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Angiotensin II-mediated increases in Damage Associated Molecular Patterns during acute mental stress

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Damage Associated Molecular Patterns (DAMPs), which are particles that operate within cells but also initiate an inflammatory response outside of the cell when needed, can initiate and maintain an inflammatory response in the absence of infection (for reviews see (1-5)). A wide range of investigations has shown that a state of heightened systemic inflammation can be intensified by acute and chronic psychological stress and distress both in adolescent and adult individuals, especially in African-Americans (6). A recent editorial in this journal by Cho and Irwin (7) discusses the value of interventions that target immune/inflammatory systems for prevention of disease. This editorial commented on observations by Cohen and colleagues that lower self-rated health was progressively associated with the development of clinical manifestations of colds, independent of potential confounding factors (8). We propose a modified version of the hypothesis that connects psychological factors with immune system dysregulation to explain the association between psychological distress and disease risk based on the findings of two recent studies in African-American (AA) men and women. Specifically, we postulate that a series of acute episodes of mental stress result in a sustained activation of the innate immune system throughout the day. Furthermore, we identified a treatment strategy that potentially can reduce this response. Towards this end, we first examined changes in DAMPs in response to an acute stress task in a cohort of AA adolescents.

Measures of DAMPs included high-mobility group box 1 protein (HMGB1) and measures of mitochondrial DNA (mtDNA). HMGB-1 is found in plasma, generally derived from necrotic cells. After binding to its receptors, the receptor for advanced glycation end products or Toll-like receptor 4, HMGB1 activates macrophages/monocytes and vascular endothelial cells to express pro-inflammatory cytokines, chemokines and adhesion molecules. MtDNA is released into the extracellular space in response to cellular necrosis and induces inflammatory responses via activation of Toll-like receptor 9 (TLR9). DAMPs are molecules released by stressed cells during necrosis signaling danger to promote and exacerbate inflammation (9-12). First observed in cancer, DAMPs are now being recognized for their role in hypertension (13-15). Animal studies have demonstrated a positive correlation between stress and DAMPs, though there is very little research in humans (16, 17). We hypothesize that cell death or injury resulting from stress-induced sodium retention and

volume-mediated blood pressure elevation leads to increased circulating DAMPs. This increase in DAMPs then contributes to the persistent inflammation often observed in hypertension, further increasing the volume expansion seen in these individuals and further extending the blood pressure elevation (18). The overall goal of this study was to determine if the innate immune system contributes to the stress-induced increase in Ang II in a subset of individuals and if its role can be attenuated by the use of an angiotensin receptor blocker (ARB). This is supported by previous research on the relationship between stroke and DAMPs(19).

The initial study we conducted was an observational study of healthy AA adolescents. Prior to the protocol, the participants were placed on a controlled sodium diet for 4 days to insure they were at similar levels of sodium balance. The mental stress protocol was a competitive video game task (one hour) preceded and followed by two hour periods of relaxation. Blood samples were obtained for the measurement of DAMPs prior to and following the mental stress protocol. The goal of the first study was to determine if mild acute stress similar to that experienced throughout routine daily activities increases circulating levels of DAMPs in AAs. Using our cohort, which consisted of a sample of young, healthy AA adolescents, we addressed this question. The results of paired t-tests are shown on the left side of Table 1, under the heading Cohort Study. Both HMGB1 and mtDNA increased significantly during the stress period.

Previous studies by our group demonstrated increases in angiotensin II (Ang II) during this same mental stress protocol in AA adults. Therefore, the goal of the second study was to determine if an angiotensin receptor blocker (ARB) effectively suppressed an increase in DAMPs. Towards this end, we analyzed available samples from twelve AA adults who were placed on an angiotensin receptor blocker (ARB; irbesartan (150 mg P.O.)) for three days prior to undergoing the same stress protocol as in the first study. A randomized double-blind, placebo-controlled crossover design was used. The effect of the ARB is shown on the right side of Table 1, under the heading Treatment Study. The ARB reduced the circulating levels of mtDNA in response to mental stress, supporting the hypothesis that suppression of Ang II prevented activation of the TLR9/nuclear factor- κ B pathway during acute mild mental stress (19).

In conclusion, findings by Cohen *et al.* (8) and other investigators examining the effects of (repeated) environmental challenges on immune system dysregulation converge with our own data, indicating activation of the innate immune system in response to acute episodes of mental stress encountered over the course of a normal day. Repeated activation of this system has been hypothesized to underlie or contribute to the development of many disorders leading to premature morbidity and mortality. For example, these findings provide proof of concept for a novel paradigm for hypertension based in part on the integration of the sympathetic nervous and renin angiotensin systems with the immune system (20). This suggests that the innate immune system plays an important role in the extended and exaggerated blood pressure elevation of these individuals, thereby contributing to the increased risk for target organ damage. As seen in our pilot data, the deleterious effects observed in this response pattern might be prevented by the suppression of Ang II. The relationship between DAMPs and blood pressure in this pilot study was positive ($r = 0.662$)

but statistically not significant ($p = 0.105$), reflecting the small sample size. Further research is needed in a larger population to validate our results and examine the mechanisms through which ARB treatment impacts DAMPs during stress.

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Table 1

Mean differences at baseline and post-treatment in untreated vs treatment group.

Variable	Cohort Study (n=10)			Treatment Study (n=12)		
	Pre-Stress	Post-Stress	P value	Pre-Stress	Post-Stress	P value
Age (years)		16.6±3.1			40.5±7.6	
BMI (kg/m ²)		28.1±4.8			29.5±9.4	
Sex		Male = 4 Female = 6			Male = 5 Female = 7	
HMGB-1 (arbitrary unit [*])	26941.767 ± 14990.172	32458.319 ± 14680.479	0.03	25253.295 ± 5847.350	24341.685 ± 4765.138	0.52
CytB (1/CT)	0.060 ± 0.006	0.063 ± 0.005	<0.01	0.060 ± 0.004	0.057 ± 0.004	0.03
NADH (1/CT)	0.061 ± 0.008	0.065 ± 0.006	0.02	0.062 ± 0.003	0.059 ± 0.004	0.01
ND6 (1/CT)	0.060 ± 0.006	0.064 ± 0.005	<0.01	0.061 ± 0.004	0.057 ± 0.004	0.01
SBP (mmHg)	118.1±12.7	119.2±11.3	0.62	110.6±10.7	112.8±12.5	0.33
DBP (mmHg)	62.7±6.7	65.4±4.5	0.13	63.3±5.6	63.3±6.4	0.96
UNaV (mEq/h)				0.249±0.118	0.385±0.178	<0.01

* Measures provided by Western blot integrated optical intensity.

Mitochondrial DNA content was measured by polymerase chain reaction method.