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Alyssa Duffy, … , Madhav V. Dhodapkar, Kavita M. Dhodapkar

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Tregs protect against combination checkpoint blockade toxicity induced by T_{PH} and B cell interactions

[1]Alyssa Duffy, [1]Maryam I. Azeem, [1]Smriti Kanangat, [1]Melinda Yushak, [1]David Lawson, [1]Madhav V. Dhodapkar*, [1]Kavita M. Dhodapkar*

[1]Emory University, Atlanta, GA

*share senior authorship

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Correspondence:

Kavita Dhodapkar, MD. Email: kavita.dhodapkar@emory.edu; or Madhav Dhodapkar, MD. Email: madhav.v.dhodapkar@emory.edu. 1365 Clifton Rd, Suite B5100, Atlanta GA 30322

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Immune-related adverse events (irAEs) have emerged as a challenge for combination checkpoint blockade (CCB) in cancer(1). CTLA-4 and PD-1 checkpoints impact both B and T-cell tolerance and both cells have been implicated in irAEs. Early changes in B cells(2) as well as baseline features of CD4+T cells(3) have been correlated with irAEs following CCB therapy. However the nature of specific T cells that help pathogenic B cells and underlying mechanisms remain unknown.

We analyzed blood specimens before/after first cycle of CCB therapy from a cohort of melanoma patients undergoing CCB (clinical characteristics in Supplementary Table1). CCB therapy led to an increase in $CD21^{lo}CD11c+$ B cells as well as plasmablasts and decline in circulating B cells in patients who developed high-grade irAEs (HG-irAE) (Supplementary Fig1A-C)(2). The phenotype of expanded $CD21^{lo}CD11c+$B cells was consistent with CXCR5-T-bet+B cells implicated in extra-follicular B cell responses and autoimmunity (Supplementary Fig1D). FlowSOM analysis identified two distinct metaclusters (MC) of Ki-67+CD4+T cells (MC3 and MC8) and a Ki-67+CD8+MC (MC4) that underwent early proliferation following therapy (Supplementary Fig1E-F). Notably, MC6 containing CXCR5+PD1+CD4+T cells (consistent with T_{FH} cells), did not change following therapy (Supplementary Fig1F-G). Proliferating CD4+T cells expressed ICOS but not CXCR5 (Supplementary Fig1H), similar to T-peripheral-helper (T_{PH}) cells(4). Next we tested the capacity of purified ICOS+ or ICOS- CD4+T cells expanded in CCB-treated patients to induce B-cell differentiation in T:B co-cultures. Addition of ICOS+CD4+T cells (but not ICOS-CD4+T cells) to B cells led to the induction of CD27+CD38+plasmablasts (Fig1A) and h-IgG secretion (Fig1B). Plasmablast induction

was significantly greater in co-cultures with memory versus naïve B-cells (Fig1C). T-cell subsets were adoptively transferred with human B-cells into MISTRG6 mice and monitored for the development of plasmablasts and Ig secretion. Co-injection of B cells with ICOS+CD4+T cells (but not ICOS- counterparts) led to plasmablast differentiation (Fig1D,E) and hIgG production (Fig1F). Together these data demonstrate that CCB therapy leads to proliferation of $CD21^{lo}$CD11c+B cells as well as ICOS+T_{PH}-like cells that help B cells, illustrating enhanced T:B cooperation following therapy.

ICOS+CD4+T-cell activation was associated with secretion of sCD40L, IL21 and interferon-γ (Supplementary Fig2A-C). Plasmablast induction in T:B cocultures was inhibited by the blockade of CD40 (Supplementary Fig2D), IL21 (Supplementary Fig2E) and interferon-γ–mediated signaling (Supplementary Fig2F). Increase in circulating ICOS+CD4+T cells was similar in patients with/without HG-irAE (Fig1G). However ICOS+T cells from patients with HG-irAE had greater capacity to provide help to B cells, suggesting functional differences in ICOS+T cells (Fig1H). FlowSOM analysis of ICOS+CD4+T cells (Supplementary Fig3A-B) revealed that patients without HG-irAEs had a higher proportion of MC6 and MC10 in the post-treatment ICOS+CD4+ T cells which co-expressed FOXP3 and CD25, consistent with Treg phenotype (Fig1I). Patients with HG-irAEs trended towards a higher proportion of a CXCR3+ MC(MC7), expressing PD1 at baseline (Supplementary Fig3B-C). Depletion of Tregs from ICOS+CD4+T cells led to increased plasmablasts (Fig1J). Together these data suggest that Tregs may suppress T:B interactions in patients without HG-irAEs.

The proportion of proliferating CD4+/CD8+ T cells did not differ between patients with/without HG-irAE (2) (Supplementary Fig3D). However, patients with HG-irAE had higher expression of CXCR3 in proliferating CD4+/CD8+T cells and $CD21^{lo}$CD11c+B cells, but not myeloid cells (Supplementary Fig3E). The expression of CXCR3 in ICOS+ CD4T cells correlated with more effector/activated phenotype with higher expression of T-bet and HLA-DR (Supplementary Fig3F).

In summary, we show that CCB therapy leads to expansion of ICOS+T_{PH} cells and $CD21^{lo}CD11c^{+}$ B cells. Both cell types express PD-1 and play an outsized role in human autoimmunity(4). However, irAE development depends on the induction of Tregs, which inhibit T_{PH}:B interactions. Expansion of ICOS+T cells by CTLA-4 blockade has also been implicated in mediating anti-tumor effects, suggesting potential overlap with mechanisms underlying irAEs. CXCR3 has been previously implicated in homing into autoimmune tissues(4). Strengths of this work are analysis of uniformly treated patients and inclusion of in-vitro/in-vivo studies to dissect mechanisms. Limitations include small sample size and lack of analysis of autoimmune tissues. Underlying mechanisms may also differ by specific types of irAEs, which were not studied here. Blockade of specific pathways such as IL21/IL21R-mediated signaling and boosting Tregs may provide novel strategies to prevent or treat CCB-induced irAEs.

Methods:

Biospecimens from melanoma patients undergoing therapy with combination checkpoint blockade were collected following informed consent approved by institutional review board. Details of methods are noted in the supplementary appendix.

Figure Legends:

Figure 1. Mechanisms of T:B interactions. (A-C) T:B cocultures: Purified B cells were cultured alone or with flow-sorted ICOS+CD4+, ICOS-CD4+T cells from melanoma patients post CCB treatment as described in methods. (A) Representative flow plot of CD38+CD27+ plasmablast. (B) Human IgG levels by ELISA (n=9). Kruskal-Wallis with Dunn's multiple-comparisons correction. (C) Impact of B cell subsets: Flow-sorted post-treatment T cells were cultured with either memory(n=5)/naïve B cells (n=6). Bar graphs indicate fold change compared to cultures with B cells alone. One-way Anova with Tukey's multiple-test correction. (D-F) Human T:B interactions in vivo: Sorted ICOS+ or ICOS-CD4 T cells from melanoma patients (n=5) were injected into MISTRG6 mice along with purified human B cells, as described under methods. (D) Representative flow plots of plasmablast frequency in the spleen at two weeks post-injection, quantified in (E). One-way Anova with Dunn's multiple correction. (F) Human IgG levels detected by ELISA in mice from panel E. Wilcoxon matched-pairs signed rank test. (G) Bar graph shows expansion of ICOS+CD4+T cells post-CCB therapy in patients with no-HG-irAEs and those with HG-irAEs. Wilcoxon matched–pairs rank test. (H) B cells were cultured with flow-sorted ICOSpos/neg CD4+T cells from patients with no-HG or HG-irAE. Bar graph shows fold change in plasmablasts in ICOS+ versus ICOS- co-cultures. Mann Whitney (I) FlowSOM performed on ICOS+CD4+T cells obtained following one cycle of combination therapy identified 10 metaclusters (MC). Bar graph shows proportions of these MCs in patients with HG-irAEs or no-HG-irAEs. Mann-Whitney. (J) Depletion of Tregs in patients with no-HG-irAEs leads to enhanced B cell activation by ICOS+CD4+T cells. Purified B cells were cultured with either ICOS-, bulk ICOS+ or Treg-depleted

ICOS+CD4T cells from patients with no-HG-irAEs. Dot plot shows plasmablast frequency as % of B cells. Bar graph shows data for 7 different experiments. Wilcoxon matched–pairs rank test. For all figures *p<0.05, **p<0.01, ***p<0.001 and ns=not significant.

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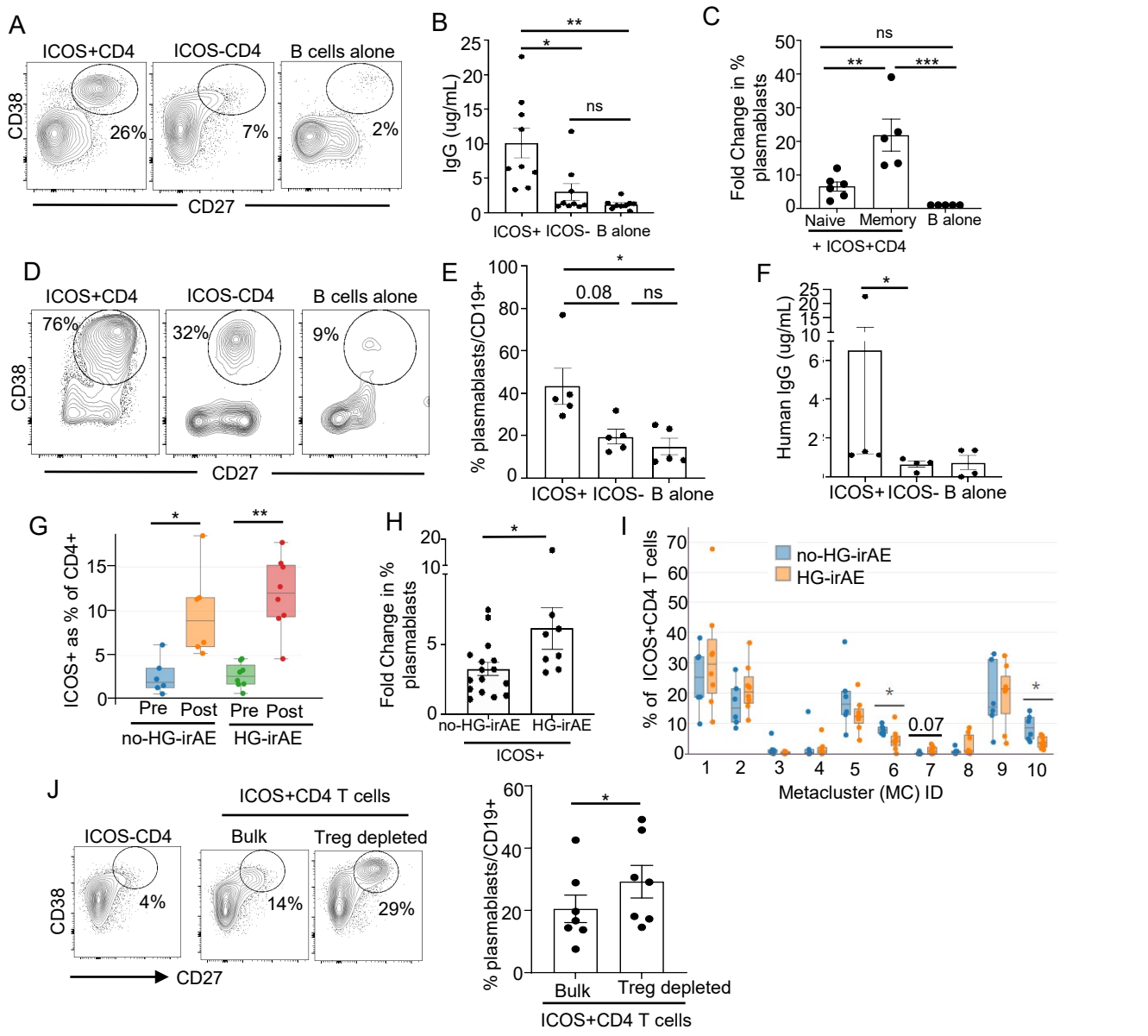
Figure 1



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