investigate whether excitatory ECS of the infarcted cortex or inhibition of the
noninfarcted cortex combined with daily impaired-for-limb rehabilitative
training (IT) 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 synapto-
phsin), 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.

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

Jamie L. White, Jerry C. Hu, Dori L. Borjesson and Kyriacos A. Athanasiou

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 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 showed superior compressive
properties and IACG 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.

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

Lillian F. Zhang, Fabian Rivera-Chavez, Hirotaka Hiyoshi and Andreas J. Baumler

OBJECTIVES/SPECIFIC AIMS: Our long-term goal is to elucidate the molecular
mechanisms and virulence factors that control the differential presentation
of *Salmonella* typhimurium and wild-type *Salmonella* typhi. Binding of LPS to recombinant intelectin
is proposed to function in innate immunity and that is known to be able to bind
specific moieties within LPS. Conversely, LPS isolated from eptB mutant
*Salmonella* typhimurium exhibit decreased expression of inflammatory cytokines in the spleen compared to mice infected
with the wild type *Salmonella* typhimurium, suggesting that loss of eptB function allows a
non-typhoidal *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 *Salmonella*
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 *Salmonella* typhimurium-induced gastroenteritis and *S. typhi*-induced typhoid fever.

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

Adam Grippin, Elias Sayour, Jon Dobson and Duane A. Mitchell

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 catonic liposome 1,2-
dioleoyl-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 transfected 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.

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

Sara Maimouni, Mi-Hye Lee and Stephen Byers

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: Anokiy assay and annexinV labeling were
used to assess drug resistance and apoptotic phenotype in RARRES1-depleted
epithelial cells. Metabolomics, AMP kinase activity, mioto-tracker, and extracellular
flux assays were used to examine the bioenergetic profile of