HELPING THE KILLERS: INNOVATIVE CANCER IMMUNOTHERAPY HARNESSING QUASI-UNIVERSAL TUMOR ANTIGEN-SPECIFIC CD4 T CELLS


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TUMOR IMMUNE MICROENVIRONMENT IN ADULT MICE ASYNCHRONOUSLY CROSS-FOSTERED AS PUPS

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Background Immune responses to cancer are highly variable and influenced by genetic and environmental factors.1 Synergistic tumor models in mice with intact immune systems are required to study anti-tumor immune responses but are unable to adequately modeled varied immune responses. Classically, different mouse strain backgrounds have been used to compare different immune responses to cancer immunotherapy, but this approach is limited by the inability to administer identical tumor cell lines, keeping constant the tumor while experimentally varying the immune response. Proper establishment of the immune system begins in early life and is regulated by environmental cues from maternal breast milk and the developing microbiota. To disrupt these cues prior to weaning, newborn pups can be cross-fostered to dams that delivered their litters asynchronously, either 2 weeks earlier or later, a model referred to as asynchronous cross-foster (ACF).2 We previously demonstrated that ACF can profoundly skew the immune profile of genetically identical offspring.2 Young ACF mice exhibited enhanced Th2 immunologic skewing and reduced peripheral tolerance in response to antigen, which resulted from impaired development of peripheral-induced regulatory T cells (pTreg). Adult mice that underwent ACF also exhibited altered systemic cytokine expression even in the absence of immunologic stimuli, suggesting that ACF has lasting impact on the immune system. Because peripheral tolerance and immune skewing directly impact anti-tumor immunity,3 we hypothesized that ACF would also impact the immune response to tumor growth.

Methods To measure impact of ACF on tumor growth and tumor infiltration, we introduced EL4 lymphoma cells into 7-week-old mice with the following foster schemes: conventionally reared mice, 1-day-old pups cross-fostered with 10-day post-partum dam (ACF1 to ppd10), and 13-day-old pups cross-fostered with 1-day post-partum dams (ACF13 to ppd1). Immune infiltration at tumor endpoint was measured using flow cytometry.

Results EL4 tumor growth was increased in ACF mice compared to conventionally-reared controls. Further, the immune infiltrate at endpoint was altered, with ACF mice having fewer natural killer (NK) cells, dendritic cells, and activated cytotoxic CD8+ T cells in the tumor microenvironment.

Conclusions Our observations support the hypothesis that ACF impacts tumor growth and tumor infiltration. Future directions include phenotyping the immune infiltrate with finer resolution, the study of additional tumor models, and investigation of the effects of ACF on spontaneous tumor incidence and immunotherapy efficacy. Development of this novel model could provide valuable insight into early life factors that influence anti-tumor immunity.

Ethics Approval The study was approved by Mayo Clinic’s IACUC approved all uses in this study, approval number A00004845.

REFERENCE
TERMINALLY EXHAUSTED CD8+ T CELLS POTENTIATE THE TOLERGENIC TUMOR MICROENVIRONMENT AS FUNCTIONAL SUPPRESSORS

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Background Blockade of co-inhibitory ‘checkpoint’ molecules, PD-1 and CTLA-4, has induced impressive clinical responses in advanced tumors; yet only in a subset of patients.1–3 Limited success with checkpoint blockade therapy suggests other cell extrinsic or intrinsic mechanisms may be dampening an effective immune response. Cytotoxic CD8+ T cells (CTL) encountering chronic antigen and metabolic restriction can differentiate to a terminally exhausted state (Texh), marked by hyporesponsiveness and metabolic, epigenetic, and transcriptional dysfunction.4–8 When enrichment of this population in tumor is a negative prognostic factor,9–10 it remains unclear whether Texh are simply non-functional or instead possess tolerogenic or suppressive properties. Transcriptional profiling of tumor-infiltrating PD-1int (progenitor exhausted) CTL versus PD-1hiTIM-3+ (terminally exhausted; Texh), reveals that exhausted cells express a pattern of genes associated with immune suppression. We hypothesize that Texh potentiate the suppressive microenvironment of solid tumor by autoregulation and inhibition of local immune responses.

Methods T cell populations were isolated from murine melanoma–B16-F10 or a lab-generated melanoma clone of the spontaneous BREF/PTEN model—by expression of inhibitory receptors and assayed in tandem in microsuspension assays. Murine melanoma clones with inhibited oxidative metabolism were generated by CRISPR-Cas9 deletion and validated for ablated mitochondrial respiration by extracellular flux analysis. Enforced expression of CD39 in effector T cells was attained by murine retroviral vector delivery.

Results When sorted directly from tumor, PD-1hiTim3+ Texh, but not progenitor exhausted PD-1int CTL, induce marked suppression of T cell effector responses, comparable to Foxp3+ Treg from the same environment. Expression of the ectonucleotidase, CD39, is uniquely expressed in Texh and increases as T cells differentiate towards exhaustion. Genetic deletion of CD39 in Texh eliminates the regulatory phenotype of tumor-infiltrating Texh and enforced CD39 expression on effector T cells can inhibit T cell receptor signaling and downstream function. CD39 expression correlates with exposure to hypoxia and Texh sorted from tumors engineered to be less hypoxic displayed a significant loss of suppressive capacity. Our data suggest that tumor hypoxia enforces Hif1α-dependent expression of CD39 which depletes extracellular ATP contributes to generation of immunosuppressive adenosine, and has been previously associated with terminal exhaustion.11–13

Conclusions Our data support a model that as CTL progress to terminal exhaustion, hypoxic exposure enforces the upregulation of CD39, providing Texh a mechanism to suppress proinflammatory processes. These findings suggest Texh are not solely dysfunctional but rather are deleterious to antitumor immunity and may need to be drastically reprogrammed or deleted in order to alleviate immunosuppressive functions.

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CD26 ENZYMATIC ACTIVITY MODULATES EFFICIENT MIGRATION OF ADOPTIVELY TRANSFERRED T CELLS TO SOLID TUMORS

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Background The inadequate ability of adoptively transferred T cells to eradicate solid tumors limits their use in treatments for patients afflicted with those cancers. Efforts to improve ACT for solid tumors aim to identify strategies that poise T cells for optimal response. We have previously identified a specific subset of CD4 T cells which express high levels of the ubiquitous ectoenzyme dipeptidyl peptidase-4 (DPP-4), also known as CD26, that produce a tremendous antitumor response in solid tumor models. We therefore sought to investigate the importance of CD26 on T cells destined for ACT.

Methods We adoptively transferred tumor specific CD26+ T cells into melanoma tumor-bearing CD26-/- mice, and continuously blocked the CD26 enzymatic activity of the donor cells in vivo with sitagliptin, an established competitive inhibitor of CD26.

Results Tumors in sitagliptin-treated mice eventually reached study endpoint, while tumors untreated mice were regressed for 130+ days. Tumor infiltration of donor cells and host CD8 and CD4 cells was diminished with sitagliptin treatment. A 32-plex cytokine array of blood plasma revealed a diminished profile of cytokines and chemokines, indicating that the inflammatory response of the T cells was dampened with sitagliptin treatment. Further experiments characterized the ability of CD26+ T cells to respond to tumor trafficking signals with not solely dysfunctional but rather are deleterious to anti-tumor immunity and may need to be drastically reprogrammed or deleted in order to alleviate immunosuppressive functions.

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