motor command at the level of the spinal motoneuron in people with multiple sclerosis (MS). This information will provide insight into neural mechanisms of motor dysfunction and their heterogeneity among patients with MS. METHODS/STUDY POPULATION: Due to advances in high-density surface EMG (HDsEMG) decomposition and the recent development of a paradigm for reverse engineering of motor unit population discharge, we can feasibly estimate aspects of excitatory, inhibitory, and neuromodulatory components of the voluntary motor command in humans on a person-specific basis. We tested 11 ambulatory patients with MS and mild-moderate disability. We recorded HDsEMG from tibialis anterior (TA) and soleus (SOL) during isometric plantarflexion and dorsiflexion, performed as slow triangle contractions. EMG was decomposed into motor unit spike trains using blind source separation. We calculated a number of motor unit variables, most notably delta-F, which estimates motoneuron excitability and the balance of neuromodulatory and inhibitory inputs. RESULTS/ANTICIPATED RESULTS: There were consistent differences in MS patients vs. controls. For TA, values were decreased for delta-F (3.9 vs. 5.9 pps), initial firing rate acceleration (5.8 vs. 7.1 pps), firing rate range (9.3 vs. 11.9 pps), and max firing rate (12.3 vs. 15.0 pps). SOL had more modest decreases in delta-F (3.0 vs. 3.8 pps) and firing rate range (4.8 vs. 5.6 pps). Self-sustained firing was longer for MS patients. Within a patient, abnormalities in motor unit variables were not consistent across muscles and legs. Interestingly, there were several abnormalities in the patients with a normal clinical motor exam, indicating that perhaps our measures are sensitive to subclinical changes in processing of voluntary motor commands. DISCUSSION/SIGNIFICANCE: Excitatory, inhibitory, and neuromodulatory components of the voluntary motor command must be appropriately balanced for skilled motor output. This study is the first to characterize how they are disrupted in MS, providing foundational information to inform the development of mechanistically-based rehabilitation interventions.

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## Hydroxypropyl beta cyclodextrin barrier prevents respiratory and eye viral infections

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OBJECTIVES/GOALS: Susceptible mucocutaneous membranes of the eye and nasal cavity are easily infected by viruses leading to pink eye or respiratory infections whose direct cost has been estimated as $16 billion annually in the United States. We have developed a novel and effective barrier that will be agnostic to variants enveloped viruses like coronaviruses. METHODS/STUDY POPULATION: We evaluated the efficacy of hydroxypropyl cyclodextrin barrier in preventing respiratory coronavirus infections using 25 humanized angiotensin converting enzyme-2 receptor (hACE-2) mice under a BSL3 laboratory setting. We have shown the barrier is safe and efficacious in preventing coronavirus infections in in vitro respiratory cell lines. We instilled 10 uL aliquot of the barrier into the nostril of the mouse 30 minutes before exposing them to a 10uL titer containing 10,000 plaque forming units of the SARS-CoV-2 delta variant. The control mice received the SARS-CoV-2 infection but not the barrier. The mice were observed for 5 days after which they were sacrificed. We analyzed the lungs and nasal palates for viral load using reverse transcription-polymerase chain reaction. RESULTS/ANTICIPATED RESULTS: We observed our barrier to be effective in preventing SARS-CoV-2 delta variant infection in hACE2 mice models. The lungs and nasal secretions of treated mice were less infectious with lower viral load than the control mice. The lungs of treated mice showed decrease in IFN gene expression and many cytokines and chemokines that regulate virally induced inflammatory responses such as IL-1b, IL-8, CXCL9, CXCL10, and the CCLs. We observed the plasma Angiotensin I and Angiotensin II decreased with barrier treatment, correlating with the viral load observed in the lungs. These peptides may be useful biomarkers for monitoring viral load within the lungs of virally infected individuals. DISCUSSION/SIGNIFICANCE: This supports the barrier's efficacy to reduce transmission and prevent infections of SARS-CoV-2. This easy to use barrier can augment the mucocutaneous layers of the eye and nasal cavity. Our agnostic barrier will reduce the economic and public health burden of seasonal respiratory and eye viral infections and their related deaths amongst the public.

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## Characterizing the single-cell transcriptomes of fetal natural killer cells isolated from the umbilical cord of fetuses exposed to human cytomegalovirus during gestation†

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OBJECTIVES/GOALS: Congenital cytomegalovirus (cCMV) remains to be the leading infectious cause of fetal anomalies. The role of fetal natural killer (NK) cells during cCMV remains largely unknown. The objective of this study is to define the transcriptomes of fetal NK cells exposed to human cytomegalovirus (HCMV) infection during gestation. METHODS/STUDY POPULATION: Four sets of umbilical cord blood and matching umbilical cord tissues were collected from two HCMV seropositive (HCMV+) and two HCMV seronegative (HCMV-) fetuses that did not experience any complications during gestation. These samples were provided by the Medical College of Wisconsin Tissue Bank and were processed within 24 hours following live birth. CD7+ CD3e-CD14-CD19-CD20- fetal NK cells were isolated, using the BD FACSAria sorter. Following cell sorting, single-cell RNA sequencing (scRNA-seq) was performed, and cDNA libraries were constructed and sequenced via NextSeq 550. Cell Ranger was then used to algin the cDNA reads and the Seurat R package was used to analyze the transcriptional data. Cells were filtered and clustered based on the number of uniquely expressed genes. RESULTS/ANTICIPATED RESULTS: Four sets of umbilical cord blood and matching umbilical cord tissues were collected from two HCMV+ and two HCMV- fetuses. We were able to successfully sort and capture fetal NK cells and perform


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scRNA-seq on these samples. Following unbiased clustering, we observed and characterized five distinct fetal NK cell subsets in the umbilical cord blood and four fetal NK cell subsets in the corresponding umbilical cord tissue. Our findings revealed that HCMV+ fetal NK cells primarily consisted of mature NK cell subsets, while HCMV- fetal NK cells constituted the majority of the immature subsets. Importantly, we identified a unique subset of NKG2CHi fetal NK cells that were significantly elevated in the HCMV+ fetuses. Finally, we defined a group of transcription factors involved in the formation of antiviral fetal NK. DISCUSSION/SIGNIFICANCE: Here, we demonstrate that HCMV infection can induce the formation of distinct NK cell subsets and drive their unique transcriptional profiles. These findings have the potential to guide the development of an innovative NK cell immunotherapy that could help prevent fetuses from developing symptomatic cCMV.

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## BiP knockdown decreases antibody production in malignant and non-malignant plasma cells

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OBJECTIVES/GOALS: Numerous diseases, including AL amyloidosis, are due to expression of aberrant antibodies. Significant effort has gone into plasma cell toxic therapies with varying degrees of success, but no therapies preventing antibody synthesis have been developed. The goal of this study is to assess BiP targeting to prevent antibody secretion in plasma cells. METHODS/STUDY POPULATION: Using 4 multiple myeloma cell lines (KMS11, RPMI8226, ANBL-6, U266), we knocked down BiP expression with RnaseH dependent siRNA or subA toxin, a bacterial toxin that specifically cleaves BiP, and measured changes in unfolded protein and intracellular light chains by flow cytometry during drug induced ER stress created by the intracellular calcium depleting agent thapsigargin. BiP is the master regulator of the unfolded protein response (UPR), an ER stress pathway important for protein folding. BiP is also an ER resident protein folding chaperone important for proper antibody folding. We hypothesized that BiP downregulation will lead to decreased folded antibody in the cell, increased unfolded antibody and constitutive activation of the UPR. RESULTS/ANTICIPATED RESULTS: 1 to 4 hours after treatment with thapsigargin plus siRNA against BiP, levels of BiP are significantly decreased. The levels of intracellular light chains decrease, and the level of unfolded protein within the cells increases dramatically. Interestingly, in alignment with the UPR literature, 24 hours post treatment, these levels have normalized again in surviving cells. SubA treatment increased BiP expression by 4 hours, contrary to our hypothesis, and minimally increased unfolded proteins and minimally decreased intracellular light chains. We expect that further functional testing of antibody secretion by ELIspot assays will show decreased secretion of antibody with BiP siRNA treatment. Combination therapies with other UPR stressing agents may act synergistically to affect antibody production. DISCUSSION/SIGNIFICANCE: BiP knockdown reduces antibodies and boosts unfolded proteins. SubA toxin ineffectiveness likely stems from increased BiP due to feedback loops. Combining anti-BiP treatments with UPR stressing drugs like bortezomib may halt antibody synthesis and induce cell death. These findings support BiP as a viable drug target for antibody-related diseases.

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## Unitary neural correlates of self-control in pediatric transdiagnostic psychopathology*†

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OBJECTIVES/GOALS: Childhood psychopathology is a worsening public health crisis leading to negative life outcomes, including self-harm and suicide. Difficulty in self-control as early as 3 years old predicts psychopathology, but the mediating mechanisms of brain function are unknown. Here, we tested one mechanism: functional connectivity (FC) integration. METHODS/STUDY POPULATION: We studied a sample of 204 children [53 F/149 M/2 NC; mean age (SD)=11 years (1.7)] with diverse self-control difficulties (e.g., attention deficit disorder [n=80]; autism spectrum disorders [n=91]). We extracted a general factor of psychopathology ("p-factor") from the parent-reported Child Behavior Checklist. For participants with high quality fMRI data on 3 self-control tasks (n=79), testing flexibility, working memory, and inhibition, we calculated FC connectomes reflecting a general self-control state, and applied connectome predictive modeling (CPM) to reveal connections predicting overall task impairment. We then measured individual variance in cross-network integration of regions with the most predictive connections and tested for association with p-factor in a multiple linear regression. RESULTS/ANTICIPATED RESULTS: We repeated CPM 1,000 times with 10-fold cross validation to generate a distribution of accuracies for predicted vs. observed task impairment scores (mean r=0.25, permutation p=0.02). Connections selected a maximum of 10,000 times (10 folds * 1,000 repetitions) were strongly predictive of task impairment (r=-0.5, p<0.001), highlighting connectivity of canonical executive networks as well as the default mode network. Regions (n=22) with the top 5% most selected connections were in lateral parietal and frontal cortices and implicated motor control. Between-network integration, operationalized with the graph theory metric participation coefficient, of one of these regions in left posterior superior frontal gyrus significantly predicted p-factor ($R2=0.26$, $F(22,56) = 0.87$; $B = -0.49$, $p<0.05$). DISCUSSION/SIGNIFICANCE: A portion of dorsolateral prefrontal cortex, associated with executive control, explained individual variance in p-factor. We plan to test alternative predictive models. Identification of such a neuro behavioral mechanism underlying psychopathology may lead to novel intervention targets.

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## Beyond Antibiotics: Monensin and its Derivatives as Promising Anti-Breast Cancer Agents

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OBJECTIVES/GOALS: Although the approval of immune checkpoint inhibitors (ICIs) revolutionized the treatment of metastatic


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