre-clinical models, mechanistic insight into irAEs, and potential approaches to enhance ICB efficacy while mitigating irAEs.

Introduction

Fig. 1 Inflammation-prone mice show exaggerated intestinal inflammation upon αCTLA-4 therapy. (A)Principal component analysis of all biopsies. (B) Estimation of immune subset abundance using expression of cell-type specific marker genes. Data represent relative abundance scores. (C) Heatmap of pathway scores, summarized from data representing pathway-related gene expression with a single score. (D) Volcano plot showing differential gene expression.

Fig. 2 Immune signatures in human intestinal irAE.

Fig. 3 αCTLA-4 remolds the intestinal immune repertoire in inflammation-prone conditions. (A) Stat3mice bearing B16-OVA melanoma were treated biweekly for 2 weeks with IgG or αCTLA-4, beginning 4-6 hours after tumor establishment. (B) Body weight change over time. (C-D) Colon histology by H&E staining. (E) Colon cytokines levels determined by multiplex assay.

Results

Fig. 4 αCTLA-4 drives systemic cytokine release and myelopoiesis in inflammation-prone mice.

Fig. 5 Therapeutic interventions to enhance αCTLA-4 efficacy and suppress irAE.

Fig. 3 (A-C) Stat3mice bearing B16-OVA tumors were treated biweekly for 2 weeks with IgG or αCTLA-4, as indicated in Fig. 1. Colon LP immune cells were subjected to scRNA-seq. (A) UMAP plot showing distinct clusters. (B) Proportion of individual clusters in each experimental group. (C) Expression-module scores of Gene Ontology terms.

Fig. 5 Stat3mice bearing B16-OVA tumor were treated biweekly for 2 weeks with IgG or αCTLA-4, as indicated in Fig. 1. (A, B) Plots were collected prior to or following αCTLA-4 treatment; fecal microbiota composition was determined by 16S ribosomal RNA profiling. (C) (A) B16-OVA bearing Stat3mice on αCTLA-4 were treated with or without broad-spectrum antibiotic (Abx) and an IL6 blocking antibody (IL6), as indicated. Colon histopathological scores and immune profiles were analyzed in mice 4 days following conclusion of therapy.

Fig. 4 (A-E) Stat3mice bearing B16-OVA tumors were treated biweekly for 2 weeks with IgG or αCTLA-4, as indicated in Fig. 1. (A) Mean concentration of differently expressed cytokines in serum. (B) qRT-PCR plot showing tissue-specific expression of key cytokines in cecum, colon, and liver. (C) UMAP plots showing differential gene expression.