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.

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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.

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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.

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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

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