investigate whether excitatory ECS of the infarcted cortex or inhibition of the noninfarcted cortex combined with daily impaired-forelimb rehabilitative training (RT) results in greater motor functional recovery compared to RT alone. Immunohistochemical (IHC) analyses and unbiased stereological techniques will be performed to investigate changes in proteins associated with dendritic restructuring (MAP2), synaptic plasticity (PSD95 and synaptophysin), and alteration in the expression of BDNF and NOGO-A. RESULTS/ANTICIPATED RESULTS: We expect that inhibitory ECS of the noninfarcted motor cortex will improve behavioral outcomes in moderate to severe stroke animals compared with excitatory ECS or no stimulation (RT alone) animals. We predict that the ECS condition that improves motor performance most significantly compared with RT alone will have a corresponding greater increase in remaining ipsi-infarct motor cortical dendritic and synaptic plasticity (demonstrated by a greater density of MAP2, synaptophysin, and PSD-95 immunoreactivity), and greater expression of BDNF. It is unknown, but also expected that better behavioral recovery will coincide with a greater reduction in NOGO-A in the injured motor cortex. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies will aid in creating a model that will allow for a better understanding of the relationship between brain stimulation, severity of injury and, in future studies, aging. These studies will also help clarify previous conflicting brain stimulation results.

2395

Self-assembling cartilage from equine mesenchymal stem cells: A comparison of bone marrow and cord blood-derived MSCs

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OBJECTIVES/SPECIFIC AIMS: Joint injury is a common cause of premature retirement for many equine athletes. Implantation of engineered cartilage offers the potential to increase the success rate of surgical intervention and hasten recovery times. Mesenchymal stem cells (MSCs) offer a particularly attractive cell source for cartilage engineering. Although bone marrow-derived MSCs (BM-MSCs) have been most extensively characterized for musculoskeletal tissue engineering, studies suggest cord blood MSCs (CB-MSCs) may elicit a more robust chondrogenic phenotype. The objective of this study was to determine superior equine MSC source for cartilage engineering via a self-assembling process (SAP). METHODS/STUDY POPULATION: MSCs derived from bone marrow or cord blood were stimulated to undergo chondrogenesis via 3D culture and then used to generate cartilage via SAP. The resulting neocartilage produced from either BM-MSCs or CB-MSCs was compared by measuring biochemical, mechanical, and histological properties. RESULTS/ANTICIPATED RESULTS: We found that while BM-MSCs possessed higher tensile properties and collagen content, CB-MSCs had superior compressive properties and GAG content. Moreover, CB-MSCs had lower alkaline phosphatase activity and higher collagen type II, suggesting a more hyaline cartilage-like phenotype. DISCUSSION/SIGNIFICANCE OF IMPACT: In conclusion, while both BM-MSCs and CB-MSCs were able to form neocartilage, CB-MSCs resulted in tissue more closely resembling native equine articular cartilage, and is therefore the superior MSC source for purposes of cartilage self-assembly.

2420

Loss of eptB decreases systemic inflammation during *Salmonella* infection and allows for evasion of the host immune response

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OBJECTIVES/SPECIFIC AIMS: Our long-term goal is to elucidate the molecular mechanisms and virulence factors that control the differential presentation of infection with Salmonella typhimurium and Salmonella typhi. The objectives of this study are to study the mechanisms that enable S. typhi to trigger a decreased inflammatory response in comparison with S. typhimurium and evade detection by the immune system, leading to the development of asymptomatic chronic carriage of S. typhi. METHODS/STUDY POPULATION: A loss of function eptB mutant S. typhimurium strain was generated. Lipopolysaccharide (LPS) was isolated from wild-type and eptB mutant S. typhimurium and wild-type S. typhi. Binding of LPS to recombinant intelectin was tested by dot blot and enzyme-linked immunosorbant assay (ELISA). C57BL/6 mice were infected with wild-type or eptB mutant S. typhimurium by oral gavage and inflammatory cytokines in the spleen, liver, and Peyer's patches were measured by qPCR. RESULTS/ANTICIPATED RESULTS: LPS isolated from wild-type S. typhimurium is not bound by intelectin, a protein that has been proposed to function in innate immunity and that is known to be able to bind certain moieties within LPS. Conversely, LPS isolated from eptB mutant S. typhimurium and wild-type S. typhi, which lacks a functional eptB, is bound by intelectin. Mice infected with an eptB mutant S. typhimurium exhibit decreased expression of inflammatory cytokines in the spleen compared to mice infected with the wild type S. typhimurium, suggesting that loss of eptB function allows a nontyphoidal Salmonella serovar to mimic the stealth phenotype of typhoidal serovars. Together, these results suggest that loss of eptB function allows intelectin to bind to and detoxify Salmonella LPS, leading to decreased systemic inflammation during infection. DISCUSSION/SIGNIFICANCE OF IMPACT: These results have broad implications for how pathogens such as S. typhimurium induce systemic shock during infection and may also help to explain a mechanism for how S. typhi is able to evade immune detection and enhance dissemination to systemic sites, leading to development of the asymptomatic chronic carrier state. Further investigation of this novel virulence mechanism will mark a decisive step forward in understanding the mechanisms underlying the differential pathogenesis of S. typhimurium-induced gastroenteritis and S. typhi-induced typhoid fever. Additionally, these results contribute to our understanding of the interactions between host and pathogen in affecting disease presentation, which will have wide appeal among researchers interested in microbial pathogenesis and the contribution of host-pathogen interactions to health and disease.

2422

Magnetic nanoparticles facilitate tracking of dendritic cells for treatment of malignant brain tumors

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OBJECTIVES/SPECIFIC AIMS: Immune-based therapies hold great promise for treatment of refractory tumors. However, development is limited by a lack of identified immune correlates to vaccination. We recently showed that dendritic cells (DCs) prolong progression-free survival (PFS) and overall survival (OS) in patients with glioblastoma, and that DC migration to site draining lymph nodes robustly correlates with both PFS and OS. While this appears to be a reliable immune correlate, the complexity of routine labeling for PET and SPECT prohibits validation in a large clinical study. We therefore seek to develop a safe, translatable reporter that can be imaged with a widely available imaging modality. METHODS/STUDY POPULATION: The cationic liposome 1,2-doleoyl-3-trimethylammonium-propane (DOTAP) was loaded with MRI-imageable iron oxide nanoparticles (IONPs) with or without the neutral molecules PEG and cholesterol. The resulting nanoparticles were loaded with RNA to form RNA-NPs that were characterized with transmission electron microscopy (TEM) and used to transfect DCs in vitro; 4.7 T MRI was then used to image particles or cells in agarose gel phantoms. RESULTS/ANTICIPATED RESULTS: TEM images of RNA-NPs indicate the presence of IONP-loaded liposomes. In vitro transfection experiments demonstrate that iron oxide does not reduce RNA-NP-mediated transfection of DCs. Additionally, small amounts of either PEG or cholesterol within RNA-NPs increased transfection of DC2.4s and enhanced T-cell priming by bone marrow-derived dendritic cells. A series of 4.7 T MRI images of particles in cells, spleens, and LNs demonstrated quantifiable differences in particle density between groups. DISCUSSION/SIGNIFICANCE OF IMPACT: This data suggests that IONP-loaded RNA-NPs can be imaged with MRI and manipulated to augment DC function. Future work will include in vivo imaging in mice and safety studies to facilitate translation into first-in-human studies. Successful completion of this project would provide a powerful clinical tool to improve and track patient responses to immune therapy.

2427

Metabo-therapy for RARRES1-depleted epithelial cells using repurposed mitochondrial metabolism inhibitor, metformin

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OBJECTIVES/SPECIFIC AIMS: The goal of this study is to examine bioenergetic phenotype of retinoic acid receptor responder 1 (RARRES1)-depleted epithelial cells and to facilitate the discovery of personalized metabo-therapeutics in the context of cancers characterized with loss of or low expression of RARRES1. METHODS/STUDY POPULATION: Anoikis assay and annexinV labeling were used to assess drug resistance and apoptotic phenotype in RARRES1-depleted epithelial cells. Metabolomics, AMP kinase activity, mito-tracker, and extracellular flux assays were used to examine the bioenergetic profile of

https://doi.org/10.1017/cts.2017.49 Published online by Cambridge University Press

RARRES1-depleted epithelial cells. Extracellular flux assays were used to assess the phenotype of RARRES1-depleted epithelial cells treated with or without metformin. RESULTS/ANTICIPATED RESULTS: RARRES1 is a major regulator of mitochondrial function. Its depletion in tumors induces an oxidative phosphorylation dependent phenotype and subsequently increases ATP abundance in the cell, enhances anabolic pathways and increases survival. Treatment with FDA approved mitochondrial respiration inhibitor, metformin, reversed the metabolic phenotype of RARRES1 depleted-epithelial cells. Metformin could be the ideal therapeutics to reduce tumor burden in cancers with loss of or low expression of RARRES1. DISCUSSION/SIGNIFICANCE OF IMPACT: Bioenergetic dynamics are emerging as a basis for understanding the pathology of cancer. The malignancy progresses as its metabolic pattern and mitochondrial respiration become more dysfunctional. The regulatory pathways of bioenergetic dynamics are currently poorly understood, and the characterization of proteins implicated in those processes must be assessed. One understudied protein and tumor suppressor is RARRES1. RARRES1 is induced by retinoic acid (a major metabolic regulator) and functions as a putative carboxypeptidase inhibitor. Understanding the connection between this carboxypeptidase inhibitor and intermediary metabolism will enlighten our understanding of the bioenergetic profile of cells and facilitate the discovery of personalized metabo-therapeutics in the context of cancer.

2436

Disagreement in middle ear volume values between tympanometry and 3-dimensional volume reconstruction

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OBJECTIVES/SPECIFIC AIMS: Middle ear volume (MEV) is a clinically relevant parameter in the treatment of many common conditions including otitis media, tinnitus, and conductive hearing loss. A growing number of studies have shifted from using tympanometry to 3-dimensional volume reconstruction (3DVR) to calculate MEV; however, MEV values between these methodologies have never before been directly compared. Here, our objective is to characterize agreement between MEV measurement methods across disease states and middle ear sizes. METHODS/STUDY POPULATION: Middle ears were identified from 36 patients ranging 18–89 years of age who underwent tympanometry testing during preoperative workup for tympanic membrane (TM) perforation, up to 1 month prior to a standard-of-care temporal bone computed tomography (CT) between October 15, 2005 and October 15, 2015. MEV values calculated by both tympanometry and 3DVR were analyzed for agreement using Bland and Altman plots. A correction factor was calculated where ear canal volumes were available for contralateral middle ears without TM perforation (n = 12), and was applied to a second Bland and Altman plot in the corresponding patient subgroup. MEV agreement was characterized across MEV quartiles (1 = smallest; 4 = largest) and across increasing states of middle ear disease using Kruskal-Wallis and Wilcoxon testing with Bonferonni correction. RESULTS/ANTICIPATED RESULTS: A Bland Altman plot demonstrated significant disagreement of MEV differences as compared to a priori clinical thresholds. Absolute MEV difference was significantly greater in the average MEV fourth to first quartile ($p = 0.0024$), fourth to second quartile ($p = 0.0024$), third to first quartile ($p = 0.0048$), and third to second quartile ($p = 0.048$). Absolute MEV difference was not significantly different across varying states of middle ear disease ($p = 0.44$). DISCUSSION/SIGNIFICANCE OF IMPACT: Statistically evident and clinically significant disagreement was demonstrated across tympanometric and 3DVR MEV estimates. This lack of agreement was most pronounced at higher average MEV and was persistent yet not appreciably different across varying severities of middle ear disease. These findings may limit the generalizability of studies of the middle ear that differ in MEV estimation methodology, particularly in pathophysiological states where MEV is increased.

2504

Defining peripheral B cell tolerance in pemphigus vulgaris

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OBJECTIVES/SPECIFIC AIMS: Pemphigus vulgaris (PV) is a potentially fatal blistering disease caused by autoantibodies to the keratinocyte adhesion protein desmoglein 3. Several other autoimmune diseases have defective B cell tolerance checkpoints, resulting in the accumulation of self-reactive and polyreactive B cells. METHODS/STUDY POPULATION: The present work aims to determine whether PV patients develop normal tolerance to self-antigens other than desmoglein 3, as a potential "first hit" in the development of autoimmunity. We use FACS to isolate single B cells at 4 developmental stages from 8 PV patients. We perform single-cell RT-PCR to amplify each B cell receptor, produce monoclonal antibodies, and screen these for autoreactivity using ELISA/IF to several self-antigens. At each B cell stage, we compare the frequencies of self-reactive and polyreactive B cells to those found in healthy controls. RESULTS/ANTICIPATED RESULTS: We anticipate similar frequencies between PV patients and controls, suggesting that the B cell repertoire in PV patients develops normally at early checkpoints. DISCUSSION/SIGNIFICANCE OF IMPACT: The absence of generalized reactivity would distinguish PV from other autoimmune diseases and would show that PV arises from a specific break in tolerance to a single self-antigen (desmoglein 3) during late B cell maturation. Such a result would further support PV as an ideal candidate for targeted immunotherapy.

2508

The role of platelet factor-4 (PF4 or CXCL4) in B cell differentiation

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OBJECTIVES/SPECIFIC AIMS: To investigate the role of platelet factor-4 (PF4) in B cell differentiation and develop strategies to better modulate B cell differentiation in vitro and in vivo. METHODS/STUDY POPULATION: We use tissue culture and flow cytometry to examine the role of PF4 in B cell differentiation. We use wild type (WT) and $PF4^{-/-}$ mice on a C57Bl6/J background. $PF4^{-/-}$ mice have reduced in vivo B cell differentiation responses. RESULTS/ANTICIPATED RESULTS: We anticipate that our studies will demonstrate that PF4 promotes B cell differentiation in the bone marrow microenvironment. DISCUSSION/SIGNIFICANCE OF IMPACT: The significance of this project may be valuable in developing efficient methods and strategies to increase or limit B cell numbers in vitro and in human disease.

2509

Estimation of HIV viral load using quantitative measurement of HIV-p24 on lateral flow immunoassays

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OBJECTIVES/SPECIFIC AIMS: High-sensitivity diagnostics for early infant diagnosis (EID) of HIV at the point of care (POC) are not widely available. Lateral flow immunoassays (LFA) can detect HIV-p24, but are not sensitive enough in practice. With improvements, LFA are a compelling platform for POC in EID. We used functionalized magnetic beads and immunocomplex dissociation to improve sensitivity of HIV-p24 LFA. Here, we evaluate the utility for LFA to quantitatively report HIV-p24 concentration and estimate HIV viral load. Using purified p24 protein and virion constructs, we determined the limits of detection for HIV-p24 using LFA rapid tests. Using measurements from HIV-p24 ELISA, laboratory-developed RT-qPCR, droplet digital PCR, and gold standard clinical viral load, we further characterized the relationship between HIV-p24 concentration, HIV genomic RNA, and LFA test line signal. METHODS/STUDY POPULATION: We measured HIV-p24 concentration by ELISA (R&D Systems) and LFA (Alere Determine HIV-1/2 Ab/Ag Combo). An LFA reader instrument was used to image test lines and measure test line signal on the LFA. HIV viral loads were measured using RT-qPCR and droplet digital RT-PCR protocols adapted in our lab. We obtained gold standard viral load measurements using the Roche Cobas TaqMan system at Vanderbilt University Medical Center. Data analysis was performed using Prism 7 and Stata 14. RESULTS/ANTICIPATED RESULTS: LFA test line signal increases in a predictable, dose-dependent manner and correlates with concentration of purified HIV-p24 with a linear range between 50 and 1000 pg/mL (Spearman $r = 1$; $p = 0.0004$). We compared p24 concentration (ELISA). We evaluated the utility of LFA to quantify HIV-p24 from virions suspended in human plasma, which increased the limit of detection for HIV-p24 to 100 pg/mL and shifted the linear range 100–10,000 pg/mL (Spearman $r = 0.77$; $p < 0.001$). To evaluate the relationship between HIV-p24 concentration and concentration of HIV RNA, we employed 3 molecular techniques. The LFA is capable of detecting HIV-p24 concentrations that correspond to a range of viral loads between 653,000 and

https://doi.org/10.1017/cts.2017.49 Published online by Cambridge University Press