methods to define TCR specificity after melanoma patients treated with TCR engineered T-cells suffered from fatal cardiovascular toxicity arising from the unpredicted recognition of a muscle-specific peptide. METHODS/STUDY POPULATION: To address this drawback to T-cell-based immunotherapies, we developed a novel protein engineering approach to define the peptide specificity of a given TCR. Here, directed evolution in a yeast display system produced a large scale peptide library, where recognition by the melanoma reactive DMF5 TCR acted as the guiding selective pressure. After this technique identified a panel of putative cross reactive peptides, sequence analysis and computational modeling followed by kinetic binding experiments and structural analysis determined the DMF5 TCR recognizes 2 distinct classes of peptides through chemically distinct mechanisms. RESULTS/ANTICIPATED RESULTS: This information led to the rational, structure-based design of additional cross reactive peptides and introduced a unique approach to screen the human proteome and identify the TCR targets which triggered undesired autoimmunity when this molecule was used in clinical trials. DISCUSSION/SIGNIFICANCE OF IMPACT: The distinct chemical nature of the 2 peptide classes suggest TCRs are more cross reactive than previously thought, presenting an obstacle to cell-based immunotherapy. Defining the peptide specificity of TCRs is of high interest to the immunology community, and will lead to improved approaches to designing engineered TCRs for cell therapy.

2074

Comparing the properties of human umbilical cord-derived mesenchymal stromal cells from preterm Versus full-term infants

Alvaro Moreira, Caitlyn Winter, Saloni Balgi, Shamimunisa Mustafa, Lauryn Winter and Peter Hornsby

OBJECTIVES/SPECIFIC AIMS: To compare functional differences in WJ-MSCs-derived from term Versus preterm infants. METHODS/STUDY POPULATION: WJ-MSCs were enzymatically digested from umbilical cord tissue from Term (gestational age ≥37 wk, n = 4) and Preterm (gestational age ≤32 wk, n = 5) neonates. Cells were characterized by (1) surface antigen markers using flow cytometry, (2) ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages following in vitro stimulation, (3) colony forming unit efficiency, (4) proliferation rates, and (5) cell motility assay. RESULTS/ANTICIPATED RESULTS: WJ-MSCs were successfully isolated from both Preterm and Term groups. Cells adhered to plastic and displayed characteristic spindle-shaped morphology when cultured under standard conditions. WJ-MSCs from both groups expressed surface antigen markers CD73, CD90, and CD146 (≥90%) and did not express hematopoietic markers HLA-DR, CD79, or CD117 (<5%). Preterm and Term cells were capable of differentiating into osteogenic, chondrogenic, and adipogenic lineages. There were no significant differences between the groups when evaluated by colony forming efficiency, proliferation rates, or cell motility. DISCUSSION/SIGNIFICANCE OF IMPACT: These preliminary findings suggest that WJ-MSCs derived from full-term or preterm neonates have similar functional characteristics. Future studies will focus on the regenerative potential of WJ-MSCs from preterm and term infants following changes in the microenvironment (eg, oxygen tension, inflammatory stimulation).

2078

NGF and TNF-α contribute to oral cancer pain by regulating pro-inflammatory cytokines

Yi Ye, Jihwan Kim, Brian L. Schmidt, Donna G. Albertson and Bradley E. Aouizerat

H+H Clinical and Translational Science Institute, New York, NY, USA

OBJECTIVES/SPECIFIC AIMS: We hypothesize that both NGF and TNF-α contribute to oral cancer pain by upregulating pro-nociceptive inflammatory cytokines. METHODS/STUDY POPULATION: In total, 48 oral cancer patients were evaluated and their pain scores were measured using a validated oral cancer pain questionnaire. Presence of perineural invasion (PNI) was identified from patients' pathology reports. We utilized The NIH Cancer Genome Atlas (TCGA) Head and Neck Cancer cohort to investigate the association between pain and genes related to NGF, TNF-α, and their receptors (TRKA, P75, TNF-α receptor 1, and TNF-α receptor 2) in oral cancer samples by employing PNI as a surrogate for pain. Demographic characteristics, clinical characteristics, and genes were analyzed using logistic regression models. A xenograft cancer pain model was created by inoculating human oral cancer cells (HSC-3) into the mouse hind paw. Mice (n = 6 per group) were treated with anti-NGF alone, anti-TNF-α alone, a combination of anti-NGF and anti-TNF-α, or PBS (vehicle control). Nociceptive behaviors were measured using an electronic paw withdrawal assay. Paw volume was measured using a plethysmometer. Cytokines in the paw tissues were measured using a multiplex assay kit with 28 cytokines. RESULTS/ANTICIPATED RESULTS: Oral cancer patients with PNI report significantly more pain compared with patients without PNI in our patient cohort ($p < 0.05$). From analysis of TCGA data, we found that PNI is significantly associated with lymphovascular invasion, pathological nodal invasion, and pathological tumor staging (all $p < 0.05$). In adjusted models, we observed that the NGF receptor p75NTR (NGFR) and the TNF-α receptor 1 (TNFRSF1A) were associated with PNI (both $p < 0.05$) and significantly correlated to each other ($r = 0.25$, $p < 0.001$). High levels of TNF-α were present in HSC-3 cell lines and the mouse xenograft cancers. In mice with cancer pain, combined treatment with anti-NGF and anti-TNF-α together provided more effective pain control compared with either anti-NGF or anti-TNF-α treatment alone ($p < 0.05$). We found significantly increased levels of MIP3a, IL-1b, IL-2, IL-4, IL-28b, IL-23, IL17a, IL-31, and IL-33 in cancer mice compared with normal mice (all $p < 0.05$). The combination therapy significantly reduced cytokines MIP3a, IL-1b, IL-4, IL-28b, IL-31, and IL-33 (all $p < 0.05$). DISCUSSION/SIGNIFICANCE OF IMPACT: We show that targeting both NGF and TNF-α provides more effective pain relief in an oral cancer model. These results suggest that therapeutic strategies aimed at both pathways could yield improved pain management for oral cancer patients.

2080

Therapeutic potential of mesenchymal stromal cells for hypoxic ischemic encephalopathy: A systematic review of preclinical studies

Alvaro Moreira, Jamie Archambault, Dawn McDaniel and Peter Hornsby

OBJECTIVES/SPECIFIC AIMS: To assess the efficacy of exogenous administration of MSCs in animal models of HIE. METHODS/STUDY POPULATION: Adhering to the Systematic Review Protocol for Animal Intervention Studies, a systematic search of English articles was performed using MEDLINE, Web of Science, CINAHL, and Google Scholar. Search term items included mesenchymal stem/stromal cell, hypoxic ischemic encephalopathy, asphyxia, cerebral ischemia, and neonatology. We selected randomized and nonrandomized studies that examined in vivo neonatal models of induced HIE. We excluded studies that combined MSCs with an adjunct therapy. Data were collected on study specifics, MSC characteristics, and outcome measurements. The primary outcome was efficacy of MSC treatment, assessed by functional neurologic measures (cognitive, motor, sensory). Risk of bias was assessed using the SYRCLE's risk of bias tool and we used the ARRIVE guidelines to describe the quality of study reporting. RESULTS/ANTICIPATED RESULTS: A total of 17 preclinical publications focusing on MSC therapy for HIE met our inclusion criteria. Fifteen of the studies (88%) induced HIE in rodents by ligating the common carotid artery followed by a period of hypoxic exposure. Nine (53%) studies derived their MSCs from rodent bone marrow, whereas the other investigators provided xenografts from human bone marrow or umbilical cord-derived MSCs. Range of MSC dose was between 0.25 and 3.5 × 106 cells with 71% of the experiments transplanting the MSCs intranasally or intracerebral. Three studies (18%) administered multiple doses. The cylinder rearing test was the most common (73%) sensorimotor functional outcome performed in the first month following the establishment of HIE. All studies demonstrated a reduction in asymmetrical paw preference after receiving MSC therapy. Lesional size was assessed, using neuroimaging or histologic evaluations, and the majority of studies showed a decreased insult following MSC therapy. DISCUSSION/SIGNIFICANCE OF IMPACT: MSC treatment demonstrates improved functional and structural outcomes that are encouraging for future translational studies.

2081

Phenotypic characterization of the CD4+ T-cell response during anti-CTLA4 therapy with ipilimumab in melanoma patients

Farha Sherani, Duane Moogk, Anuj Bapodra, Karolina Malecek, Una Moran, Yesung Lee, Iman Osman and Michelle Krogsgaard

OBJECTIVES/SPECIFIC AIMS: To characterize the CD4+ T-cell response during CTLA-4 blockade immunotherapy with ipilimumab in patients with metastatic melanoma by correlating cytokine profiles with phenotypic changes in the intratumoral lymphocyte compartment of tumor biopsies obtained before and after treatment. METHODS/STUDY POPULATION: Peripheral

https://doi.org/10.1017/cts.2017.199 Published online by Cambridge University Press

blood mononuclear cell samples were obtained from patients with metastatic melanoma undergoing monotherapy with ipilimumab via the Interdisciplinary Melanoma Cooperative Group at New York University Langone Medical Center. We isolated CD4+ T-cells and used a cytometric bead array assay following in vitro activation with anti-CD3, anti-CD28 antibodies to characterize cytokine expression profiles by quantifying IFN gamma, IL-2, IL-4, IL-6, IL-10, IL-17, and TNF-α at 5 time points during therapy. In total, 53 peripheral blood samples were included from 12 patients. To correlate cytokine profiles with CD4+ T-cell phenotypes in the intratumoral lymphocyte compartment, multiplex immunofluorescence was performed using CD4, CD8, CCR7, CD45RO, and FOXP3 antibodies on tumors before and after treatment with ipilimumab. RESULTS/ANTICIPATED RESULTS: Patients with evidence of clinical benefit (CB), as defined by having achieved partial response or stable disease, were compared with nonresponders (NR). All patients had an increase in IFN-γ, IL-2, and IL-10 secretion by CD4+ T-cells during ipilimumab therapy. NR had a statistically higher increase in all 3 cytokines. Mean IL-10 secretion was 22.3-fold higher compared with patients with CB (p value 0.0458; 95% CI = 0.6676–43.89). Mean IFN-γ secretion was 12.4-fold higher from baseline levels in NR compared with CB (p value 0.046; 95% CI = 0.3589–24.35). Mean IL-2 secretion was 6.9-fold higher in NR compared with CB (p value 0.032; 95% CI = 0.9688–12.75). There were no statistically significant differences seen in the secretion of IL-4, IL-6, IL-17, or TNF-α. Multiplex immunofluorescence for immune profiling of 20 pre and post treatment tumor biopsies is ongoing. We expect to see distinct intratumoral lymphocyte compartment changes which correlate with clinical response and the above described differential cytokine profiles. Specifically, we anticipate CB patients will have increased intratumoral effector T-cells and decreased regulatory T-cells when compared with their NR counterparts. DISCUSSION/SIGNIFICANCE OF IMPACT: Cytokine expression profiles of peripheral blood CD4+ T-cells have not been previously correlated with patient response in patients undergoing treatment with ipilimumab. We describe distinct secretion profiles for IFN-γ, IL-2, and IL-10 for CB Versus NR patients. NR had a statistically higher increase in IL-10, an inhibitory cytokine which typically indicates upregulation of regulatory T-cells and consequent immune escape. Increased secretion of IL-2 and IFN-γ suggests skewing towards a Th1 type, anti-tumor effector T-cell response; these cytokines increased with ipilimumab treatment in both patient groups. However, the mean increase was several fold higher in NR. Recent evidence suggests loss of the interferon gamma pathway in tumor cells confers resistance to anti-CTLA4 therapy. Chronic IFN-γ secretion is associated with an exhausted T-cell phenotype and impaired tumor rejection. Therefore, higher increases in IFN-γ secretion by CD4+ T-cells in NR suggest impaired IFN-γ dependent tumor rejection in these patients. Our findings suggest IFN-γ, IL-2, and IL-10 cytokine expression profiles can be useful as biomarkers for response to ipilimumab treatment.

2092

Chronic branched-chain amino acid ingestion aggravates hilar neuron loss in a rodent model of temporal lobe epilepsy

Shaun Evan Gruenbaum, Roni Dhaher, Amedeo Rapuano and Tore Eid

OBJECTIVES/SPECIFIC AIMS: We previously developed a translationally relevant model of temporal lobe epilepsy (TLE) in which glutamine synthetase is irreversibility inhibited by methionine sulfoximine (MSO), resulting in spontaneous seizures and dentate hilar neuron loss. The objective of this study was to determine the effects of chronic BCAA ingestion on neuronal viability in the dentate hilus in the MSO model of TLE. METHODS/STUDY POPULATION: Sixteen rats were randomly divided into 2 groups: 8 rats drank a 4% aqueous solution of all 3 BCAAs (BCAA group) ad libitum for 31 days, and the other 8 rats drank regular water (control group) for the same period. After 10 days of drinking, a microinfusion cannula (Alzet osmotic pump, model 2004) was surgically implanted in the right dentate gyrus to continuously infuse MSO at a rate of 0.625 g/hour for 28 days. After 31 days of drinking, rats were perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde in phosphate buffer. The brains were removed and fixed, sectioned on a Vibratome at 50-μm thickness, and were mounted on a gelatin-coated slides and stained with NeuN. Neuron counts in the hilar region were performed ipsilateral and contralateral to the infusion site using a stereological technique. RESULTS/ANTICIPATED RESULTS: Rats in the BCAA group had 37% fewer neurons in the ipsilateral dentate hilus than the control group ($5.8 \times 10^{-4} \pm 6.8 \times 10^{-5}$ vs. $8.9 \times 10^{-4} \pm 5.6 \times 10^{-5}$ cells, respectively, $p < 0.01$). Similarly, rats in the BCAA group had 39% fewer neurons in the contralateral dentate hilus than the control group ($5.0 \times 10^{-4} \pm 5.8 \times 10^{-5}$ vs. $7.0 \times 10^{-4} \pm 3.4 \times 10^{-5}$ cells, respectively, $p = 0.01$). DISCUSSION/SIGNIFICANCE OF IMPACT: This study demonstrates that chronic ingestion of BCAAs aggravates hilar neuronal loss in a translationally relevant rodent model of MTLE. This study gives important insight into how BCAAs may affect neuronal viability. Although the role of BCAAs on seizure activity is poorly understood, these results suggest that BCAAs may play an important role in neurochemical modulation and neurotoxicity.

2097

Aging-associated increases in platelet granzyme A regulate pro-inflammatory gene synthesis by monocytes

Matthew Thomas Rondina, Robert A. Campbell, Anish Bhatnagar, Zechariah Franks, Jesse W. Rowley, Bhanu Kanth Manne, Mark A. Supiano and Alistair N. Ward

The University of Utah School of Medicine, Salt Lake City, UT, USA

OBJECTIVES/SPECIFIC AIMS: Platelets govern signal-dependent inflammatory responses by leukocytes. Although dysregulated inflammation is common in older adults, platelet-leukocyte signaling events and downstream inflammatory gene synthesis in aging is not known. METHODS/STUDY POPULATION: Highly-purified platelets and monocytes were isolated from healthy older (age > 60, n = 27) and younger (age < 45, n = 36) adults and incubated together in autologous and nonautologous conditions. Inflammatory gene synthesis by monocytes, basally and in the presence of activated platelets, was examined. Next-generation RNA-sequencing allowed for unbiased profiling of the platelet transcriptome in older and younger adults. Differentially expressed candidates in aged platelets were validated and recombinant granzyme A (in the presence and absence of TLR4 and Caspase-1 inhibition) identified putative ligands controlling inflammatory gene synthesis. RESULTS/ANTICIPATED RESULTS: In unstimulated or activated conditions, monocyte chemoattractant protein 1 (MCP-1) and interleukin-8 (IL-8) synthesis by monocytes alone did not differ between older and younger adults. However, in the presence of autologous activated platelets, monocytes from older adults synthesized significantly greater MCP-1 (867.150 vs. 216.36 ng/mL, $p < 0.0001$) and IL-8 (41.5 vs. 9.2 ng/mL, $p < 0.0001$) than younger adults. Nonautologous, or switch experiments, demonstrated that aged platelets were sufficient for upregulating MCP-1 and IL-8 synthesis by monocytes. Surprisingly, classic platelet proteins known to signal to monocytes and induce MCP-1 synthesis (p-selectin, RANTES, and PF4) were not increased in platelets from older adults. Using RNA-seq followed by validation via RT-PCR and immunoblot, we identified candidate platelet molecules increased in aging that mediate platelet-monocyte signaling and pro-inflammatory gene synthesis. We confirmed that granzyme A (GrmA), a serine protease not previously identified in platelets, is present in human platelets at the mRNA and protein level. GrmA is secreted by activated platelets in signal-dependent fashion. Moreover, GrmA in platelets is significantly increased in aging (~9-fold vs. younger adults). Blocking GrmA inhibited MCP-1 and IL-8 synthesis in older adults. Finally, we uncovered that platelet GrmA signaling to monocytes is regulated through TLR4 and Caspase-1. DISCUSSION/SIGNIFICANCE OF IMPACT: Human aging is associated with reprogramming of the platelet transcriptome. A previously unrecognized protein in platelets, GrmA, is increased in aging and causes increased MCP-1 and IL-8 gene synthesis by target monocytes in a TLR4 and Caspase-1 dependent mechanism. Increased platelet GrmA in aging may contribute to injurious inflammatory responses common in older adults.

2098

Endogenous reverse transcriptase (LINE-1) in human platelets regulates cell morphology and protein synthetic events

Hansjorg Schwertz, Jesse W. Rowley, Larry W. Kraiss, John V. Moran, Robert A. Campbell, Guy A. Zimmerman, Andrew S. Weyrich, Matthew Thomas Rondina, Gerald G. Schumann and Ulrike Thorack

The University of Utah School of Medicine, Salt Lake City, UT, USA

OBJECTIVES/SPECIFIC AIMS: Endogenous RT (eRT) is necessary for the function of retrotransposons, elements that replicate via an RNA intermediate. One source of eRT activity is long interspersed elements (LINE). LINEs, of which there are several subgroups (L1, L2, L3), are retrotransposons that regulate cellular growth and gene expression. Given their diverse and important roles, we hypothesized that L1 elements regulate functional responses in megakaryocytes and platelets; a concept not yet examined in the field. METHODS/STUDY POPULATION: To study eRT in human platelets we used RT activity assays, PCR, and Western blot approaches. Furthermore, we used an RT-inhibitor to dissect the function of eRT, analyzed RT-dependent protein synthetic capacity, and immunoprecipitated RNA-DNA hybrids. RNA-DNA hybrids were also detected by means of ICC and automated analysis using CellProfiler software. RNA-DNA hybrids were validated by PCR and eRT

https://doi.org/10.1017/cts.2017.199 Published online by Cambridge University Press