

506 **FC γ RIIB EXPRESSED ON CD8 T CELLS LIMITS RESPONSIVENESS TO PD-1 CHECKPOINT INHIBITION IN CANCER**

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Background Immune checkpoint inhibition (ICI) using Fc-containing monoclonal antibodies has emerged as a powerful therapeutic approach to augment anti-tumor immunity. While ICI has drastically improved patient outcomes in melanoma, there is still variability in patient response.^{1, 2} We recently showed that Fc γ RIIB, the only inhibitory IgG-Fc receptor, is expressed on differentiated effector CD8 T cells in mice and humans^{3, 4}, raising the possibility that CD8 T cell responses may be directly modulated by checkpoint inhibitor binding to T cell-expressed Fc γ RIIB.

Methods Flow cytometric phenotyping was performed on PBMCs isolated from melanoma patients and healthy donors. For *in vitro* functional experiments, healthy human PBMCs were stimulated with CD3/28 Dynabeads and/or PMA/ionomycin. Effector function was assessed through intracellular cytokine staining. Anti-PD1 (clone J116) or anti-CTLA4 (clone BN13) were used where mentioned and the anti-PD1 F(ab) was generated from Nivolumab. *In vivo* experiments were performed in mice with B16-hgp100, B16-OVA, or LLC-OVA tumors. Where mentioned, WT OT-I, *Fcgr2b*^{-/-} OT-I, or WT pmel-17 CD8 T cells were adoptively transferred into these mice. For treatment, 250 μ g of anti-PD1 (clone RMP1-14) and anti-Fc γ RIIB (clone 2.4G2) or isotype antibodies were administered for blockade experiments.

Results Here, we show that despite exhibiting strong proliferative and cytokine responses at baseline, human Fc γ RIIB⁺ CD8 T cells exhibited reduced responsiveness to both PD-1 and CTLA-4 checkpoint inhibition compared to Fc γ RIIB⁻ CD8 T cells *in vitro* ($p < 0.05$). Moreover, frequencies of Fc γ RIIB⁺ CD8 T cells were reduced following treatment of human melanoma patients with nivolumab *in vivo* ($p < 0.05$). This reduced responsiveness was Fc γ RIIB-dependent, because conditional genetic deletion of Fc γ RIIB on tumor-specific CD8 T cells improved response to checkpoint blockade in a B16 mouse melanoma model ($p < 0.01$). The limited responsiveness of Fc γ RIIB⁺ CD8 T cells was dependent on an intact Fc region of the checkpoint inhibitor, in that treatment with Fc-devoid anti-PD-1 F(ab) fragments resulted in a significant increase in proliferation of Fc γ RIIB⁺ CD8 T cells, without altering the response of Fc γ RIIB⁻ CD8 T cells ($p < 0.05$). Finally, blocking Fc γ RIIB in the context of PD-1 blockade significantly improved anti-tumor CD8 T cell responses in B16 melanoma and in Lewis lung cancer mouse models ($p < 0.05$, $p < 0.001$).

Conclusions These results illuminate an Fc γ RIIB-mediated, cell-autonomous mechanism of CD8 T-cell suppression which limits the efficacy of checkpoint inhibitors *in vivo*. The data presented here support the novel conclusion that CD8-expressed Fc γ RIIB is both a factor to consider in the development of therapeutic antibodies, and a new potential target for immunotherapeutic intervention.

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Ethics Approval Patients undergoing treatment at Emory University Hospital for advanced stage II-IV melanoma between 2009 and 2019 were enrolled in an immune monitoring protocol approved by Emory University's Institutional Review Board (IRB #00046593). Healthy controls were enrolled after informed consent.

This study was also carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol (PROTO201700558) was approved by the Institutional Animal Care and Use Committee of Emory University. All surgery was performed under general anesthesia with maximum efforts made to minimize suffering.

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