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Murine lung cancer induces generalized T-cell exhaustion

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ABSTRACT

Background: Cancer is known to modulate tumor-specific immune responses by establishing a microenvironment that leads to the upregulation of T-cell inhibitory receptors, resulting in the progressive loss of function and eventual death of tumor-specific T-cells. However, the ability of cancer to impact the functionality of the immune system on a systemic level is much less well characterized. Because cancer is known to predispose patients to infectious complications including sepsis, we hypothesized that the presence of cancer alters pathogen-directed immune responses on a systemic level.

Materials and methods: We assessed systemic T-cell coinhibitory receptor expression, cytokine production, and apoptosis in mice with established subcutaneous lung cancer tumors and in unmanipulated mice without cancer.

Results: Results indicated that the frequencies of programmed death-1-positive, B and T lymphocyte attenuator-positive, and $2B4^+$ cells in both the $CD4^+$ and $CD8^+$ T-cell compartments were increased in mice with localized cancer relative to non-cancer controls, and the frequencies of both $CD4^+$ and $CD8^+$ T-cells expressing multiple different inhibitory receptors were increased in cancer animals relative to non-cancer controls. Additionally, $2B4^+CD8^+$ T-cells in cancer mice exhibited reduced interleukin-2 and interferon-γ, whereas B and T lymphocyte attenuator-positive $CD8^+$ T-cells in cancer mice exhibited reduced interleukin-2 and tumor necrosis factor. Conversely, $CD4^+$ T-cells in cancer animals demonstrated an increase in the frequency of annexin V^+ apoptotic cells.

Conclusions: Taken together, these data suggest that the presence of cancer induces systemic T-cell exhaustion and generalized immune suppression.

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1. Introduction

The mechanisms by which tumors escape the immune system and become invasive is a major focus of cancer research. Such mechanisms include an immune suppressive microenvironment, which may contain T-regulatory cells, myeloid-derived suppressor cells, impaired antigen presentation, and tumor-specific immune cell effector function [1–5]. Tumor microenvironments can lead to the upregulation of inhibitory receptors such as B and T lymphocyte attenuator (BTLA), programmed death-1 (PD-1), and 2B4 (CD244) on T cells [6] resulting in the progressive loss of cell function and eventual death of tumor-specific T-cells. The relative and coordinate expression levels of these and other coinhibitory receptors serve to fine-tune T-cell functionality and determine the profoundness of T-cell exhaustion. However, the ability of cancer to impact the functionality of the immune system on a systemic level is much less well characterized.

Previously, we showed that during an acute systemic bacterial infection, the presence of preexisting pancreatic adenocarcinoma tumors (localized to the inner thigh) resulted in increased phenotypic exhaustion and impaired differentiation of bacterial antigen-specific $CD8^+$ T cells [7], suggesting that cancer may indeed function on a systemic level to impair pathogen-specific T-cell responses. In addition, it is known that the presence of preexisting malignancy is a major risk factor for increased mortality during sepsis. Specifically, preexisting malignancy was noted to be the most common co-morbidity in human septic patients and is associated with a mortality that is nearly 50% higher than patients without cancer [8–10]. Furthermore, septic mice with pancreatic adenocarcinoma tumors have a 24% increase in mortality after sepsis [11]. Because the integrity of the immune system is well known to play a critical role in survival during sepsis, these data suggested that the presence of malignancy may fundamentally compromise the integrity of the immune system on a systemic level and thus affect the pathophysiology of sepsis.

Given this context, we sought to determine the impact of malignancy on phenotypic and functional exhaustion within the $CD4^+$ and $CD8^+$ T-cell compartments, with the hypothesis that localized tumors may function to modulate systemic cellular immunity. Using a murine model of lung cancer, we found that cancer fundamentally altered $CD4^+$ and $CD8^+$ T-cell coinhibitory receptor expression profiles and impaired T-cell functionality on a systemic level.

2. Methods

2.1. Ethics statement

All experiments were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Emory University School of Medicine (Protocol 2001875-082815BN).

2.2. Mice

Adult male 6-wk-old C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). This study was conducted before the NIH mandate that both genders be examined during animal experimentation. After allowing the mice to acclimate for 1 wk, they were randomized to cancer and no cancer groups. Animals were sacrificed at 3 wk after tumor injection, at which point all had palpable tumors, using asphyxiation by CO_2. All animals were housed in the animal facility and had access to chow and water.

2.3. Cancer model

A syngeneic mouse lung cancer line, Lewis lung carcinoma 1 (LLC1), was used to induce tumors (American Type Culture Collection, Manassas, VA). Cells were maintained in 1640 RPMI culture medium supplemented with 10% fetal bovine serum, 1% glutamine, penicillin and/or streptomycin, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. All mice in the cancer group received a subcutaneous injection of 250,000 LLC1 cells suspended in 0.1 mL of phosphate-buffered saline at the inner right thigh. Mice were then housed in the animal facility with free access to water and chow for 3 wk to allow for tumor growth, and all mice with palpable tumors were used for experimentation. Tumor sizes ranged between 1.5–1.7 cm. During the 3 wk of tumor growth, mice were assessed using Emory University's Institutional Animal Care and Use Committee tumor burden scoring guidelines. At no time did any animals meet criteria for euthanasia. Mice in the no cancer group were unmanipulated.

2.4. Phenotypic flow cytometric analysis

Three weeks after injection of cancer cells in the CA group, spleens were collected from WT and CA mice, and single-cell suspensions were prepared for flow cytometric analysis. Cells were stained with anti-CD4-PO (Invitrogen, Waltham, MA), anti-CD8-PB and anti-2B4-APC (eBioscience, San Diego, CA), anti-PD-1-FITC and anti-BTLA-PE (BD Pharmingen, San Jose, CA), and Fc Block (BioLegend, San Diego, CA). TruCount Beads from BD Pharmingen were prepared using manufacturer's instructions and used to determine absolute cell numbers where shown.

2.5. Intracellular cytokine staining

For intracellular cytokine staining, $2–3 \times 10^6$ splenocytes were plated in a 96-well plate. Cells were suspended and incubated in culture medium consisting of RPMI 1640 containing 10% fetal bovine serum (Mediatech, Herndon, VA), 2 mM L-glutamine, 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, 100 mg/mL gentamicin (Mediatech), and 5×10^{-5} M 2-mercaptoethanol (Sigma–Aldrich, St. Louis, MO). To measure production of interferon (IFN)-γ, tumor necrosis factor (TNF), and interleukin (IL)-2, cells were stimulated for 4 h with phorbol 12-myristate 13-acetate (30 ng/mL) and ionomycin (400 ng/mL) with 10 μg/mL of Brefeldin A. After