

Project Title: Biomarkers-Driven Experimental Therapeutics

Principal Investigator: Raymond A. Dionne, D.D.S., Ph.D.

ABSTRACT

Biomedical science has greatly improved our understanding of TBI in recent decades, but no specific pharmacologic therapy for TBI is available that improves patient outcomes, despite significant research in this area. Based on their review of the literature, Beauchamp et al. conclude that most promising therapeutic strategies derived from animal studies have failed to translate to the clinical treatment of TBI. They attribute these failures to several factors: missing their target, not reaching an adequate concentration in the intracranial compartment or by missing the therapeutic 'window of opportunity.' The failure of the CRASH study (Roberts et al., 2004) suggests that future interventions will have to be more targeted and possibly be administered as early as possible following the traumatic injury. This line of investigation is informed by these prior studies by a) characterizing the early up-regulation of gene expression and cytokine release in the acute period following injury to identify novel targets for pharmacologic intervention, b) using microarray techniques to identify sub-groups that may be more amenable to targeted therapies, and c) identify the optimal timeline for administration in order to preempt the initiation of deleterious pathophysiological cascades that are not amenable to reversal at later time points. We will accomplish these objectives through two specific aims.

Specific Aim #1: Characterize changes in gene expression and cytokine responses due to acute TBI by comparison of gene expression, molecular-genetic risk factors and cytokine levels in blood, neuronal tissue, and cerebrospinal fluid (CSF) between blast/head injury victims and matched controls.

Specific Aim #2: Design interventional studies that are based on the biomarker profile with targeted pharmaceutical agents.

Military and civilian patients with a moderate or severe head or blast injury (Glasgow Coma Score 12 or less) and controls will be screened for eligibility. Collection of samples will be initiated within the first 72 hours following head injury when feasible and collected for up to 7 days or until the subject is discharged. To minimize potential circadian effects, samples will be collected during a two-hour time interval in the morning (8-10 am). Samples will include peripheral blood for DNA and RNA extraction and later analyses, CSF as clinically appropriate and brain tissue when available. The control subjects will be individuals matched by age, gender and ethnicity who have not experienced a blast or head trauma. Brain tissue and CSF will be immediately frozen in liquid nitrogen; blood samples will be collected into Paxgene blood RNA tubes and ACD tubes for DNA extraction. The level of head trauma, the time from the injury and related demographic and medical variables will be collected at the time of sample collection. Samples will be analyzed (as described below) using methods that are routinely performed in the Symptoms Management Branch laboratory of the NINR.

Gene expression analysis using Affymetrix microarray: Affymetrix arrays (HG-U133) and reagents will be used following procedures as described by our laboratory (Wang XM et al., 2006, 2007, 2008, 2009) and in a recent molecular biology protocol (Wang D et al., 2010). Verification by qRT-PCR: The changes in gene expression selected from microarray analysis will be validated using qRT-PCR as previously described and more recently in Wang D et al, 2010.

Genome-wide sequencing to identify epigenetic changes associated with acute or repeated head trauma: High throughput DNA sequencing will be used to identify genetic polymorphisms and epigenetic changes that may be initiated by TBI and contribute to the development of chronic symptoms and post-traumatic stress disorder. An Illumina Genome Analyzer in our lab uses a 'sequence by synthesis' approach with a proprietary microfluidic cBOT and glass flow cell with attached oligonucleotides complementary to specific adapters that are ligated into library fragments. At the end of each sequencing run, the sequence of each cluster is computed and subjected to quality filtering to eliminate low-quality reads and generates sequences of 250 million clusters in a single run for comparison between TBI cases and matched controls.

Project Title: Neuro-Glial Antibodies in TBI

Principal Investigator: Michael J. Iadarola, Ph.D.

ABSTRACT

In both civilian and military populations, TBI contributes significantly to disability and death. Studies of nervous system injury implicate the immune system in exacerbation of the injury, inhibition of recovery, physiological dysfunction and inflammation at the site of injury and have led to efforts to identify informative biomarkers, which could serve to identify, classify and better understand TBI. One possible source of biomarkers of TBI-induced neural or glial damage is autoantibodies. In a mouse model of TBI, Stein et al. observed IgG antibody binding to dying neurons four hours following a cortical lesion. Autoantibodies directed at neuronal proteins have also been detected in the serum of rats following brain injury. In a model of spinal cord injury, Ankeny et al. demonstrated that following lesion of the spinal cord, pathogenic autoantibodies are produced and mice lacking B cells exhibited better recovery of locomotion in comparison to wild type mice following injury.

These results strongly implicate a humoral immune response subsequent to CNS injury, and suggest that treatments targeting humoral responses to CNS injury could have therapeutic benefit. In peripheral nervous system injury, which is frequently seen in conjunction with TBI, autoantibodies have also been observed. For example, Kohr et al. observed reactivity in the sera of neuropathic pain patients, but not healthy control sera, with the surface of autonomic neurons, implicating a possible autoimmune etiology.

Although larger studies are needed, neuropathic pain patients have been shown to respond to intravenous immunoglobulin (IVIg) therapy, suggesting a pathogenic role for autoantibodies. Taken together, these observations support the idea that aberrant innate and humoral immune responses contribute to disease burden following nervous system injury. In addition, the presence of autoantibodies in both CNS and PNS injury could represent important biomarkers for detecting and assessing neuronal damage. We are examining the hypothesis that damage to neural tissue can initiate a humoral immune response, resulting in the production of antibodies against self proteins, including neural- and/or glial-specific proteins and other proteins which may be over-expressed, upregulated or altered as a result of tissue damage. This project employs a new high definition antibody profiling technology, Luciferase Immunoprecipitation Systems (LIPS), to detect known autoantigens and to identify novel autoantigens, which arise following damage to neural tissue.

One major goal of this project is to generate a panel of informative autoantibody biomarkers that can be employed to better understand the pathogenesis of immune-mediated neural damage and which could be used to monitor and identify neural damage resulting from TBI. To accomplish this goal, the project involves assembling several patient cohorts to better understand humoral immune responses to TBI, CNS brain damage, and peripheral nerve injury.

Establishing specificity and the range of nervous system damage that elicits autoantibody production is a critical element. Thus, in addition to screening a cohort of TBI patient serums, we will evaluate autoantibodies in conditions involving other types of brain and nerve injury in which autoantibodies have been previously detected, including stroke, complex regional pain syndrome (CRPS) and limb amputation. This type of systematic approach may yield a new level of insight into the clinical presentation of TBI and potential new avenues for intervention in appropriate patient populations.

Project Title: Characterization of the Human Blood Transcriptome Response to TBI

Principal Investigator: Hyung-suk Kim, Ph.D.

ABSTRACT

Many Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) veterans present with symptoms consistent with mild TBI (mTBI) and post traumatic stress disorder (PTSD) that were unrecognized post-deployment¹. Both PCS and PTSD significantly impair physical and psychological health, resulting in long-lasting effects on the ability of service members to function in civilian settings and in future in-theater settings. Specifically, service members with PTSD and PCS face increased incidence of sleep disorders and cardiovascular (CV) risks². The importance of differentiating PCS from stress reactions (e.g. post-traumatic stress disorder (PTSD)) has been recognized, because treatments differ³, and comorbidity influences treatment responses.⁴ However, the overlap in clinical symptoms makes this distinction difficult.

The human response to TBI is multi-factorial and mediated by specific interplays between genes, gene products and their interactions with the environment. Given this complexity, the nature of human variability requires a platform that can assess several molecular pathways simultaneously and integrate that knowledge into information that can be interpreted with clinical relevance. A novel approach to the study of TBI is the use of gene expression profiling to unveil the molecular pathways involved in brain recovery and health.

This study aims to identify a molecular fingerprint of mTBI and PTSD via Transcriptome profiling (gene expression, protein expression, and genetic regulation) in a post-deployment military population. This study also seeks to determine responses to a comprehensive sleep evaluation in OIF/OEF combat exposed soldiers with PTSD (with or without mTBI), assess the prevalence of sleep disorders in this convenience sample of soldiers with PTSD, and determine sleep architecture in soldiers with new onset PTSD secondary to recent combat trauma.

The primary aim of the study is:

1. To identify peripheral blood transcriptome fingerprints that differentiate the following four groups: mild TBI exclusive (mTBI-PTSD) and co-morbid with PTSD (mTBI+PSD), PTSD exclusive of mTBI (PTSD-mTBI) and controls with no PTSD or TBI.

The secondary aims of this study are to:

1. Characterize alterations in peripheral blood transcriptome and inflammatory markers associated with sleep disorders and disturbances in soldiers with PTSD, and the relation to co-morbid mTBI symptoms.
2. Determine if a comprehensive sleep evaluation affects peripheral blood Transcriptome and inflammatory markers within the three groups of mTBI-PTSD, mTBI+PSD, PTSDmTBI.
3. Assess the prevalence of sleep disorders in soldiers with mTBI-PTSD, mTBI+PSD, PTSD-mTBI and a control group of redeployed soldiers.

This is a prospective cohort study of mild TBI exclusive (mTBI-PTSD) and comorbid with PTSD (mTBI+PSD), and PTSD exclusive of mTBI (PTSD-mTBI) in male and female active duty soldiers with recent return from either OIF/OEF. Patient groups will be matched on age and rank to n=75 non-TBI non-PTSD post-deployment military personnel for a total of four groups and n=300 subjects from the Sleep Medicine Clinic at Fort Lewis, Madigan Healthcare System Tacoma, Washington. Data will be obtained from clinical procedures and medical records as part of standard of care. Blood samples for research purposes will be taken when phlebotomy is performed for evaluation and/or treatment at the Sleep Medicine Clinic and otherwise as part of separate blood draw as part of the research protocol. Patients will not be treated as part of this protocol, except as clinically indicated for their underlying sleep disorders, and the research performed will not interfere with the clinical evaluation or treatment of patients. Patients will be followed up to 90 days (3 months) to determine the development of PTSD/PCS.

Project Title: Plasma biomarkers of blast-related brain edema

Principal Investigator: Fabio Leonessa, M.D.

ABSTRACT

Brain edema is one of the most critical complications of TBI. Brain edema is particularly fast and severe in blast-related TBI, though the mechanisms by which blast causes this edema remain to be defined. Accurate biomarkers in easily accessible biofluids, not presently available, would be particularly useful for the evaluation of risk and early detection of brain edema at the lower echelons of care, and they may assist in later therapeutic decisions and monitoring.

Specific Aims of this research project are:

1. to evaluate the primary (shock wave, but perhaps also other physical components) and tertiary (acceleration) mechanisms of blast injury, and of their combination, in producing brain edema in rats;
2. to use the rat model of blast-related brain edema to identify new potential biomarkers.

As part of these specific aims, we are carrying out evaluations on a new rat model of explosive-driven primary blast TBI and we are working on a novel model for the evaluation of the interaction of blast-related overpressure and acceleration on brain injury and edema. Preliminary results of ongoing evaluations do not support an independent role of primary blast as an independent cause of blast-related brain edema even at intensity (peak overpressure) levels that approach lung injury related lethality in the presence of chest protection, and exceed it in its absence.

Additionally, and unexpectedly, no clear correlation has been observed so far between blast intensity and extent of brain injury at intensities ranging from 18 to 50 p.s.i. (peak static pressure), suggesting the possible role of blast-related mechanisms other than blast's overpressure wave. The observed evidence of primary blast-related brain injury includes: subdural, subarachnoid, intraventricular, and intracerebral and perivascular hemorrhages, macroscopic discoloration of the brain surface facing the blast source, corresponding to microscopic evidence of microvessel congestion and, sometimes, albumin staining of microvessel walls. However, pathological and behavioral evidence of neuronal injury has been so far very limited.

Moreover, reproducibility of the observed injuries is poor. Experiments are ongoing, extending pathological evaluation to multiple time points, and adding multiple behavioral endpoints. Establishment of a novel rat model for the evaluation of the interaction of blast-related overpressure and acceleration is presently being aided by computational modeling. Experimental conditions for in vivo studies on combinations of blast-induced overpressure and acceleration will be further optimized by preliminary evaluations on a surrogate model of a rat head. We expect to observe brain edema as a consequence of the combination of blast overpressure and acceleration mechanisms. Using antibody-based assays, several protein candidates, selected on the basis of known biological significance, or of evidence obtained from in vitro models of astrocytic swelling, will be tested as potential biomarkers of vasogenic and cytotoxic brain edema, respectively, in plasma samples collected in the course of the in vivo studies.

Project Title: Evaluation of Putative Biomarkers in TBI

Principal Investigator: Gregory P. Mueller, Ph.D.

ABSTRACT

Aim 1. Obtain and use commercially available assay kits to evaluate putative blast TBI biomarker proteins in plasma pools.

Status: completed: A systematic evaluation of commercially available assay kits has revealed that good assays exist for only a very small number of candidate TBI biomarkers. Moreover, these assays are not multiplexed and their overall costs are high. Having determined that the needs of the CNRM for investigating TBI-specific biomarkers cannot be met though the limited availability of commercially assay kits, the decision was made to develop and multiplex our own assays.

Aim 2. Develop assays for putative biomarker proteins for which assay kits are not available commercially.

Status: ongoing, phase 1 completed We have selected the ELISA technology developed by Meso Scale Discovery (MSD), Inc., Gaithersburg, MD, as the foundation platform for the development of multiplex TBI biomarker assays. We have successfully developed and multiplexed assays for the following six biomarker candidates: glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), brain derived neurotrophic factor (BDNF), intercellular adhesion molecule-5 (ICAM-5), S100 beta and monocyte chemotactic protein-1 (MCP-1). Continuing work under this aim will focus on the development of assays for additional biomarker candidates. Several attractive targets have already emerged from the work carried out under AIM 5.

Aim 3. Use newly developed assays to evaluate putative blast TBI biomarker proteins in plasma pools.

Status: ongoing: While samples of human TBI serum/plasma have yet to be available for our evaluation, substantial progress has been made using commercially obtained serum and plasma samples from healthy control subjects. These samples have allowed us to refine and define the performance of our assays in both singleplex and multiplex format. Importantly, we have determined that multiplexing does not appreciably alter the performance of assays developed in singleplex format. Additionally, we have determined the contribution made by platelets to the blood borne TBI biomarker signature.

Aim 4. Initiate validation of selected blast TBI biomarker proteins using individual samples of human and rat plasma.

Status: planned: As noted above, human TBI samples have yet to become available for this work. We anticipate that samples will be available in the near future as they are now beginning to arrive into the CNRM Biospecimen Repository. Work validating the applicability of our assays to rodent samples is underway. Our initial effort involves analysis a rat brain as a sample that is enriched in the candidate biomarker proteins being studied here.

Aim 5. Discover novel TBI biomarkers by identifying brain autoantigens that arise in response to TBI. In addition to the originally approved Aims 1-4, we have expanded the scope of this project to include this new aim.

Status: ongoing: This aim represents a novel approach for the discovery of TBI biomarkers where we use the humoral immune response to brain injury as a pathway for the discovery of brain-specific proteins that may serve as early biomarkers for mild TBI. The underlying hypothesis for this approach is that brain-specific autoantibodies can be used to identify proteins that will serve as biomarkers for assessing the severity of TBI and monitoring its response to therapy. To date, research carried out in rats has produced compelling evidence for the identity of six candidate TBI biomarker proteins. Importantly, two of these candidates are established as TBI biomarkers. This adds to the potential for the other four novel candidates as being clinically relevant TBI biomarker proteins.

Project Title: Proinflammatory Biomarkers for Mild Blast TBI

Principal Investigator: Harvey B. Pollard, M.D., Ph.D.

ABSTRACT

The coincidence of inflammation and coagulation is a common response of vertebrate tissues to damage, and thus could be a compelling origin for mild blast TBI biomarkers. The diagnosis of mild blast TBI is often missed immediately because, aside from transient confusion and memory deficits, there are little or no objective signs or symptoms of illness. However, multiple experiences with mild blast TBI are cumulative. Our goal is therefore to develop a set of biomarkers in a biological fluid, such as CSF or plasma, which will provide a timely identification of the occurrence, severity, and progression of blast-induced TBI, as well as its response to therapeutic intervention. Preliminary data indicate that non-blast damage to the brain can be accompanied by release of contents from broken cells and by expression of proinflammatory mediators from surviving cells in the vicinity of the damage. Such expressed mediators include TNF, IL-6, and IL-8, as well as leukotrienes and other metabolites. These molecules will flow physiologically into the systemic circulation, and may also be discharged into the general circulation through the compromised blood brain barrier (BBB). In addition, extrinsic and intrinsic coagulation processes are activated by exposure of collagen in the subendothelium and by exposure of phosphatidylserine (PS) on membrane fragments. We therefore hypothesize that mild blast TBI is accompanied by elevated levels of proinflammatory biomarker proteins in the cerebrospinal fluid (CSF) and general circulation. To test this hypothesis, we have proposed the following Specific Tasks:

Specific Task 1: To determine changes in TNF α and related proinflammatory mediators in CSF and plasma from rats exposed to mild blast TBI.

Specific Task 2: To determine the low abundance signaling proteome of CSF and plasma from rats exposed to mild blast TBI.

Specific Task 3: To determine the metabolomic biomarkers in CSF and plasma from rats exposed to mild blast TBI.

Specific Task 4: To determine candidate biomarkers for mild blast TBI in CSF and plasma from exposed humans.

Successful identification of biomarkers for mild blast TBI will be significant as objective diagnostics of exposure; significant as indicators of the biological basis for brain-specific responses to blast exposure; and significant as objective surrogates to follow experimental therapeutics.

Project Title: Non-Invasive Sweat Patch Technology for Determination of Neuroimmune Biology

Principal Investigator: Esther M. Sternberg, M.D.

ABSTRACT

We have established a methodology to simultaneously evaluate a large array of neuroimmune biomarkers in sweat, collected through commercially available and FDA approved cutaneous sweat patches, and measured by recycling immunoaffinity chromatography (RIC). The sweat patch is a non-invasive, unobtrusive method to collect biomarker data over an extended time period (24 h) and is a valid alternative when blood collection is unfeasible or undesirable, such as in the field. RIC is a non-commercial methodology that is highly sensitive and specific and allows quantification of up to 30 analytes in minute volumes of fluid. The combined sweat patch-RIC methodology allows simultaneous and repeated assessment of a battery of biomarkers reflecting different physiological systems. In our initial studies using the combined sweat patch-RIC methodology, we showed that cytokines and neuropeptides are detectable in sweat and are positively correlated with plasma levels in a group of healthy subjects and in a group of women with major depressive disorder (MDD) in clinical remission.

Biomarker patterns in these women also strongly correlated with depressive and anxiety symptoms on the Hamilton Anxiety and Depression scales, indicating their functional significance. Specifically, pro-inflammatory cytokines were elevated, as was the sympathetic neuropeptide NPY and the sensory/pain-related neuropeptides, SP and CGRP, while the parasympathetic neuropeptide VIP was significantly decreased. This pattern is consistent with a shift in MDD from parasympathetic to sympathetic tone, and an underlying proinflammatory state that could account for enhanced susceptibility to conditions known to be comorbidly expressed with MDD. A similar biomarker profile has been found in post-traumatic stress disorder (PTSD) patients. Moreover, patients with co-morbid PTSD and MDD are at higher risk for concurrent medical illnesses as well as higher rates of suicide.

In TBI, CNS inflammation may be one risk factor in the consequential development of MDD, PTSD and/or cardiovascular disease. However, not all TBI patients develop these downstream disorders, suggesting that identification of signature biomarker patterns may assist in identifying subjects at risk for inflammatory and behavioral sequelae of TBI. One drawback of the RIC method is that it is labor-intensive and not high-throughput. To further develop and apply the sweat patch method of biomarker profiling, we are establishing a sensitive, high-throughput analytic method, using a glass chip-based static antibody microarray system. This technology can provide an important and high-impact tool for clinical and translational research, associating specific biomarker patterns to identify susceptibility to and progression of long-term inflammatory and behavioral sequelae in conditions such as MDD, PTSD and TBI, as well as treatment efficacy in preventing such downstream detrimental consequences. Ultimately, this novel methodology could be used for diagnostic and prognostic purposes and for the optimization of individualized treatment regimens. The expression of a particular biomarker profile can serve as a “molecular signature” of a specific subtype within a heterogeneous disorder, to identify patients at risk and treat before they become symptomatic. The primary aims of this pilot project are:

Specific Aim: To develop/validate a fast, sensitive and reliable assay for high-throughput analysis of multiple analytes (neural, endocrine, immune, metabolic) from a single low volume sample of eluate extracted from sweat patches using a glass chip-based antibody microarray.

Long-term aim of this study: To apply the sweat patch methodology to elucidate the pathophysiology of TBI, to identify persons at risk for developing long-term inflammatory and behavioral sequelae of TBI, including MDD and PTSD, and to assess the efficacy of therapeutic interventions, as well as preventative measures, to limit the development and/or progression of long-term inflammatory and behavioral sequelae of TBI.

Project Title: Brain Region Specific Biomarkers in Animal Models of TBI

Principal Investigator: Amina S. Woods, Ph.D.

ABSTRACT

The purpose of the project is to identify endogenous chemicals present in an accessible body fluid, whose presence or change in concentration might indicate the occurrence and/or severity of brain damage or functional impairment following exposure to blast or other mechanical injuries to the head and brain. A secondary objective is to determine if there is any regional specificity of chemical “biomarkers” potentially linked to such brain injuries, since knowledge of injury location might be helpful in evaluating prognosis and therapeutic approaches. This project will focus on changes in lipid expression in brain after exposure to TBI. To more fully understand the molecular consequences, progression, and ultimately, treatment of these injuries, we are qualitatively and quantitatively characterizing a range of lipid species from controlled cortical impact (CCI) brain injuries and other TBI models in a rat model, utilizing imaging mass spectrometry (IMS) to identify the regional location of selected lipids, and electrospray ionization mass spectrometry (ESI) of extracts containing internal standards to facilitate quantification of specific lipids in dissected regions of brain.

Project Title: Evaluating a Novel Peptide Therapy for TBI; a Biomarkers-Based Approach

Principal Investigator: Amina S. Woods, Ph.D.

ABSTRACT

The long-term objective of the project is to test a peptide, dynorphin decoy peptide (DDP), designed to complex with and inactivate the endogenous neuropeptide dynorphin, for its effectiveness when given post-trauma in preventing injury-induced neuronal cell death in vivo. This peptide has previously been successfully tested in an NMDA-induced injury model in which dynorphin-mediated neurotoxicity results in paralysis. In this model, DDP treatment prevented paralysis. In the rat middle carotid artery occlusion model of stroke, treatment with the same peptide greatly reduced the area and volume of dead brain tissue. Since trauma to the brain causes release of high local concentrations of dynorphin that contributes to neurotoxicity, we propose that the administration of one or more doses of DDP following trauma will prevent or diminish trauma-induced cell death, and facilitate recovery from the injury.