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.1 Work by us identified that checkpoint blockade immunotherapy resistance in T-cell-inflamed lung cancer is driven by lung cancer-specific CD8+ T-cell dysfunction, characterized by reduced cytolytic capacity and established during priming in the mediastinal lymph nodes (mLN).2 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 MHCII-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+ T111-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+ T111-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.

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REFERENCES

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