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THE SIGNIFICANCE OF ACUTE PAIN AND MOOD IN INFLAMMATION THROUGH COMPLEMENT WITHIN RHEUMATOID ARTHRITIS

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder that degrades the synovial joints of the skeletal system and is estimated to affect 2.1 million individuals within the United States alone. In women with RA, the degree to which both acute and recent pain and stress were associated with two key components of the complement system was investigated. The key molecules of interest were: C-reactive Protein (CRP) and complement protein C5a. C5a is a downstream protein fragment and prostaglandin precursor with no previous measurement or linkage with stress in this population to my knowledge. CRP is known to be an indicator of RA severity and is linked to psychological stress. The goals of the present investigation were to determine if a) it is possible to obtain levels of C5a in this population, b) whether C5a is associated with CRP and c) if these molecules are correlated with pain and stress indicators. I hypothesized that C5a and CRP would be associated and individually linked with markers of pain and stress, particularly acute pain and stress as opposed to recent stress or RA severity. These questions were examined within a larger study of the effects of manipulated emotional state (sadness, anger, or happiness, vs. a control condition) on inflammation among nine women with RA. Participants completed four separate study visits of four hours each (which varied only by emotion condition) during which the inflammatory response was acutely stressed via a pain threshold test. Blood was drawn via a catheter at baseline, 10, 60, and 100 minute post pain and C5a and CRP were assayed from frozen stored blood samples using commercially available kits. Results suggest that C5a is detectable in the study's population of interest and may be more indicative of pain responses than CRP in RA. Of greatest significance in predicting C5a were acute recent pain measures. In addition, the more long term indicator of pain interference was marginally correlated with later C5a time points and predicted 100 minute C5a over and above baseline values. Further investigation on the value of C5a as a predictor of pain responses in RA is imperative to shed light on triggers of pain severity and new methods of intervention.

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Table of Contents

INTRODUCTION

Rheumatoid arthritis (RA) is estimated to affect 2.1 million individuals within the United States (U.S. Department of Health and Human Services, 2004). It is an autoimmune chronic inflammatory disorder that focuses on the synovial joints of the skeletal system. The autoimmune characteristics of RA make it unique from osteoarthritis, the most common form of arthritis that typically develops in older adults (National Institute of Health, 2009). In an autoimmune disorder, the body is unable to differentiate between self and non-self which results in the body's immune system to recognize itself as foreign. In the case of rheumatoid arthritis, the tissues within the joints of the patient are recognized as non-self and overtime pursued for destruction by the person's immune system. This property of the disease results in life-long chronic inflammation and pain within the individual's joints (U.S. Department of Health and Human Services, 2004). Emerging evidence suggests that stress and emotion are strongly linked with both pain and inflammation (Bruehl, Donahue, & Burns, 2006; Graham et al., 2006; Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). There is a strong need for further research into the underlying factors influencing inflammation and their degree of impact within rheumatoid arthritis. Through an analysis of the role of acute and long term psychosocial variables within the complement system, an innate inflammatory activator, a more well-rounded and dynamic understanding of inflammation can be established. My research is focusing on the role of emotion-specific conditions on the complement system of the innate inflammatory immune response. The molecules under investigation are the key complement system activator C-reactive protein and the downstream protein fragment and prostaglandin precursor complement protein C5a.

The Immune System and Inflammation

Innate immunity is referred to as the body's first line of defense. The first response of the immune system to the danger of foreign substances is inflammation. When the body recognizes a non-self component, a series of signals cause dramatic changes to the area of impact such as vasodilation, increased blood vessel permeability, and blood flow. This allows the rapid entry of

neutrophils that elicit a non-specific response through the release of reactive oxygen species (ROS). In addition to the release of ROS, neutrophils produce antimicrobial peptides and pro-inflammatory cytokines such as IL-6, tumor necrosis factor α (TNF- α), gamma interferon (IFN-gamma) and IL-1β. The entry of neutrophils is followed by an influx of a variety of leukocytes to combat the intruding foreign and non-self-components (Kindt et al, 2007).

Chronic inflammation is a result of long term stimulation of the inflammatory response because of the body's inability to remove the agent recognized as foreign. After the maturation of monocytes into more resilient macrophages, the perpetual release of prostaglandins and pro-inflammatory cytokines IL-6, tumor necrosis factor α (TNF- α) and IL-1β in the synovial fluid and the peripheral blood causes the chronic joint pain and inflammation in those diagnosed with rheumatoid arthritis (Kindt et al, 2007).

One of the end products of pro-inflammatory cytokine cascade is C-reactive protein (CRP). CRP is currently used as the primary indicator of the level of severity of rheumatoid arthritis in a clinical setting. The use of this indicator is supported by the fact that with proper long term rheumatoid arthritis treatment, levels of CRP have been consistently seen to decrease (Feldmann et al., 2001).

CRP is also a preliminary activator of a cascade of events known as the complement system, more specifically the classical pathway (Kindt et al, 2007). The complement system is a critical component of the innate immune response. CRP is an opsonin and acute phase protein that originates in the liver and is regulated by the cytokine IL-6 (Mcdade et al., 2004). Its primary purpose is to create a membrane attack complex through the sequential cleavage of nine proteins and ultimately lyses harmful bacteria and targets it for destruction (Salyers et al., 2010).

The presence of the anaphylatoxin C5a indicates that the complement pathway has been activated. C5a is a protein fragment downstream of CRP common to all three types of complement activation (classical, lectin and alternative) that has been found to have chemotactic and vascular permeability functionality when guiding leukocytes to a site of infection within the peripheral

tissue. In addition, C5a is a known precursor to the production of prostaglandin within the body, a known emotion regulator (Shiho et al., 2007).

Prior studies show elevated levels of CRP within the peripheral blood and C5a within the synovial tissue of the rheumatoid arthritis patients. Due to technical limitations of the procedure used in 1990, researchers were unable to determine the presence of C5a within the peripheral blood (Jose et al., 1990). Within the blood, the C5a complement fragment is cleaved into the C5a-desArg form by a specific serum carboxypeptidase and the C5a ELISA used within this analysis is able to quantify this alternate form of C5a (Bokisch, 1970). The available C5a ELISA is significantly more sensitive and should provide more conclusive results on the presence of C5a within the peripheral blood stream in RA patients. The presence of C5a within the bloodstream would indicate that the activated complement protein is circulating and not localized in specific tissues with our study population. The systemic nature of C5a and C5a's role as a precursor to the regulatory hormone prostaglandin could be a potential direct link between psychological factors and inflammation. Hence, both CRP and C5a are target molecules of the present investigation.

The complement system is activated by bacterial antigens and is a primary immune response against bacterial infection. If it can be justified that the complement system is activated within this study with or without emotion-specific stimulation, it provides additional support for a bacterial etiology for rheumatoid arthritis. Many recent studies have supported the hypothesis that a citrullinated protein of the bacterium *Porphyromonas gingivalis*, the primary cause of gingivitis and perdonitis of the mouth, as the most likely etiological agent of rheumatoid arthritis (Wenger et al., 2010). These psychosocial stressors could serve as triggers for RA development in the presence of bacteria of interest. There have been no previous studies of which we are aware that have focused on the responses of specific cytokines and biomarkers such as C-reactive protein to acute controlled changes in emotion. In rheumatoid arthritis conditions where chronic pain and inflammation are prevalent, the impact of emotional stressors may magnify the conditions and result in severely detrimental long term effects on the body (Davis et al., 2008)

Although CRP levels are more stable than most other pro-inflammatory cytokines, the use of high-sensitivity ELISA techniques to measure CRP changes on the scale of hours have been successful (Veldhuijzen et al., 2005). More research has been done supporting that anger and hostility are associated with inflammatory markers, especially CRP (Graham, 2006). A better understanding of the relevance of C-reactive protein changes due to emotional manipulation within collected blood serum of rheumatoid arthritis patients could lead to better means of chronic pain reduction. Lastly, C-reactive protein has also been shown to have clinical significance in cardiovascular diseases such as stroke and heart disease. A further investigation of impact of emotional manipulation on C-reactive protein levels could provide insight in the creation of new preventive measures for combating certain cardiovascular diseases (Mcdade et al., 2004).

In addition to C5a and CRP, an exciting target of investigation would be prostaglandin E2 (PGE2), an arachidonic acid lipid derived hormone-like molecule that is highly involved with emotional regulation within the body (Shiho et al., 2007). PGE2 originates from the complement system's anaphylotoxin C5a protein fragment. In times of psychological stress and illness, PGE2 is created and released within the brain and in a variety of cell types within the body (Furuyashiki et al., 2010). An exciting analysis we hope to perform in the near future would be testing for elevated levels of PGE2 within the peripheral blood through direct radio-immunoassay techniques to see how they map onto complement and CRP activation as well as stress.

Goals of Present Investigation

The goals of the present investigation were to determine if a) it is possible to obtain levels of C5a in this population, b) whether C5a is associated with CRP and c) if these molecules are correlated with pain and stress indicators. I hypothesized that C5a and CRP would be associated and that each would be linked with markers of pain and stress, particularly acute pain and stress as opposed to recent stress or RA severity. To assess potential acute and long term causes of cytokine and inflammatory biomarker fluctuation, a variety of scales were used to better quantify and understand implications of environmental stressors. Within this analysis, seven long term and acute

pain and mood measures were focused on and are further elaborated within the methods section.

These goals were investigated within a larger study of the effects of emotion manipulation (anger vs. positive mood vs. negative mood vs. control) and psychological stress on inflammation called the Rheumatoid Arthritis Multidimensional Project (RAMP). Each session of the RAMP study is a four hour block of time specific to a single emotion condition for each participant. During the visit, an acute emotional response is stimulated through writing and verbal communication and the inflammatory response is acutely stressed through the use of a pain threshold test. The purpose of these portions of the protocol was to focus on responses to acute stressors. For a more all-encompassing outlook, each participant underwent a comprehensive analysis of long term indicators that may impact their overall mood, level of general stress and tendency for a heightened inflammatory response.

METHODS

Participant Selection

Nine participants were recruited from local State College rheumatology clinics through the distribution of flyers and advertisements. The study screened for women between the ages of 18 and 80 whom had been diagnosed with adult-onset rheumatoid arthritis by a certified physician based on the standards set by the American Rheumatic Association (Arnett et al., 1988). Once a participant agreed to participate and both HIPPA and consent forms were signed, a clinician confirmed the rheumatoid arthritis diagnosis. Prior to the first visit, potential participants were screened using an online questionnaire about medication use, initial baseline assessment for rheumatoid arthritis severity, demographics, and a variety of psychosocial scales.

In regards to medication use, selection criteria excluded participant currently using anti-tumor necrosis factor medication or greater than 7.5mg/day of prednisone. Additional selection criteria required that participants have a Body Mass Index less than 40 and do not report an excess of alcohol or caffeine use (greater than 21 drinks per week or 10 cups of coffee per day respectively). In terms of simultaneous illness conditions, individuals with additional chronic illnesses or those admitted to a hospital within the last 3 months were not allowed to participate.

Lastly, women within 3 months of any child bearing activity were excluded from the study.

If a participant fulfilled the selection criteria, she was asked to fast upon the arrival to each visit at 8:00 am. Each participant was asked to refrain from any medication for ten hours prior to the visit. These included over the counter or prescribed pain medications. Due to the schedule of its administration, participants taking methotrexate had not taken it for at least 48 hours before each visit.

RAMP Study Overview

Once selected, each participant scheduled a total of four 4 hour visits at the University Park General Clinical Research Center (GCRC). Figure 1 illustrates the participant's standardized timeline for each visit. The only change occurring across visits was the mood designation for the emotion focusing exercise. Each visit began at 8:00 am and was randomly pre-assigned one of the following four conditions: happiness, sadness, anger and control. The designated emotion was targeted during the emotion focusing exercise that was followed by a standardized pain stressor. Each visit had four scheduled blood draws: at baseline (T_0) and 10, 60, 100 minutes after the participant received the pain stressor. Over the course of the visit, the participant was asked to complete a variety of self-evaluations on their current mood and pain as well as more long term stressors based on previously tested scales. The scales used in this analysis are discussed in a subsequent section of the methods.

CRP ELISA protocol

The principle assay used to measure the concentration of C-reactive protein was Biocheck Inc.'s hsCRP ELISA. This high-sensitivity C-reactive protein ELISA was used to assay a total of 412 samples of collected serum at two time points: the baseline and the last 100 minute post pain time point. The patient's serum sample was reacted with an immobilized mouse monoclonal anti-CRP antibody and a mobile goat anti-CRP antibody within a horseradish peroxidase solution responsible for the assay's color change. Upon the addition of the two antibodies, the CRP molecule of interest are sandwiched between the solid phase and mobile antibodies and washed to remove any antibody excess after a 45 minute incubation period. Once washed, a tetramethylbenzidine

reagent (TMB) is added and incubated for twenty minutes to induce the production of a blue color within the well plates. Lastly, the addition of hydrochloric acid was used as a stop solution and to change the solution color to yellow. The concentration of CRP was then determined based on color intensity using spectrometry at 450 nm.

C5a ELISA protocol

The BD OptEIA TM Human C5a ELISA Kit II was utilized to determine levels of complement C5a. Each blood sample of 100 microliters was placed in a 96-well plate coated with monoclonal antibody for human C5adesArg. Next, the C5a molecules were bound. After a wash, a solution of streptavidinhorseradish peroxidase and biotinylated anti-human C5a antibody was added to produce a C5a antigen sandwiched by C5a antibodies. A second wash and substrate solution created a spectrum of blues based on the concentration of C5a within solution. This is followed by the addition of a stop solution to change the color of the solutions to a spectrum of yellows which is then quantified into concentrations by passing a 450 nm light through the solution.

Self-Report Measures

Acute Pain and Stress Indicators

The following acute pain and stress indicators are more representative of the conditions the participants experienced during the visit itself, such as the emotion-stimulating exercise and the palpometer pain threshold test during the visit.

Current Pain. A Visual Analog Scale (VAS) was created to measure current levels of acute pain. The original scale used a visual horizontal bar where participants would mark their level of pain while being able to observe the entire spectrum of potential acute pain (Carlsson, 1983). The RAMP protocol modified this scale to a numerical 0 to 10 scale that increased in increments of 0.5.

Recent Pain. The West Haven-Yale Multidimensional Pain Inventory (MPI) was developed for patients experiencing chronic pain. This scale of 0 to 6 for three items measures the severity of current pain experienced during the visit as well as the most extreme pain experienced within the past week. These three items are averaged to determine total recent pain severity (Kerns et al., 1985).

Current Mood. Current anxiety and sadness were assessed with items pulled from the widely used POMS (Usala & Hertzog, 1989) and PANAS-X (Watson & Clark, 1994) standard adjective checklist measures of current mood. Items selected from these larger adjective scales were those of clearest theoretical relevance to health and pain. Both subscales include five items on which participants are asked to rank how they feel right now on a 1-5 scale. Anxiety items were nervous, worried, uneasy, tense and on edge; sadness items were sad, blue, depressed, glum and gloomy. Using these scales, current anxiety and sadness were measured four times within the study at baseline, 10, 60 and 100 minutes post pain.

Longer Range Recent Pain and Stress Indicators

The following recent pain and stress indicators are better representative of factors that impact the participant outside the range of each visit. These indicators are expressive of conditions with which the participant came to the visit (i.e., those which could not have been elicited of affected by the visit itself).

Perceived Stress. Recently perceptions of psychological stress were assessed with the 10-item version of the Perceived Stress Scale (Cohen et al., 1988; PSS). The PSS is a widely used scale depicting a participant's perceptions of their stress expand coping. Each item question was scaled from 0 (*never*) to 4 (*very often*). After the reverse scoring and summation of the ten items, a total perceived stress score was identified. This question set was administered to the participant once per visit prior to the first blood draw.

Arthritis Severity. The Modified Health Assessment Questionnaire (mHAQ) was used as an indicator of the each participant's rheumatoid arthritis severity. The scale asks participants to rate the difficulty of 8 specific tasks on a scale from 1 (*not much difficulty*) to 4 (*cannot do*). Items included “able to dress yourself; laces and buttons?” and “walk outdoors”. The mHAQ is a widely used and validated measure of RA severity (Pincus, Summey, Soraci, Wallston, & Hummon, 1983). The mHAQ was administered once at each visit prior to the first blood draw.

Pain Interference. The Pain Interference subscale of the West Haven-Yale Multidimensional

Pain Inventory (Kerns, Turk, & Rudy, 1985; MPI) was used to assess pain interference, or the degree to which disruption of daily activities participants perceived were caused by their pain. The MPI is a widely-used self-report measure developed exclusively for use with chronic pain patients (Tait, 1999). There is substantial support for the reliability and validity of these subscales based on heterogeneous samples with a variety of pain complaints (Tait, 1999).

Data Analysis

All of the long term and acute pain and mood measures were first coded and screened for normality. The same screening took place for the two time points of CRP assayed for within all the emotion conditions and the four C5a time points assayed for within the sadness condition. Change in CRP by emotional condition was examined via repeated measures ANOVA.

Correlations

The statistical software SPSS version 18 was used for all analyses. Bivariate correlations were calculated between the two biomarkers of interest CRP at baseline and 100 minutes post pain and C5a at all 4 blood draw time points (baseline, 10 minutes, 60 minutes and 100 minutes post pain) within the sadness condition. A second set of correlations were computed for CRP at the two time points within all emotion conditions and the acute and recent pain and mood scales. The last set of bivariate correlations was calculated for the four C5a time points and the pain and mood scales with the sadness condition.

Regression

To further investigate all marginally significant and significant correlations, two step hierarchical regressions were run to determine how well the variable of interest predicted the CRP or C5a levels controlling for baseline levels; in each of these analyses, baseline levels of the dependent variable were entered in the first step and the variable of interest (e.g., a pain or stress indicator) was entered in the second step.

RESULTS

Correlation Analysis

Comparing the Change in CRP Over the Four Experimental Conditions

As shown in Table 1, there was no significant change in CRP from baseline to 100 minutes in any of the conditions for the sample as a whole. The mean of serum CRP at baseline was 8.53 mg/L and the mean of serum CRP at 100 minutes post pain was 8.36 mg/L. This change in control condition serum CRP was not significant with $F(9) = 0.07$, $p=0.80$. Within the sadness/depressed mood condition, the mean of serum CRP at baseline was 12.46 mg/L and the mean of serum CRP at 100 minutes post pain was 12.0 mg/L. The change in serum CRP in the sadness/depressed mood condition was not significant with $F(9) = 0.08$, $p=0.79$. Within the happiness condition, the mean of serum CRP at baseline was 20.71 mg/L and the mean of serum CRP at 100 minutes post pain was 21.32 mg/L. The change in serum CRP within the happiness condition was not significant with $F(9) = 0.20$, $p= 0.67$. Lastly, the mean of serum CRP at baseline within the anger condition was 8.96 mg/L and the mean of serum CRP at 100 minutes post pain was 6.26 mg/L. The change in serum CRP in the anger condition was not significant with $F(9) = 2.02$, $p=0.20$.

Changes in CRP was not correlated or predicted by any of the acute emotion conditions elicited within the study. Due to budget limitations, C5a could only be assayed in one condition. The sadness condition was selected for several reasons. To our current knowledge, the last that C5a was measured in the peripheral blood of rheumatoid arthritis patients was in 1990 where the results were inconclusive due to the lack of assay sensitivity. (Jose et al., 1990). Due to the low concentration of C5a in the previous inconclusive analysis, it was hypothesize that the obtained C5a levels would be low and potentially undetectable, thus indicating that activated C5a did not circulate within the peripheral blood. Due to the estimate that C5a would not be detectable, the most emotionally stimulating condition was chosen to gauge if emotion or pain had a role in complement activation. The sadness condition was determined to be the most emotionally stimulating condition based on changes in current sadness. Further, it was the cleanest in terms of eliciting the target emotion without exuding increases in other emotions. Thus, C5a analyses are presented on the

sadness condition only.

CRP and C5a Within the Sadness Condition

As shown in table 2, within the sadness condition (the only time point at which C5a was obtained), there were non-significant trends for serum CRP 100 minutes post pain to be correlated with serum C5a at the 60 minute and 100 minute post pain time points (r = .704, p= 0.119, and r=.649, p=0.163, respectively).

CRP and C5a With Acute Pain and Mood Indicators Within the Sadness Condition

Current Pain and CRP

Serum CRP at baseline was marginally correlated with 60 minute post pain manipulation current pain (r=0.671, p=0.068, N=8) 8. CRP at 100 minutes was most strongly (but still marginally) correlated with current pain at 60 minutes (r=0.719, p=0.068). There was also a non-significant trend for CRP at 100 minutes post pain to be associated with current pain at 10 minutes post pain (r=0.553, p=0.198). Baseline current pain was not significantly correlated with either CRP time point. In addition, current pain at 10 minutes post pain was also not significantly correlated with baseline CRP.

Current Pain and C5a

Baseline serum C5a did not significantly correlate with baseline, 10 minutes or 60 minutes post pain current pain. Serum C5a at 10 minutes post pain manipulation was marginally correlated with the baseline, 10 minute and 60 minute current pain ratings(r=0.672, p=0.144; r=0.736, p=0.096; and r= 0.063, p=0.151, respectively). It is important to note that current pain 10 minutes post pain was most strongly yet marginally correlated with C5a at 10 minutes post pain.

Recent Pain Severity and CRP

Baseline serum CRP and 100 minutes post pain CRP did not significantly correlate with recent pain severity as measured by the MPI.

Recent Pain Severity and C5a

Baseline serum C5a did not significantly correlate with recent pain severity. Recent pain severity marginally correlated with serum C5a at 10 minutes, 60 minutes and 100 minutes post pain (r=0.729, p=0.162, r=0.800, p=0.104) and r=0.689, p=0.199. It is important to note that recent pain severity was most strongly yet marginally correlated with C5a at 60 minutes post pain.

Current Sadness CRP

Baseline CRP was marginally correlated with 60 min acute sadness (r=0.538, p=0.169). All other Sadness and CRP correlations were not statistically significant.

Current Sadness and C5a

Baseline C5a was inversely marginally correlated with 10 minutes post pain Sadness with r=0.523, p=0.184. All other sadness and C5a correlations were not statistically significant.

Current Anxiety and CRP

All anxiety and CRP correlations were not statistically significant.

Current Anxiety and C5a

Baseline and 10 minutes post pain serum C5a and baseline current anxiety were marginally correlated (r=0.522,p=0.184 and r=0.644, p= 0.168). All other anxiety and C5a correlations were not statistically significant.

CRP and C5a With Recent Pain and Stress Within the Sadness Condition

None of the recent pain and stress indicators were significantly associated with either CRP or C5a with the exception of a marginally significant correlation between CRP at baseline and RA severity (r=0.550, p=0.158).

Additional Correlations Within Remaining Emotional Conditions

Correlations between CRP and both current acute and long term pain and mood indicators for all the remaining emotional conditions (anger, happiness and control) were not significant. The only two correlations of marginal significance was within the anger condition between serum CRP 100 minutes post pain and the 10 min post pain current sadness (r=0.654, p=0.079) and baseline

CRP within the happiness condition and the 10 min post pain current sadness (r=0.548, p=0.159).

Regression Analyses Controlling for Baseline Levels

CRP at 100 Minutes Post Pain as a Predictor of C5a at 100 Minutes Post Pain

Controlling for baseline levels of C5a, CRP at 100 minutes post pain marginally predicts C5a at 100 minutes post pain, ΔR^2=0.20, β=-0.47 , p=0.17.

Acute Pain as a Predictor of C5a and CRP

Table 5 details the two step hierarchical regression analysis of how powerfully acute pain predicts the 10, 60 and 100 minute C5a time points and the 100 minute CRP time point while controlling for baseline.

Controlling for baseline levels of CRP, acute pain at 10 minutes post pain manipulation did not predict CRP at 100 minutes post pain, ΔR^2=0.0, β=0.01 , p=0.71. Similarly, current pain at 60 minutes did not predict CRP at 100 minutes, with ΔR^2=0.0, β=-0.10, p=0.64.

Acute Mood Predictors: Sadness and Anxiety as Predictors of CRP and C5a

The two step hierarchical regression of 10 minute post pain Sadness did not predict C5a at 100 minutes post pain with ΔR^2=0.03, β=-0.197 , p=0.671. The same regression analysis of Sadness at 60 minutes post pain did not predict CRP at 100 minutes post pain with ΔR^2=0.01, β=-0.12 , p=0.453.

The two step hierarchical regression of baseline anxiety marginally predicted C5a at 100 minutes post pain with ΔR^2=0.19, β=-0.71 , p=0.184.The same regression analysis for C5a at 10 minutes post pain did not predict CRP at 100 minutes post pain with ΔR^2=0.0, β=-0.00 , p=0.968

Acute Pain Predictors: VAS as a Predictor of CRP at 100 Minutes Post Pain within the Sadness Condition

The two step hierarchical regression of VAS at 10 minutes post pain did not predict CRP at 100 minutes post pain with ΔR^2=0.0, β=0.01 , p=0.71. The same regression analysis for VAS at 60 minutes post pain did not predict CRP at 100 minutes post pain with ΔR^2=0.0, β=-0.10 , p=0.64.

Long Term mHAQ as a Predictor of CRP at 100 Minutes Post Pain within the Sadness Condition

The two step hierarchical regression of the mHAQ, long term indicator of disease severity taken at baseline did not predict CRP at the 100 minute time point with ΔR^2=0.0, β=-0.01 , p=0.94.

DISCUSSION

A key goal of the present project was to determine if C5a levels could be detected in circulating human serum and to confirm that it was associated with CRP. The presence of C5a within the bloodstream would indicate that the activated complement protein is circulating and not localized in specific tissues with our study population. In addition, observing C5a trends in relation to specific psychosocial variables could shape novel future rheumatoid arthritis treatment methodology. Contrary to expectations that C5a might be difficult to detect, we obtained consistently high and detectable levels within the spectrum of measurement capable by the ELISA. Indeed, one participant showed C5a levels that were undetectably high with the dilution in place; anecdotally, the experimenter for that day had noted that this particular participant had a particularly strong emotional response compared to the other participants. Further, within the sadness condition (the only condition in which we had C5a data to analyze), CRP at the 100 minute time point expressed a marginal correlation with C5a at the 60 and 100 minute time points. Further, controlling for baseline values, CRP at the 100 minute time point marginally predicted both 60 and the 100 minute C5a. These results fit the knowledge that CRP is upstream of the C5a complement protein within the process of complement activation. With a larger sample size, I would anticipate a significant correlation and predictive trend relating CRP and C5a.

The present project is the first to show that the complement protein C5a appears to be strongly activated by acute pain. Importantly, baseline levels of C5a were not associated with recent or acute pain; instead, both acute pain caused during the visit and perceptions of recent pain were marginally correlated with C5a at every time point, with the strongest correlations being in the measures closest in time to one another. Further supportive of a link between pain and C5a

activation, after controlling for baseline levels, all three later C5a time points were associated by acute pain experienced at baseline, 10 and 60 minutes post pain manipulation. In particular, acute pain at baseline and 10 minutes post pain each significantly predicted 14 and 15% of the variance, respectively, in C5a at 10 minutes. Even more dramatically, approximately 30% of the variance in C5a at 100 minutes was predicted by acute pain at baseline. Recent pain (MPI) taken at three time points within the study was also marginally correlating with all C5a time points excluding the baseline. The strongest association between recent pain and C5a was between average recent pain and 60 minute C5a; after controlling for baseline values, 26% of the variance in C5a at 60 minutes was predicted by the average recent pain of the visit. Within a small sample size of nine individuals, this strong association between pain and C5a suggests that C5a is strongly activated by pain experience and requires further investigation.

CRP was also somewhat, although perhaps not as strongly, associated with the pain indicators in the present research. Acute current pain was marginally correlated with CRP at the later 60 and 100 minute time points. After controlling for baseline values, however, acute pain did not predict CRP at baseline or 100 minute time point.

The only longer term variable that predicted the inflammatory complement markers of interest was pain interference, an indicator of pain related distress. Pain interference specifically did associate strongly with these inflammatory markers, particularly with C5a and was marginally correlated with both CRP and C5a at the baseline and 100 minute time points. After controlling for baseline levels, pain interference significantly predicted 36% of the variance in 100 minute C5a, whereas it did not predict significantly more variance in CRP over and above baseline levels.

Importantly, two longer range indicators of distress did not predict CRP or C5a over and above baseline levels. As expected, given the strong extent to which CRP is related to disease severity, RA severity was associated with CRP at baseline. However, RA disease severity did not predict CRP production at the 100 minute time point after controlling for baseline. Neither CRP nor C5a were predicted by overall perceived stress.

Although acute pain and negative mood are often inextricably linked, the present study suggests that pain itself is a stronger trigger of the activation in C5a. Acute anxiety and sadness as manipulated in the current study were correlated with C5a and CRP at various time points. However, neither sadness nor anxiety was significantly predictive of either biomarker after controlling for baseline values. Perhaps pain is a more immediate activator of C5a such that its role was able to be captured in the short time frame observed in the present study. It is possible that the influence of mood on inflammatory response may take longer to unfold or may occur via more indirect routes.

Limitations

The primary weakness within this study was the small sample size of participants. To further support the assertions made within this paper a larger sample size of women are needed. If trends continue the way the analysis is anticipating, a larger sample will lead to significant results and additional significant regressions and stronger correlations.

Missing data is anticipated in any clinical study. Data was not able to be collected for the following reasons: blood draw was unsuccessful during the experimental procedure, participant could not continue participating in the study and out of range data within the C5a ELISA. A lack of funding prevented a second assay from being run.

Conclusions

Of greatest predictive power in predicting C5a over the course of the 4 hour session investigated in the present research were acute pain and recent pain. In addition, the more long term indicator of pain interference was marginally correlating with later C5a time points and significantly predictive of the 100 minute C5a measure. Within the sadness condition, acute sadness was also strongly correlated variables to CRP and C5a concentrations. My analysis of nine participants found acute pain and acute sadness to be marginally correlated with C5a and CRP concentrations at all time points of the study (excluding the baseline) and most strongly correlated with the closest respective time point within the most emotionally stimulating sadness condition.

These results indicate that the degree of complement activation within the RAMP analysis is more likely to be dependent on acute short term pain and mood stressors of the sadness visit itself rather than the pain and mood indicators of long term events occurring prior to the visit.

This investigation inspired a variety of new analyses we hope to begin in the near future. The significance of prostaglandin's precursor C5a in estimating acute pain has lead to an interest in testing for elevated levels of PGE2 within the peripheral blood through direct radio-immunoassay techniques. Based on our current analysis, it may be that the emotion regulating lipid based prostaglandin E2 would result in statistically significant correlations with the complement protein C5a, acute pain and potentially acute mood variables.

A better understanding of the relevance of C-reactive protein changes due to emotional manipulation within collected blood serum of rheumatoid arthritis patients could lead to better means of chronic pain reduction. C-reactive protein has also been shown to have clinical significance in cardiovascular diseases such as stroke and heart disease. A further investigation of impact of emotional manipulation on C-reactive protein levels could provide insight and allow for the creation of new preventive measures for combating certain cardiovascular diseases (Mcdade et al., 2004).

Overall, these results suggest that C5s is an important molecule that can be assayed among humans and appears to be relevant to pain responses among individuals with RA, potentially more so than CRP for short-term activation. Further investigation of complement, more specifically C5a, may help shed light on pain severity and stress within RA and could be used to better understand the etiology of the disease as well as novel possible treatments. A timely expansion of this study's protocol has the potential for a variety of potential clinical improvements for the treatment of rheumatoid arthritis and a variety of other illnesses involving chronic pain.

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Table 1: Comparing the Change in CRP (From Baseline to 100 Minutes Post Pain) Over the Four Emotion Conditions

Emotion Condition	Control	Depressed	Happiness	Anger
Mean Serum CRP, baseline (mg/L)	8.53	12.46	20.71	8.96
Mean Serum CRP, 100 min post pain (mg/L)	8.36	12.00	21.33	6.23
F value of CRP change	0.07	0.08	0.20	2.02
Significance of CRP change	0.80	0.79	0.67	0.20

Table 2: Correlating CRP with the Complement Protein C5a Within the Sadness/Depressed Condition

Pearson Correlation (r)	Serum C5a, baseline	Serum C5a, 10 min post pain	Serum C5a, 60 min post pain	Serum C5a, 100 min post pain
Serum CRP, baseline	-0.08	0.11	0.51	0.47
Serum CRP, 100 min post pain	0.16	0.30	0.70┼	0.65┼

┼ Correlation shows a non-significant trend, with p value less than 0.2 (2-tailed)

Table 3: Correlating CRP and Complement Protein C5a With Acute Pain and Mood Indicators (Sadness, Anxiety, VAS, and MPI) Within the Sadness/Depressed Condition

	Pain and Mood Indicators									
	Sadness			Anxiety			Current Pain			MPI
Serum CRP, baseline	B	10 min	60 min	B	10 min	60 min	B	10 min	60 min	
	-0.08	-0.03	0.54┼	-0.45	-0.41	0.365	0.28	0.46	0.67┼	0.31
Serum CRP, 100 min	-0.34	-0.08	-0.38	-0.47	-0.46	-0.581	0.29	0.55┼	0.72┼	0.21
Serum C5a, baseline	0.26	0.52┼	0.01	0.52┼	0.28	0.188	0.24	0.24	0.41	0.11
Serum C5a, 10 min	0.53	0.57	-0.09	0.64┼	0.47	0.299	0.67┼	0.74┼	0.66┼	0.73┼
Serum C5a, 60 min	0.33	0.40	-0.22	0.38	0.08	-0.217	0.66┼	0.71┼	0.71┼	0.80┼
Serum C5a, 100 min	0.24	0.32	0.15	0.36	0.30	0.147	0.74┼	0.75┼	0.76┼	0.70┼

Note. B= Baseline

┼ Correlation shows a non-significant trend, with p value less than 0.2 (2-tailed)

Table 4: Correlating C-Reactive Protein and Complement Protein C5a With Long Term Pain and Mood Indicators (PSS, mHAQ and PI) Within the Sadness/Depressed Condition

	Recent Pain and Stress Indicators		
	PSS	mHAQ	Pain Interference
Serum CRP, baseline	-0.33	0.55┼	0.97┼
Serum CRP, 100 min	0.01	0.20	0.88
Serum C5a, baseline	0.05	0.16	-0.66
Serum C5a, 10 min	0.42	0.61	0.41
Serum C5a, 60 min	0.23	0.60	0.59
Serum C5a, 100 min	0.35	0.39	0.97┼

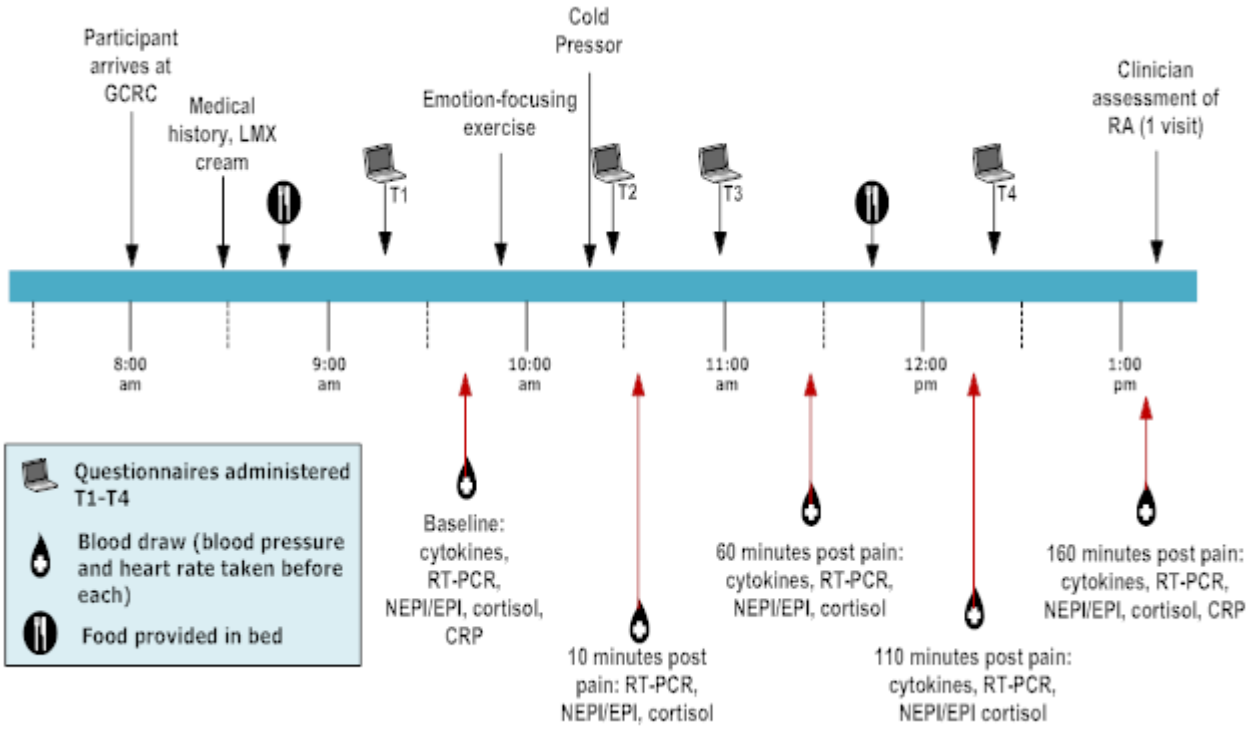
┼ Correlation shows a non-significant trend, with p value less than 0.2 (2-tailed)

Table 5: Predictive Value of VAS Measure For Baseline, 10, 60 and 100 Minutes Post Pain and Averaged MPI 1-3 Scale on C5a at 10, 60 and 100 Minutes Post Pain within the Sadness Condition

	C5a 10 min post pain			C5a 60 min post pain			C5a 100 min post pain		
VAS	ΔR^2	ΔF	β	ΔR^2	ΔF	β	ΔR^2	ΔF	B
baseline	0.15*	22.90*	0.41*	0.19^{+}	8.84^{+}	0.46^{+}	0.30*	11.49*	0.57*
VAS 10 min	0.14*	13.90*	0.41*	0.15^{++}	3.94^{++}	0.42^{++}	0.22^{++}	3.94^{++}	0.51^{++}
VAS 60 min	--	--	--	0.17^{+}	5.50^{+}	0.44^{+}	0.24^{++}	5.16^{++}	0.53^{++}
MPI average	0.13^{+}	8.19^{+}	0.40^{+}	0.26*	372.0*	0.55*	0.16	1.96	0.44

$^{++}$p< 0.20, $^{+}$ p< 0.10, *p<0.05, **p<0.01

Figure 1: General Participant Timeline for GCRC Visit



Appendix A-RAMP Study IRB Approval

All the necessary IRB approvals were in place prior to September 1st 2009. The Rheumatoid Arthritis Multidimensional Project (RAMP) has Penn State IRB approval (IRB #27409), Penn State Biohazard Safety Approval (IBC#27962), and Penn State GCRC approval (G229). In addition, this research study has permission from the Geisinger Health System IRB (#2008-0186) for study referrals from Dr. Ayoub's practice.

Appendix B- Acute and Longer Term Measure Questionnaires

MORA Mood

Please circle the appropriate number to indicate how you feel right now. Try not to take too long with any one item.

	Not at all	A little	Moderately	Quite a bit	Extremely
Tired	○	○	○	○	○
Angry	○	○	○	○	○
Sad	○	○	○	○	○
Happy	○	○	○	○	○
Nervous	○	○	○	○	○
Proud	○	○	○	○	○
Alert	○	○	○	○	○
Content	○	○	○	○	○
Worried	○	○	○	○	○
Furious	○	○	○	○	○
Fatigued	○	○	○	○	○
Blue	○	○	○	○	○
Pleased	○	○	○	○	○
Lively	○	○	○	○	○
Uneasy	○	○	○	○	○
Grouchy	○	○	○	○	○
	Not at all	**A little**	**Moderately**	**Quite a bit**	**Extremely**
Weary	○	○	○	○	○

Guilty					
Active					
Depressed					
Enthusiastic					
Tense					
Bad-Tempered					
Exhausted					
Energetic					
Gloomy					
Cheerful					
On Edge					
Ashamed					
Mad					
Full-of-Pep					
Glum					
Worn Out					
Joyful					
Confident					

VAS

Please rate your CURRENT pain on the following scale. (0 = No Pain, 10 = Extreme Pain)

0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10

MPI Severity

In the following questions you will be asked about pain you may or may not have experienced recently. If you have NO PAIN answer zero to all of these questions.

	0 - No Pain	1	2	3	4	5	6 - Very Intense
1. Rate the level of your pain at the PRESENT MOMENT.	☐	☐	☐	☐	☐	☐	☐
2. On average, how severe has your pain been DURING THE LAST WEEK?	☐	☐	☐	☐	☐	☐	☐
3. How much suffering have you experienced because of pain DURING THE LAST WEEK?	☐	☐	☐	☐	☐	☐	☐

PSS

The questions in this scale ask you about your feelings and thought during the last month. In each case, please indicate how often you felt or thought a certain way.

	0 = Never	1 = Almost Never	2 = Sometimes	3 = Fairly Often	4 = Very Often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	☐	☐	☐	☐	☐
2. In the last month, how often have you felt that you were UNABLE to control the important things in your life?	☐	☐	☐	☐	☐
3. In the last month, how often have you felt nervous and "stressed"?	☐	☐	☐	☐	☐
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	☐	☐	☐	☐	☐
5. In the last month, how often have you felt that things were going your way?	☐	☐	☐	☐	☐
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	☐	☐	☐	☐	☐
7. In the last month, how often have you been able to control irritations in your life?	☐	☐	☐	☐	☐
8. In the last month, how often have you felt that you were on top of things?	☐	☐	☐	☐	☐
9. In the last month, how often have you been angered because of things that were outside of your control?	☐	☐	☐	☐	☐
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	☐	☐	☐	☐	☐

MPI Interference

Please answer the following additional questions about your chronic pain in the last week.

	0 - No interference	1	2	3	4	5	6 - Extreme interference
1. How much has your pain interfered with your day to day activities in the past week	☐	☐	☐	☐	☐	☐	☐
2. How much has your pain interfered with your ability to work in the last week?	☐	☐	☐	☐	☐	☐	☐
3. How much has your pain interfered with your ability to participate in social and recreational activities in the last week?	☐	☐	☐	☐	☐	☐	☐
4. How much has your pain interfered with the amount of satisfaction or enjoyment you have gotten from participating in social and recreational activities in the last week?	☐	☐	☐	☐	☐	☐	☐
5. How much has your pain interfered with the amount of satisfaction you have gotten from family-related activities in the last week?	☐	☐	☐	☐	☐	☐	☐
6. How much has your pain hurt your romantic and/or other family relationships in the last week?	☐	☐	☐	☐	☐	☐	☐
7. How much has your pain interfered with the amount of satisfaction or enjoyment you have gotten from work in the last week?	☐	☐	☐	☐	☐	☐	☐
8. How much has your pain interfered with your ability to do household chores in the last week?	☐	☐	☐	☐	☐	☐	☐
9. How much has your pain hurt your friendships with people other than your family in the last week?	☐	☐	☐	☐	☐	☐	☐
10. How much has your pain interfered with your ability to plan for the future?	☐	☐	☐	☐	☐	☐	☐

MHAQ

Today are you able to:

	No Difficulty	Some Difficulty	Much Difficulty	Cannot Do
1. Dress yourself; including laces and buttons?	☐	☐	☐	☐
2. Get in and out of bed?	☐	☐	☐	☐
3. Lift a full cup or glass to your mouth?	☐	☐	☐	☐
4. Walk outdoors on flat ground?	☐	☐	☐	☐
5. Wash and dry your entire body?	☐	☐	☐	☐
6. Bend down and pickup clothing from floor?	☐	☐	☐	☐
7. Turn regular faucets on and off?	☐	☐	☐	☐
8. Get in and out of a car?	☐	☐	☐	☐

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EDUCATION

Pennnsylvania State University, University Park, PA
Bachelor's of Science in Immunology and Infectious Disease
Honors in Biobehavioral Health

RESEARCH EXPERIENCE

Dr. Jennifer Graham's Stress and Health Lab at Penn State, Honors Research Assistant
(Spring 2010 to present)

- Working with Rheumatoid Arthritis patients to determine the effects of emotion and temperament on inflammation by measuring various cytokine, hormone and transcription factor levels within the blood samples drawn.
- Preparing and completing a honors thesis investigating the levels of C-reactive protein and specific transcription factors within drawn blood samples and its ties to emotional stimulation
- Skills: Trained as a lead experimenter, blood spinner, data manager and manager of other lab activities (scheduling participants and etc). Will be heavily involved in running immunoassays from participant's blood samples in the near future.

Healthwise with Linda Caldwell at Penn State, Research Assistant
(Summer 2010)

- Helping to evaluate and improve upon the Healthwise curriculum implemented in South Africa. This curriculum's goal is to help students in 8th and 9th grade to better utilize their free time and explore activities of interest. The implementation of this curriculum was aimed to reduce the practice of high risk behaviors and to help prevent the spread of HIV/AIDS through awareness/education.
- Currently adapting the curriculum for implementation within a variety of communities across the state of Pennsylvania.

The Vision, Memory and Computational Neuroscience Lab at Penn State, Research Assistant
(Summer 2010)

- Worked with a group of graduate students to be trained on conducting a variety of facial recognition vision experiments.
- Skills: Proficient in the use, application and care of EEG upon human participants, practice in following and running a variety of experimental vision experiment protocols with and without the use of the EEG and participation in experiment piloting

Dr. Anthony Schmitt's Viral Pathogenesis Lab at Penn State, Research Assistant
(Summer 2009)

- Created a series mutant genes of the mumps virus with single point mutations for protein analysis. The goal is to see which portion of the genome encodes for virus budding.
- Skills: Proficient in PCR, DNA purification, bacterial transformation and gel electrophoresis

Dr. Claude dePamphillis' Plant Genomics Lab at Penn State, Research Assistant
(Spring 2008)

- Worked with graduate student Yuannian Jiao primarily focused on literature searches of relevant plant gene family lineages
- Skills: Proficient in literature searches, quick and efficient navigation through large scientific journal databases and other important organizational skills

AWARDS AND GRANTS

The Young Award for International Agriculture-Spring 2011
Phi Beta Kappa Honors Thesis Grant- Spring 2011
Most Innovative Solution- 2010 Milking the Rhino Innovative Solutions Showcase at Penn State
Penn State Schreyer Honors College Summer Discovery Grant- Summer 2010

LEADERSHIP SKILLS

Co-founder of Prerana (Fall 2010 to present)

- Our social entrepreneurship venture is in the process of developing a tranformative education system for the women of the Self- Employed Women's Association (SEWA). Our goal is to provide an affordable education solution that creates tangible value in the lives of SEWA women.

Global Leadership Initiative at Penn State (Summer 2010- Spring 2011)

- Under the guidance of senior faculty, a group of selected students create and implement an innovative individual health related projects abroad. The group of students also attend weekly lectures on how to successfully implement and create projects, learn about current global health policy and how policy is implemented worldwide. The group of students is also responsible for mentoring future program participants.

Penn State Biology Department Lab Teaching Assistant/Tutor (Spring 2008-present)

- Lab Teaching Assistant skills: creation of a positive learning environment for all students within the class, guide students through a series of experiments relevant to lecture, provide supplemental lectures to enhance the student's knowledge of the course material
- Tutoring skills: work with students in smaller groups to review past exams and unclear concepts, provide supplemental material to aid in a more comprehensive understanding of the material

Penn State Physics Department Tutor (Spring 2009-present)

- Tutoring skills: provide individualized attention to review broad concepts and homework questions

Subway Manager and Head Overseer for locations in Ewing, Yardville and Pennington, NJ (March 2008-March 2009)

- In charge of providing all locations with appropriate materials including food, change and other goods, placing weekly food orders, inspecting the stores for any potential problems, maintaining records of cash drops and daily sales, taking weekly inventories and ultimately upholding the highest standard of service

Swim America Coach, Ewing NJ- Lauri Kemmerling and Brent Matheson **(August 2003-present)**
Private Swim Instructor (September 2005- present)

COMMUNITY SERVICE

The Penn State IFC/Panhellenic Dance Marathon (THON)- The largest student run philanthropy in the world involved in raising funds (7.6 million dollars in 2010) and providing support for children with pediatric cancer throughout the year culminating in a 46 hour dance marathon every February

- **Independent Dancer Candidate and THON Chair** – '09-'10
  - head of fund raising event organization, book keeping and maintaining the philanthropic ideals of the organization
- **Rules and Regulations Committee Member- Kids' Mail Chair**- '08-'09
  - in charge of maintaining security during THON weekend, ensuring that the rules of the facilities are upheld to guarantee the future success of THON, fund raising participation

Doctors Without Borders Office Volunteer- New York Office

- Aid with general secretarial tasks around the office and provide support to a variety of hosted events within the New York City area