Biomarker Signatures of Biological, Chemical, or Psychological Stress

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We hypothesize that biological, chemical, physical, and/or psychological stressor environmental type of exposures to humans result in alterations to the neuro-endocrine-immune axis. These changes provoke shifts in molecular components within the blood that reflect changes in levels of biomarkers responding to these stressors. Septic patients will be used as prototype stressed individuals to identify and quantify the presence of serum proteins that have increased, decreased, or epitope-modified expression. These molecular features will form the basis of specific biomarker signatures that are characteristic of stressed human individuals. In addition to enabling us to identify unique stress-related profiles and new biomarkers of a particular type of biological stressor, we will utilize the sera from septic patients to compare analytical methodologies. We have quantified the types of cytokines in septic sera by a Luminex assay, a fluorescent bead-based method with capture and detection antibodies. We will determine whether a new biosensor method described as grating-coupled surface plasmon resonance imaging (GCSPRI) can produce comparable results. GCSPRI is a microarray platform that will enable the multiplexed detection of these biomarker signatures with an automated diagnostic system. The GCSPRI system will be assayed for matrix interference and affinity of capture agents for numerous analytes. The advantage of the GCSPRI method is that no labeled antibodies are required and that hundreds of analytes can be simultaneously quantified. Additionally, the quantification of analytes can be accomplished in near real time. Our initial plans are to use well established GCSPRI instrumentation for these analyses, but two “next generation” SPR technologies are also currently under evaluation. Initial studies indicate sensitivity can be substantially improved with usage of fluorescently-tagged detection antibodies.

Preliminary experiments document that biological, chemical, and psychological stress alters plasma protein expression levels, which in part are due to inflammation and/or oxidative processes. Since we suggest that the different forms of stressors modulate the interactive pathways between the endocrine, immune, and nervous systems, we anticipate that immune, endocrine, and nervous system factors will be predictors of stress and that the profiles of these factors will both serve as markers of the particular stress and will provide prognosis for the degree of stress and the severity of the exposure. Individual biomarkers, which are predicted to relate to regulatory pathways associated with inflammation:anti-inflammation, oxidant:anti-oxidant, and with innate immune processes, will then be quantified in humanized mice after exposure to three prototype stressors: cadmium, cold-restraint, and listerial infection. Humanized mice are created by engraftment of human stem cells isolated from cord blood in mice that lack T cells, B cells, and NK cells. The mice (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}) are reconstituted by injection of human hematopoietic progenitor cells (hHPCs) into the livers of one day old neonate pups. We have experience producing these “humanized” mice and have determined that by 8-10 weeks after injection the mice have established a stable human immune system including T cells, B cells, and NK cells of human origin in their blood, spleen and lymph nodes.

Our plan is to delineate and quantify the normal basal and stress-responsive plasma concentrations of relevant human *vs*. mouse biomarkers, and to validate their analysis employing both traditional microbead based assays and GCSPRI. In addition to stressor-induced changes in plasma constituents, we will evaluate changes to blood leukocyte antigens; lymphocytes are especially sensitive to inflammatory products and oxidants. Blood products are obtained with minimal invasiveness, and they represent the best composite of the systemic response to a stressor. The biomarkers to be evaluated include blood clotting factors, cytokines, stress proteins, neuropeptides, antioxidant enzymes, and normal plasma proteins with thiol-related modifications. We will evaluate the consequences of different stress response capabilities on the character of the biomarker signatures that have been identified in this work. As an additional modifier of the stress and the host response, we will establish the human immune system in mice that over- or under-express metalliothionein (MT). MT has been shown to be a critical regulator of both adaptive and innate immune response mechanisms. The human population shows evidence of metallothionein polymorphisms, which may relate to metallothionein's potential contributions as a susceptibility factor for diseases such as neoplasia, autoimmune diseases, and infections.

At the conclusion of this work, we will have identified specific biomarker signatures that will be invaluable in both the diagnosis of stress, and the characterization of therapeutic management of stressed individuals. The comparative evaluation of chemical, biological, and psychological stress is especially relevant to the individuals living under different psychosocial conditions and being differentially exposed to environmental toxicants and/or pathogens. Our final analyses will address the relative contribution of genes and environmental exposures on health and will assist in delineating means to beneficially impact their influences on human health.