Background Although failure to respond to checkpoint blockade immunotherapies is frequently associated with a lack of T-cell infiltration into the tumor, clinical data suggests that in patients with lung cancer, T-cell-inflamed tumors can also be resistant to therapy. Work by us identified that checkpoint blockade immunotherapy resistance in T-cell-inflamed lung cancer is driven by lung cancer-specific CD8+ T-cell dysregulation, characterized by reduced cytolytic capacity and established during priming in the mediastinal lymph nodes (mLN). In this study, we sought to uncover lung-specific mechanisms that blunt priming of anti-tumor cytotoxic T-cell responses.

Methods To study T-cell priming against lung cancer, we implanted a syngeneic lung cancer cell line (KP) orthotopically in the lungs or subcutaneously in the flanks of C57BL/6 mice. Immune subsets were profiled using flow cytometry, immunofluorescence staining and RNA-sequencing. Immunological mechanism was dissected using adoptive T-cell transfers, bone marrow chimeras and ex vivo co-cultures.

Results Both lung and flank KP tumors resulted in type-1-conventional dendritic cell (DC1)-dependent expansion of tumor-reactive T-cells, however, CD8+ T-cells primed in response to lung tumors in the mLN failed to upregulate key markers of effector CD8+ T-cell differentiation, namely CD25 and Granzyme B. Comparing DC1 from tumor-draining inguinal (iLN) and mLN revealed equivalent antigen load, but reduced expression of CD80, CD86 and IL-12 on mLN-derived DC1. Regulatory T-cell (Treg) depletion rescued both stimulatory molecule expression on DC1 and cytotoxic T-cell priming in the tumor-draining mLN, suggesting that lung CD8+ T-cell dysfunction required the local presence of Treg during priming. Ex vivo co-cultures validated that DC1 and Treg were required and sufficient to induce dysfunctional CD8+ T-cells. This immunosuppression was spatially coordinated within tissue-specific LN microniches and required antigen-specific contact between DC1 and Tregs, as abrogating MHCI-dependent Treg:DC1 interactions restored DC1 capacity to prime cytotoxic T-cell responses against lung tumors. The lung-specific suppression was associated with clonally expanded CXCR3+ T\textsubscript{T\textsubscript{H}1}-like effector Tregs, which were induced upon interferon sensing in the mLN. Consequently, interferon-gamma neutralization early during tumor induction could prevent the immunosuppression and restore cytotoxic T-cell priming in the mLN. Similarly, in cancer patients, interferon-sensing CXCR3+ Tregs but not CD8+/Treg ratios correlated with resistance to checkpoint blockade immunotherapy.

Conclusions Our work suggests that the functional quality of Tregs, specifically the interferon-induced CXCR3+ T\textsubscript{T\textsubscript{H}1}-like effector state, rather than Treg quantity, is instrumental in restraining tumor-reactive T-cell responses and represents a critical barrier to productive anti-tumor immunity.

Acknowledgements This work was supported by the Pew Stewarart scholarship, the Koch Institute Frontier Research program, the Ludwig Center at MIT and the MIT School of Science Fellowship in Cancer Research.

REFERENCES


Ethics Approval All mouse experiments in this study were approved by MIT’s Committee on Animal Care (CAC) – DHHS Animal Welfare Assurance # D16-00078.