expression of total eNOS, p-eNOSS1177, total PP2A, and p-PP2AY307. For activity p-eNOSS1177/total eNOS and p-PP2AY307/ total PP2A ratio was used. A two-way ANOVA was used for statistical analysis. RESULTS/ANTICIPATED RESULTS: Irrespective of the donors' race, there was no influence of serum treatment or interaction effect in any of the measured proteins of interest. Moreover, compared to CA, HUVECs from AA had lower expression of eNOS irrespective of condition (race p=0.01). Compared to CA, HUVECs from AA tended to have lower expression of p-eNOSS1177 irrespective of condition (race p=0.07). However, there was no racial differences in eNOS activity (p=0.68). There was no racial difference in the expression of PP2A (p=0.35), p-PP2AY307 (p=0.30), or PP2A activity (p=0.97) in all conditions. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our preliminary results suggest no influence serum constituents from hypertensive donors or race on PP2A or eNOS expression and activity in HUVECS. Future research should consider conducting proteomics profiling to compare NT and HT serum.

39800

Immune Checkpoint Blockade during Periprosthetic Joint Infection

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ABSTRACT IMPACT: If immune checkpoint blockade increases bacterial clearance with or without antibiotics in vitro, clinical application would be almost immediate and dramatic creating a seismic shift in the current therapeutic paradigm of periprosthetic joint infection. OBJECTIVES/GOALS: Periprosthetic joint infection (PJI) is a major cause of failure after joint replacement. Currently, the treatment of PJI relies on removing biofilm contaminated implants. Some of the bacteria within biofilm undergo a phenotypic shift becoming small colony variants (SCVs). SCVs induce local immunosuppression through PD-1/L1 signaling. METHODS/STUDY POPULATION: We will infect cultured human macrophages and bone marrow aspirate with stable Staphylococcus aureus SVCs and treat with anti-PD-1 or anti-PD-L1 monoclonal antibodies with and without antibiotics (e.g., gentamycin, cefazolin, vancomycin, rifampicin) and assess the residual bacterial viability. We will utilize multiplexed ion beam imaging to quantify PD-1/L1 expression in human tissue from patients with a chronic PJI and compare those to patients undergoing an aseptic revision. Patients with a chronic PJI are likely to have increased expression of PD-1/L1 as their tissue samples are prospectively screened. RESULTS/ANTICIPATED RESULTS: SCVs reduce the phagocytic activity of macrophages and can survive intracellularly. SCVs also induce anti-inflammatory M2-macrophage polarization and recruit a heterogeneous group of immature monocytes and granulocytes called myeloid-derived suppressor cells (MDSC) to the periprosthetic microenvironment. M2-macrophages and MDSCs then produce an immunosuppressive cytokine milieu characterized by increased IL-10 and decreased TNF-α. Clinically isolated SCVs up-regulate the expression of PD-L1 and PD-L2 on the surface of macrophages, representing a mechanism by which SCVs induce host immunosuppression and survive immune clearance. Our preliminary data show PD-L1 expression during septic PJI, but not in aseptic revisions. DISCUSSION/SIGNIFICANCE OF FINDINGS: If immune checkpoint blockade is shown to increase bacterial clearance with or without antibiotics, host immunomodulation would represent a novel class of therapeutic adjuvants to assist surgical debridement and antibiotic administration that could be superimposed on existing treatment algorithms to improve PJI related outcomes.

41224

REDUCED FRONTOSTRIATAL FUNCTIONAL CONNECTIVITY IN 41- TO 70-YEAR-OLD ADULTS WITH HIV*

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ABSTRACT IMPACT: The knowledge acquired from my research can inform the development of early diagnostic methods for HIV-associated neurocognitive disorders. OBJECTIVES/GOALS: In the era of combination antiretroviral therapy (cART), the prevalence of HIV-associated neurocognitive disorders (HAND) remains high but the neural mechanisms are unclear. We examined whether older people with HIV (PWH) with minimal cognitive impairment have reduced functional connectivity in frontostriatal circuits compared to controls. METHODS/STUDY POPULATION: 99 PWH (mean age 56.6 years, 75% male, 62% Black, mean duration of HIV-infection 26.2 years ±9.3, 90% viral load <50 copies, 98% on stable cART) and 38 demographically-comparable controls (mean age 54.5 years, 71% male, 58% Black) participated in a cross-sectional study. A 7-domain neuropsychological battery and an Activities of Daily Living index were used to determine HAND diagnoses: 32 PWH met criteria for asymptomatic to mild HAND. Motor skill was assessed using the Grooved Pegboard Test by measuring performance speed. Structural MRI and resting-state functional MRI were collected. Seed-to-voxel analyses were conducted using 4 distinct regions in the striatum as seed regions. We used a voxel threshold of $p<0.001$ and cluster threshold of $p<0.05$ (FDR-corrected) after controlling for demographic variables. RESULTS/ANTICIPATED RESULTS: Compared to controls, PWH had lower resting state functional connectivity between the default mode region of the striatum (i.e., medial caudate) and bilateral superior frontal gyrus, supplementary motor cortex and paracingulate gyrus ($p<0.05$; cluster size: 567 voxels). Also, compared to controls, PWH had reduced resting state functional connectivity between the motor division of the striatum (i.e., posterior putamen) and anterior cingulate cortex and left supplementary motor cortex ($p<0.05$, cluster size: 405 voxels). Performance speed on the Grooved Pegboard motor test negatively correlated with functional connectivity between the motor region of the striatum and supplementary motor frontal regions in all participants (Spearman's rho=-0.18, p=0.04). DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results support the hypothesis that frontostriatal abnormalities are widely present in PWH and might play a key role in HAND development. Our data suggest that dysfunction within the frontostriatal circuits may be involved in motor impairment in PWH, and ongoing inflammation may contribute to motor impairment and frontostriatal injury.

45724

VC2 Oncolytic Virotherapy Induces Robust Systemic Anti-Tumor Immunity and Increases Survival in an Immunocompetent B16F10-derived Mouse Melanoma Model

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ABSTRACT IMPACT: Our data demonstrate that VC2 oncolytic virotherapy has significant clinical potential. OBJECTIVES/

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GOALS: Use our novel oncolytic herpes simplex virus type I (HSV-1), VC2, to understand how oncolytic virotherapy affects the immunosuppressive tumor microenvironment as a mechanism of efficacy. METHODS/STUDY POPULATION: We tested the efficacy of VC2 as an oncolytic virotherapy (OVT) in a syngeneic B16F10-derived mouse model of melanoma. We modified the B16F10 to express nectin-1 (B16F10n-1), the major receptor for HSV-1. Engrafted B16F10n-1 tumors were intratumorally treated with either phosphate-buffered saline (PBS) or 1x10^6 pfu VC2. At indicated time points, treated tumors were excised and processed for immunohistochemistry or flow cytometry analysis. For our experimental metastasis studies, mice were intravenously challenged with B16F10n-1 cells. For our depletion studies, CD4+ and CD8+ T cells were depleted in mice by treatment with mouse anti-CD4 and anti-CD8 monoclonal antibodies respectively, while the control mice were given Rat IgG2b isotype. RESULTS/ANTICIPATED RESULTS: We found that VC2 slowed tumor growth rates and significantly enhanced survival times over control treated mice. VC2-treated mice that survived initial tumor engraftment were able to reject a second tumor challenge and were also resistant to lung colonization (experimental metastasis) of tumor cells. Furthermore, VC2 treatment promoted increased intratumoral T cell infiltration and induced a strong antitumor effect that decreased growth rates of distant, untreated tumors. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our data demonstrate that VC2 OVT has significant clinical potential. Furthermore, due to the increased survival rates and CD8+ T cells dependence, our model will enable study of the immunological correlates of protection for VC2 OVT and OVT in general, as well as to inform the rational design of future OVs with improved therapeutic potentials.

47461

Regulation of the immune response in the tumor microenvironment of lung adenocarcinoma

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ABSTRACT IMPACT: This work will provide a rational approach to improve the efficacy of current immunotherapy approaches in patients that have historically responded poorly to immune checkpoint inhibitors. OBJECTIVES/GOALS: Recent evidence of immunogenic cell death as a predictor of response to therapy has increased the interest in monitoring the presence of damage-associated molecular pattern protein (DAMPs). By regulating DAMP expression, our lab is interested in discovering new ways to improve the patient response rate to immune checkpoint inhibition. METHODS/STUDY POPULATION: Using cultured cell, and a limited number of patient tumors and serum (n=4), we measured intracellular and extracellular levels of DAMP molecule, high mobility group box 1 (HMGB1) using enzyme-linked immunosorbent assays and immunoblots. Immunological assayed were compared to the expression of immune checkpoint molecules PD-1/PDL1 on patient tumors as presented in pathology reports. RESULTS/ANTICIPATED RESULTS: HMGB1 release was associated with increased levels of PD-L1 on tumor cells. Targeted inhibition of HMGB1 altered the expression of programmed death-ligand 1 (PD-L1), a target for immune checkpoint inhibition therapy. Patients with higher levels of PD-L1 possessed increased levels of HMGB1 in serum. DISCUSSION/SIGNIFICANCE OF FINDINGS: This implies that regulating the expression of HMGB1 could have an effect on the response of patients to immunotherapy. The main objective of the work is to determine the potential benefit of targeting HMGB1 to improve the efficacy of current therapeutic approaches to treating lung cancer.

48019

Create a mouse model of chronic sleep deprivation by specific-neuron targeted ablation

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ABSTRACT IMPACT: A mouse model of minimally-invasive chronic sleep deprivation is essential for elucidating the impact of sleep deprivation on various health issues, and it would lead to the possibility of sleep as a therapeutic target. OBJECTIVES/GOALS: The lack of sleep has been associated with various health conditions. In mice, sleep deprivation has been achieved mainly by physical disturbances, which raises concern about confounding effects by stresses. Without physical disturbance, targeted neuron ablation can address this methodological flaw. METHODS/STUDY POPULATION: AdultVgat-IRES-cre mice undergo a stereotaxic injection of adeno-associated virus (AAV) vector containing mCherry-dtA to bilateral parafacial zone (PZ) to perform GABAergic neuron-specific cell ablation. Control mice receive an injection of AAV vector containing hSyn-DIO-mCherry. All mice are implanted with electroencephalogram and electromyogram (EEG/EMG) electrodes for sleep-wake analysis. After 7-10 days of the postoperative recovery period, mice are kept individually in a cage for sleep-wake state recording. EEG/EMG and video recording are used to measure total wake time, total sleep time, percent of rapid eye movement (REM) and non-REM sleep, and detailed characterization with spectral analysis. RESULTS/ANTICIPATED RESULTS: We anticipate that the ablation of GABAergic neurons in bilateral PZ decreases the fraction of sleep state in mice, especially non-REM sleep. In the Vgat-IRES-cre mice that received the injection of AAV vector containing mCherry-dtA, total sleep time is expected to be decreased constantly during the 8-week observation period. Sleep-wake staging by video activity recording is anticipated to be closely correlated with the gold standard staging by EEG/EMG. Possible stresses caused by the restriction of physical activity and handling of mice for EEG/EMG recording can be further minimized by the sleep-wake staging performed with the video activity recording. DISCUSSION/SIGNIFICANCE OF FINDINGS: The lack of sleep has been associated with negatively affecting overall health and is implicated in major health conditions including obesity, diabetes, and cardiovascular diseases. This chronic sleep deprivation mouse model can be used to understand the mechanisms of such detrimental effects on health, and would improve many health conditions.

49483

Evaluating the Role of IFNLR1 Receptor Dynamics and Plasticity in Regulating Cellular Response to Interferons

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ABSTRACT IMPACT: We hope to provide a more nuanced understanding of the type-III IFN system, thereby exploring its therapeutic

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