IMMUNE SYSTEM MODULATION IN VICTIMS OF INTIMATE PARTNER VIOLENCE

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Michael Brian Cheung

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IMMUNE SYSTEM MODULATION IN VICTIMS OF INTIMATE PARTNER VIOLENCE

Michael Brian Cheung

Thesis

Approved:	Accepted:
_____	_____
Advisor Dr. James Holda	Department Chair Dr. Monte Turner
_____	_____
Committee Member Dr. Thomas Alexander	Dean of the College Dr. Chand Midha
_____	_____
Committee Member Dr. Stephanie Woods	Dean of the Graduate School Dr. George Newkome
	_____
	Date

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"If I have seen further it is only by standing on the shoulders of giants." - Sir Isaac Newton

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CHAPTER I

INTRODUCTION

The immune system protects the body from disease by identifying and destroying pathogenic microorganisms, materials and even dangerous self-cells. In addition to the bone marrow and circulating blood, cells from the immune system can be found in high concentrations in the thymus, lymph nodes, spleen, as well as other lymphatic tissues. Generally speaking, the immune system can be divided into two categories based on specificity; the adaptive immune response, made up of cells which respond to specific antigens, and the innate immune response, made up of cells which can respond to a variety of immune challenges. The innate immune cells, such as neutrophils and natural killer cells (NK cells), are often considered the first line of defense against disease causing agents since they respond to broad categories of immune challenges, such as bacteria and eukaryotic parasites. Adaptive immune cells, B and T cells, react to specific disease causing agents and therefore present a second line of defense against pathogens which have developed specific techniques of avoiding innate immune detection. If immune cells from either category are compromised in either number or effectiveness of action the body will be more susceptible to infection, disease, and other pathological processes.

Substantial evidence exists that indicates immune cells are modulated by situations not always considered by medical professionals, such as psychological state or physical activities like exercise (Boscarino & Chang, 1999; Dhabhar, 2002; Inslicht et al, 2006; Ironson et al, 1997; Kanter et al, 2001). Interactions between psychological stress and physiological status are widely studied in the fields of medical science, such as neurology and endocrinology, and yet much remains to be examined specifically with regard to immune system health. While some psychosomatic mechanisms by which the body copes with mental or physical stress are partially understood the picture is often incomplete, with the total effects of widely acting hormone or cytokine activity being a complex web of positive and negative feedback. In addition, psychological stresses may be diagnosed and grouped too broadly or inaccurately, such as a diagnosis of post-traumatic stress or depression, which can be caused by a variety of events and traumas, and may therefore exhibit different health conditions dependent on the initial triggering incident. Or, while psychological symptomology may be similar between subjects the physiological symptoms may be more dependent on the type of incident involved. Classifying the cause of the stress and then exploring the medical conditions correlated with that initial cause is vital to providing more effective care for those diagnosed. In the case of this study, the type of trauma suffered by abused women will be compared to the immune system's readiness to combat pathogenic challenges.

Intimate partner violence (IPV) is unfortunately widespread in today's world. In the US alone one out of every three women suffer violence from a

current or former spouse or partner (Tjaden & Thoennes, 2000). Increasing literature has linked IPV to numerous health risks both psychological, such as depression and post-traumatic stress disorder (PTSD), as well as physical health symptoms (Campbell et al 2002; McCauley et al 1995; Nicolaidis et al, 2004; Woods 2000). However, the classification of IPV can be applied to a continuum of abuse, from a single event to long-term ongoing battery (Tjaden & Thoennes, 2000). Additionally, four separate types of behaviors are associated with IPV; physical abuse, sexual abuse, threats of violence, and non-physical emotional abuse. To gain a better understanding of the effects abuse may have on victims physiologically each of these behaviors needs to be studied and independently linked to changes in health status.

Therefore, the objectives of this study were to examine the immune health of female victims of IPV and identify immune system modulation that correlated with PTSD symptoms and IPV classifications. Results from this thesis will be collected together with the work of other investigators to create a total health profile of victims of specific forms of IPV. Better understanding will undoubtedly lead to the development of more effective and complete therapies that take into account the physiological and psychological condition of the patient.

CHAPTER II

LITERATURE REVIEW

Post-traumatic stress and immune health

In the literature there is evidence for innate immune system modulation within patients suffering from PTSD. Ironson et al (1997) found that victims of hurricane Andrew who suffered from high levels of PTSD showed significant loss of NK cell cytolytic function. However, they also exhibited higher absolute NK cell counts, indicating the immune system was perh aps controlling for decreased NK cytotoxicity by increasing the quantity of circulating NK cells (Ironson et al, 1997). Another paper found that there were abnormal leukocyte (white blood cell) counts even twenty years after the event that caused the severe stress (Boscarino & Chang, 1999). However, little of the published research has included, let alone focused on women. One study that did focused specifically on women and the relationship of immune health with self-reported stress did find a decreased NK cell response associated with higher levels of stress, although they reported no associations with other measured immune parameters (Picardi et al, 2007). This study did not include any PTSD positive women, however. Recent research by Gill, Szanton, and Page has indicated that men and women

may face different symptoms, even when exhibiting from similar psychological disorders such as depression and PTSD (2005).

IPV-related PTSD

Some research indicates that the type of stressor, attitude, and degree of control the patient has over the stressor has an effect on the type and degree of immune system modulation (Seiber et al, 1992; Inoue-Sakurai et al, 2000). It is therefore important in this study to classify the PTSD-linked immune changes based on the type of IPV suffered by the subject. The difficulty in following this line of reasoning is that abused women often suffer multiple forms of IPV making it difficult to classify which PTSD-related symptoms are caused by which type of violence. Also, these multiple types of victimizations may have cumulative effects (Nicolaidis et al, 2004).

Natural killer cells

Natural killer cells (NK cells) are an important piece of the lymphocyte immune response. Although derived from the same cell lineage as B- and T-lymphocytes NK cells are not considered a part of the adaptive immune response. Rather, they have evolved to fill in a gap left in T cell immunity. Specifically, while T cells recognize self with non-self, NK cells are able to respond to loss of self. For example, tumors or viruses which turn off major histocompatibility complexes (MHC) would typically be able to escape detection by T-cytotoxic cells. Since the T cell receptor (TCR) relies on MHC-I with foreign ligand in order to respond to and destroy the infected target cell, a strategy

selected for in viruses and transformed tumor cells is to remove MHC-I molecules before they could “sound the alarm.” However, the MHC and MHC-like surface molecules also act as NK activity inhibitors, thus when they are missing from a cell’s membrane NK cells are able to recognize the “missing self” and respond, causing cytolysis of the infected or cancerous cell. (Herberman, 1986; Wendt et al, 2006)

Stimulation of NK cells is a complex event where certain molecules act as either inhibitors or stimulators to the lymphocyte (for more detail refer to Lanier’s reviews from 1998 and 2005). Rather than parsing through these NK cell effector molecules this particular study focused more generally on whether the conditions found in women suffering from PTSD can lead to altered NK cell activity. A decrease in NK cell activation in these experiments correlated with stress level reported in the psychological surveys could indicate new and unreported health risks such as increased susceptibility to cancer development or decrease in the immune response to viral infection.

CHAPTER III

MATERIALS AND METHODS

Participants

Participants in this study consisted of a convenience sample of women who attended battered women shelters in Summit and Medina counties, used shelter-affiliated services, or were recruited from the community. Eligible women were 18 years of age or older, in an intimate abusive relationship at the time, and were not suffering from known autoimmune disorders or cancer. For the protection of the participants confidentiality of all materials and information collected was maintained. The study was approved by the Institutional Review Board for the Protection of Human Research Participants (attached in Appendix).

Procedures

After the data collector introduced the purpose of the study and informed consent was given by the participant the researchers then administered questionnaires and conducted a trauma interview. Afterwards, blood samples were drawn using universal precautions. Two samples were collected in EDTA-coated tubes, one for complete blood count (CBC) with differential and one for lymphocyte subset analysis. In addition two heparin-coated tubes were collected, one of which was used in a cytokine assay not included in this thesis

and the other used for the lymphocyte and neutrophil activation assays. Tubes were placed in biohazard bags and immediately transferred to Akron City Hospital for processing and analysis.

Two rounds of sampling were administered to participants, the first at time one (T1) and again approximately one year later (T4). Only 132 participants of the original 157 contributed to the second T4 dataset. Scores from the self-tests and interviews were each entered twice into a database and cross-referenced to reduce the risk of entry error.

PTSD and depression evaluations

Two tests were administered: the PTSD Symptom Scale and the Trauma Symptom Inventory. Each is a well-established tool for diagnosis of PTSD and other trauma-associated psychological disorders such as depression, anger and irritability, and dissociation.

The Trauma Symptom Inventory (TSI) uses a 100-item self-report system where participants can rate how frequently they suffer from a variety of trauma related symptoms on a scale from 0 (never) to 3 (often). This test evaluates a wide variety of psychological impacts and returns scores on 3 validity scales and 10 clinical scales. The scores on the validity scales help to identify participants who's responses may fall outside the standardized sample for a variety of reasons, including over-endorsement of all items, marking all zeros rather than not completing the test, as well as random selection of answers. The 10 clinical scales report the extent to which a participant exhibits 10 different forms of

trauma-related symptoms such as anxious arousal, depression, anger, and dissociation. (Briere, 1995)

In order to diagnose post-traumatic stress in the participants a self-report version of the PTSD Symptoms Scale (PSS) was used. The PSS asks participants to rank symptoms of PTSD using a 4-point scale based on frequency. This 17-item questionnaire evaluates re-experiencing, avoidance, and arousal associated with PTSD. (Foa et al, 1993)

Intimate partner violence

Type and degree of IPV was determined using the Severity of Violence Against Women Scales (SVAWS), a 46-item scale. The SVAWS test is able to sort between the types of IPV and return scale scores for actual physical violence, sexual violence, and threats of violence (Marshall, 1992). In order to measure emotional abuse the Index of Spouse Abuse Non-Physical Subscale (ISA-NP) was used. This subscale has been utilized in similar studies (Woods et al, 2005; Campbell & Jones, 2002). In order to measure risk of homicide the Danger Assessment Scale (DA) was used. The DA is a simple test where participants answer yes or no to whether they experience certain risk factors (Campbell, 2007).

Immune system status

In order to deal with the complicated network of immune checks and balances multiple components were assayed. Since the absolute number of immune system cells is an important indicator of both effectiveness as well as

potential pathological processes the leukocytes, or white blood cells (WBCs) were counted. Also, as different cell types are responsible for different types of immunity, such as bacterial, parasitic, or viral protection, a differential WBC count are reported. In addition to cell number the actual functionality of the immune cells is important. Natural killer cells (NK cells) were chosen for a number of reasons. Firstly, they occur in high levels in circulating blood; lymphocytes (B, T, and NK cells) make up around 40% of circulating WBCs. Also, these cells are key components of human immune defense, responding to and destroying cancerous and virally infected cells. Additionally, changes in number and cytotoxicity of NK cells have been associated with PTSD in the literature (Ironson et al, 1997; Inoue-Sakurai et al, 2000; Woods et al, 2005).

White blood cell levels

In order to evaluate the immune system status of participants a CBC with differential and lymphocyte subset counts were used. The CBC returns leukocyte numbers and percentages that can inform a researcher of changes in the immune capability of the subject. The leukocyte counts and percentages were determined using the SYSMEX 9500 automated hematology analyzer (Sysmex Corporation). Lymphocyte subset counts and percentages were determined via flow cytometric methods using fluorescently labeled antibodies (BD bioscience). These results will be compared between T1 and T4 as well between subjects diagnosed clinical or nonclinical for PTSD and depression.

Natural killer cell activation assay

The NK cell activation assay was done using a flow cytometric procedure established by Britton and Alexander (Britton, 2001; Britton & Alexander, 2002). This procedure utilizes an earlier activation marker on NK cells called CD69 that can be identified by flow cytometry, along with two mitogens phytohemagglutinin (PHA) and human 60-kDa heat shock protein (HSP60). PHA and HSP60 each activate NK cells, however they do so using different mechanisms. PHA is a polyclonal activator or lymphocytes, meaning it will activate many immune cells and cause cascading activation via cytokine signaling. In contrast, HSP60 has been shown to be specific for NK cell activation (Britton, 2001) and is a typical cellular stress indicator in vivo.

Following Britton and Alexander's procedure (2002), 500μL of whole blood was pipetted into each of six test tubes. Each tube then received either 50 μL PHA (Sigma Chemical Co., St. Louis, MO) at 500μg/mL, 50μL HSP60 (Stressgen Bioreagents, Ann Arbor, MI) at 2mg/mL, or nothing (mitogen procedure on Table 1). All the tubes were then vortexed and incubated overnight in a 37C shaking waterbath.

Tubes 1 and 2 were the negative controls (no activator) and revealed the background level of NK cells expressing surface CD69. The tubes that received PHA and HSP60 (tubes 3 and 4, 5 and 6 respectively) were considered the activated cells. After incubation with the appropriate mitogen 100μL of blood from each tube was allocated into a tube and mixed with 10μL of the three-colored fluorescent labeled antibody mixture of anti-CD45 conjugated to peridin

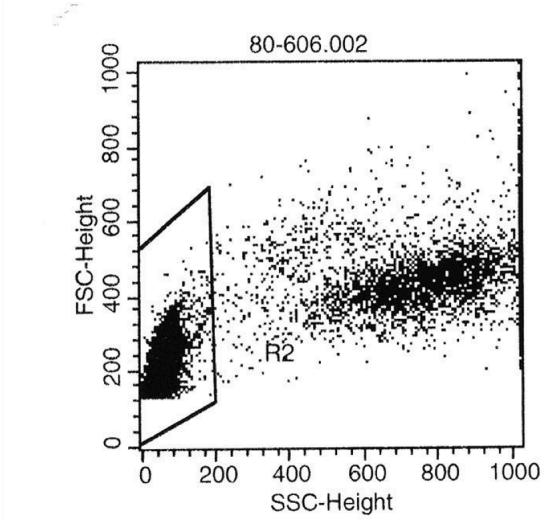
chlorophyll protein (PerCP), anti-CD56 conjugated to fluoresein isothiocyanate (FITC), and anti-CD69 conjugated to phycoerythrin (PE) (Becton Dickinson, Franklin Lakes, NJ). A dark incubation period of 20 minutes at room temperature followed to permit antigen-antibody complexes to form while preventing quenching of the fluorescent molecule. 1mL of a FACSlyse solution (Becton Dickinson, Franklin Lakes, NJ) was then added to each tube to lyse all red blood cells in the blood. After a second 20 minute dark incubation at room temperature the samples were ready to be analyzed on the flow cytometer (a Becton Dickinson FACS calibur cytometer with a 488nm argon air-cooled laser). The Worklist Manager and Cell Quest programs (BD Biosciences, Franklin Lakes, NJ) were used to create files, store data, and perform the flow analysis of the samples.

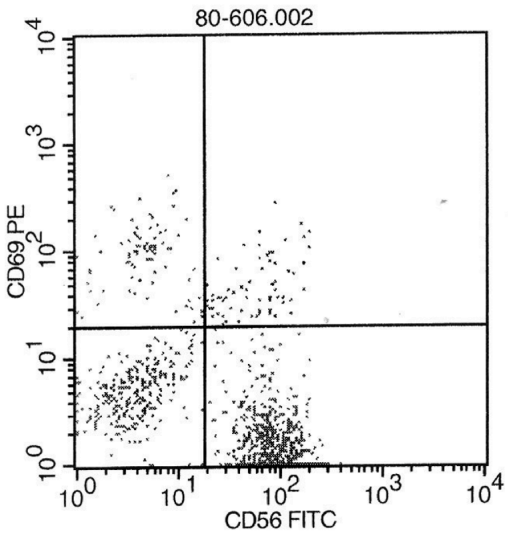
10,000 events were collected from each tube. Events were sorted first by forward-scatter and side-scatter and gated on the lymphocyte subset. These gated events were then transferred to a histogram plotting CD56 (FITC fluorescence) against CD69 (PE fluorescence). The cells with high CD56 levels were counted as NK cells, while those cells with high CD56 and CD69 levels were considered activated NK cells. Two measures of NK cell activation were used; NK cell Activity, which was calculated by dividing the absolute number of CD56+/CD69+ events by the absolute number of CD56+ events, and percent NK cell activated, in which the percent of events gated which were CD56+/CD69+ was used. An example flow cytometry output can be seen in Figure 1.

Percent activity of the NK cells was analyzed by dividing the number of activated cells by the total NK cell count. To control for background levels the stimulation index (SI) was calculated for each mitogen. The SI is the ratio of percent NK cell activity of the experimental to the negative control, reported as SI_{PHA} and SI_{HSP60}. Independent-samples t test was performed in SPSS 16.0 for Mac (SPSS Inc., Chicago, IL) to compare the mitogenic treatments to one another at each time period to determine significance. Levene's test for equality of variances was used for each comparison to determine whether or not to assume equal variances for each group. Additionally, the activity data was sorted into categories based on PTSD and depression diagnosis in order to compare participants who suffered from clinical levels of each.

Table 1 – NK cell activation assay tube setup

Tube	Blood	PHA (500µg/mL)	HSP60 (2mg/mL)
1	500µL		
2	500µL		
3	500µL	50µL	
4	500µL	50µL	
5	500µL		50µL
6	500µL		50µL





Acquisition Date: 22-Apr-09
Gated Events: 10000
Total Events: 10000

Region	Events	% Gated	% Total	Px,Py
R1	1673	16.73	16.73	2, 5
R2	6605	66.05	66.05	2, 1

Acquisition Date: 22-Apr-09
Gated Events: 1673
Total Events: 10000

Quad	Events	% Gated	% Total
UL	82	4.90	0.82
UR	69	4.12	0.69
LL	271	16.20	2.71
LR	1251	74.78	12.51

The flow cytometer recorded the first 10,000 events. These events were plotted on a forward-scatter (FSC) versus side-scatter (SSC) histogram (shown on the left) then gated for characteristics of lymphocytes. The gated events were tense are then plotted on a CD56 (FITC) versus CD69 (PE) histogram (shown on right). Events that represented CD56+ lymphocytes were considered NK cells (quadrants UR and LR), while those that were both CD56+ and CD69+ were considered activated NK cells (quadrant UR).

Figure 1 – Sample flow cytometry NK activation assay output

CHAPTER IV

RESULTS

Participant statistics

Average age of participants was approximately 34 years old with a range from 18 to 64 years (standard deviation 9.5 years). Forty-seven percent (n = 74) of the participants were African American, 46% (n = 72) were Caucasian, and 7% (n = 11) consisted of American Indian/Alaskan Native, Mexican American/Chicano, or other. Education ranged from 8-9th grade level to a Bachelor's degree in college with the average volunteer graduating high school. Average annual income was under $10,000. Complete descriptive statistics for subjects at T1 can be found on Table 2.

IPV and psychological measure changes from T1 to T4

Table 3 shows the descriptive statistics and t-test comparing IPV and the psychological symptom data from T1 and T4. The scores on all the data were significantly different from the first collection time to one year later (all p-values were less than 0.001). All of the mean scores went down. However, when sorting T4 data for whether the abuse continued (based on the IPV scores) there was a significant difference for each psychological sub-score on the PSS and the

TSI (Table 4). This indicated that change in abuse (shown on Figure 2) was linked to the change in PSS and TSI measure changes.

Immune system status statistics

Additionally, the immune system measures were compared from T1 and T4. First, to be sure the data were parametric, the CBC and lymphocyte subset counts from both T1 and T4 were plotted on a frequency histogram with the normal distribution curve (shown on Figure 3). Since the data closely resembled the normal curve, parametric statistical measures like Student's t test were used in the analysis. White blood cell, lymphocyte, and NK cell activity statistics comparing T1 and T4 are located on Table 5.

Significant differences were seen between the T1 and T4 red blood cell ($p < 0.05$), hemoglobin ($p = 0.022$), and hemocrit ($p = 0.020$). Each of these scores was highly correlated with one another (see Table 6). Out of the lymphocyte subset analysis only NK percent (CD56, CD16 positive cells) was significant with an alpha of 0.05. This can be seen on Figure 4.

NK cell activities, reported as percent of NK cells displaying CD69, were significantly different for the two time periods (all $p < 0.001$). This is shown graphically on Figure 5.

Immune system modulation due to PTSD and/or depression

Results of the lymphocyte subset counts showed significant increases in T cells ($p < 0.01$ for CD3 percent) and decreases in B and NK cells ($p < 0.05$ for CD19 percent, $p < 0.01$ for CD56 percent) between participants who displayed

clinical levels of PTSD and those who did not, although CBC and differential results were not significantly different between the two groups (results on Table 7 and Figure 6). Background (unstimulated) percent NK activity was also highly significant ($p < 0.001$). Samples activated by PHA were significantly different between the groups ($p < 0.05$) however those stimulated by HSP60 did not show significance at an alpha of 0.05 (results on Figure 7). The activation of NK cells was also grouped based on depression diagnosis, resulting in statistically significant differences for background ($p < 0.001$) and both PHA and HSP60 mitogen treatments ($p < 0.01$ for each) (see Figure 8). Additionally, NK activity was compared between participants who were not at clinical levels for either PTSD or depression and those who suffered from either or both (Figure 9). Background ($p < 0.001$) and PHA stimulated ($p = 0.008$) activity differed at statistically significant levels.

In order to measure the change in activation of the cells after stimulation with a mitogen the SI of PHA and HSP60 were calculated. These were then grouped based on PTSD diagnosis and compared using the independent samples t test. The results are shown in Figure 10. The SI_{HSP60} was significantly lower in those positive for PTSD ($p = 0.003$) and SI_{PHA} was statistically lower as well ($p < 0.05$).

Additionally, the stimulation indexes were grouped based on combined diagnoses into four groups: those who were subclinical for PTSD and depression ($n = 35$), those clinical for depression only ($n = 5$), those clinical for PTSD only ($n = 83$), and those who were clinical for both diagnoses ($n = 79$) (Figure 11). Each

of the last three was compared with the scores of those participants who were not diagnosed with PTSD or depression. SI_{HSP60} was found to be significantly different between those that were subclinical for both and those that were positive for both ($p = 0.002$) as well as those that were positive for PTSD only ($p = 0.026$). Also, SI_{PHA} was significantly different in participants at clinical levels for both PTSD and depression when compared to those negative for each diagnosis ($p = 0.009$). Unfortunately the small sample sizes, such as those who only suffered from depression, made it difficult to examine differences between the clinical diagnoses.

Table 2 – Descriptive statistics of study participants

		Min	Max	Mean	SD
Age at Study Start		18	64	33.68	9.519
			Frequency		Percent
Ethnic Category	American Indian or Alaskan Native		4		2.5%
	African American		74		47.1%
	Mexican American or Chicano		3		1.9%
	White		72		45.9%
	Other		4		2.5%
Education level	8-9th grade		10		6.4%
	10-11th grade		26		16.6%
	HS graduate		77		49%
	Partial college or technical training		38		24.2%
	College degree (bachelor's)		6		3.8%
Total annual income [a]	Under $10,000		94		59.9%
	$10,000-14,999		17		10.8%
	$15,000-19,999		12		7.6%
	$20,000-24,999		8		5.1%
	$25,000-29,999		5		3.2%
	$30,000-34,999		8		5.1%
	$35,000-39,999		4		2.5%
	$40,000-44,999		2		1.3%
	$50,000 and over		1		0.6%
Smoking habits	No		46		29.3%
	Yes		111		70.7%
Packs per day? [b]	None		46		29.3%
	1/2 or less		59		37.6%
	About 1		38		24.2%
	About 1 1/2		7		4.5%
	2 or more		5		3.2%

a: Six participants did not report on total annual income.
b: Two participants did not report on # of packs per day.

Table 3 – Participant IPV and PTSD statistics for T1 and T4

	Time One			Time 4			
	N	Mean	SD	N	Mean	SD	P
Intimate Partner Violence							
Threat of Violence	157	54.610	1.058	132	30.890	1.306	<0.001
Physical Violence	157	53.090	1.228	132	30.050	1.322	<0.001
Sexual Violence	157	11.320	0.433	132	7.900	0.373	<0.001
Index of Spouse Abuse (Non-phys)	157	63.030	1.482	132	35.140	1.736	<0.001
Homicide Risk (DangerAsst)	156	9.790	0.328	132	4.280	0.391	<0.001
PTSD Symptom Scales							
Total PTSD Severity Score (PSS)	157	33.360	0.852	132	17.420	1.245	<0.001
Re-Experiencing (PSS)	157	9.340	0.320	132	4.800	0.378	<0.001
Avoidance (PSS)	157	13.290	0.408	132	7.050	0.546	<0.001
Increased Arousal (PSS)	157	10.700	0.287	132	5.570	0.406	<0.001
Clinically PTSD Pos (PSS)	156	0.930	0.021	131	0.620	0.043	<0.001
Trauma Symptom Inventory							
Anxious Arousal (TSI)	157	64.680	0.885	132	53.160	1.068	<0.001
Anger Irritability (TSI)	157	61.740	0.864	132	54.030	1.018	<0.001
Intrusive Experiences (TSI)	157	65.400	0.893	132	56.520	1.099	<0.001
Defensive Avoidance (TSI)	157	64.660	0.695	132	57.060	0.983	<0.001
Dissociation (TSI)	157	67.280	0.980	132	55.020	1.158	<0.001
Depression (TSI)	157	64.490	0.732	132	54.110	0.930	<0.001
Clinically Depressed (TSI)	157	0.590	0.039	132	0.210	0.036	<0.001

Table 4 – Affect of continued abuse on psychological measures

	Did abuse continue?	N	Mean	Std. Dev.	p
Total PTSD Severity Score (PSS)	Abuse ended	63	13.32	13.87	0.001***
	Abuse continued	69	21.17	13.747	
Re-Experiencing (PSS)	Abuse ended	63	3.57	3.859	0.002**
	Abuse continued	69	5.93	4.49	
Avoidance (PSS)	Abuse ended	63	5.6	6.427	0.011*
	Abuse continued	69	8.38	5.866	
Increased Arousal (PSS)	Abuse ended	63	4.14	4.486	0.001***
	Abuse continued	69	6.87	4.465	
Anxious Arousal (TSI)	Abuse ended	63	50.38	12.541	0.012*
	Abuse continued	69	55.7	11.542	
Anger Irritability (TSI)	Abuse ended	63	50.63	10.735	0.001***
	Abuse continued	69	57.13	11.751	
Intrusive Experiences (TSI)	Abuse ended	63	53.62	12.409	0.011*
	Abuse continued	69	59.17	12.311	
Defensive Avoidance (TSI)	Abuse ended	63	54.71	11.516	0.022*
	Abuse continued	69	59.2	10.719	
Dissociation (TSI)	Abuse ended	63	52.37	13.425	0.028*
	Abuse continued	69	57.43	12.81	
Depression (TSI)	Abuse ended	63	51.75	11.029	0.015*
	Abuse continued	69	56.26	9.964	

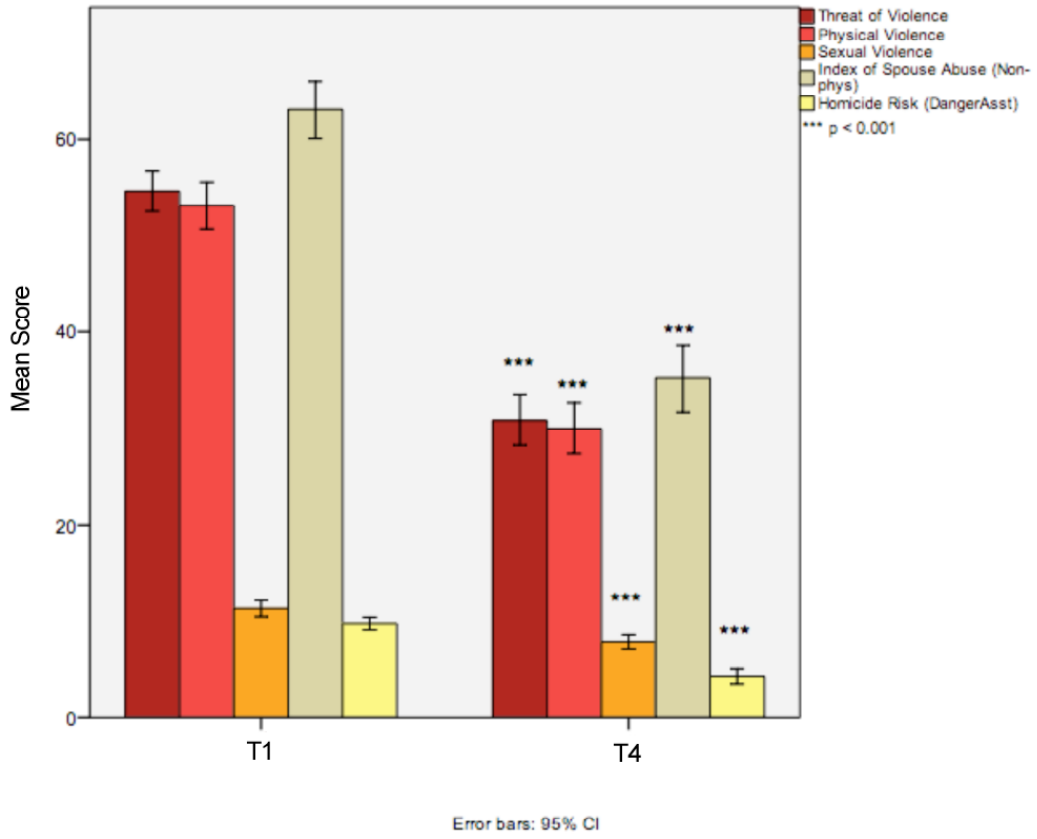


Figure 2 – Changes in IPV from T1 to T4

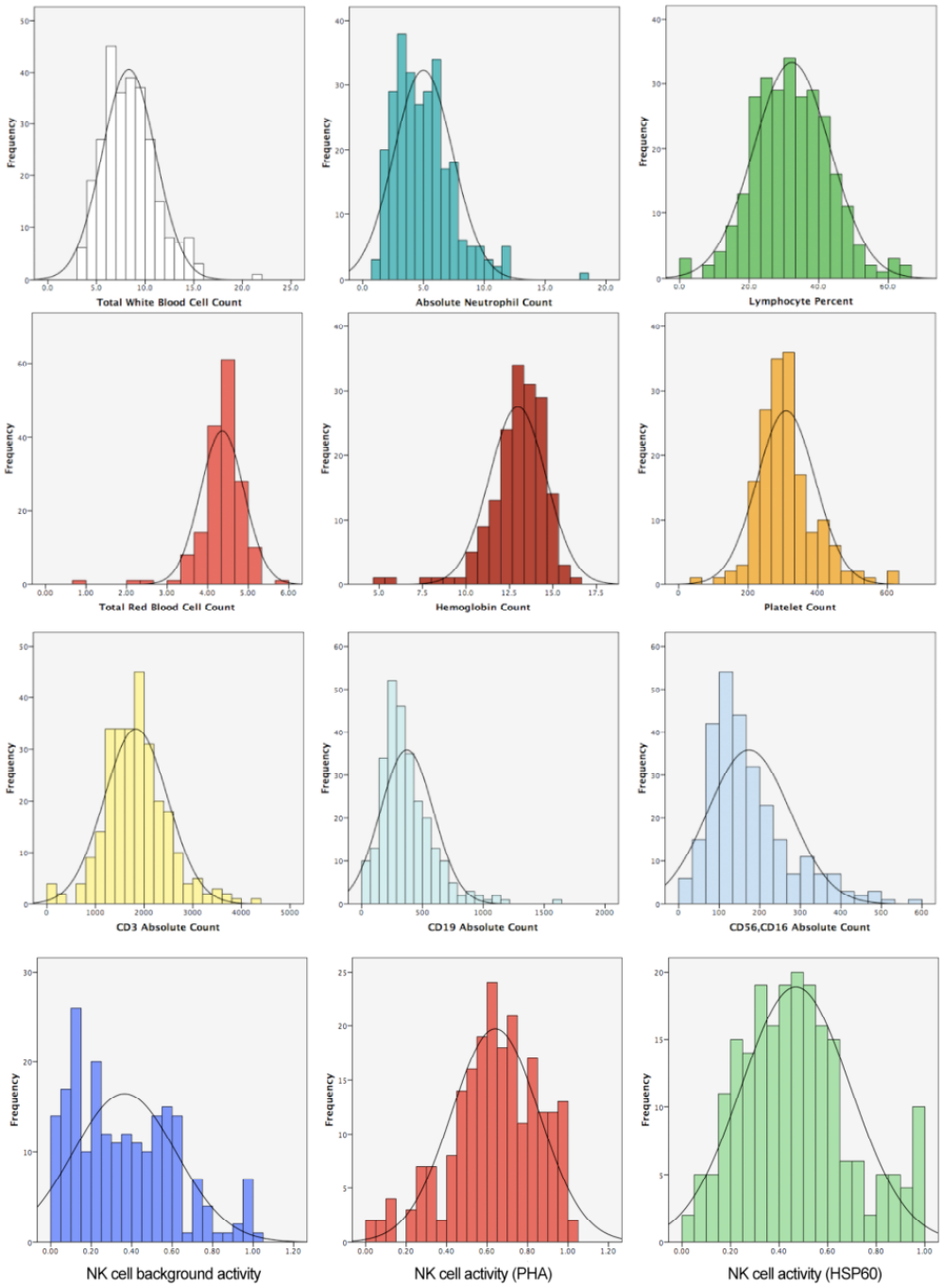


Figure 3 – Distribution of immune data

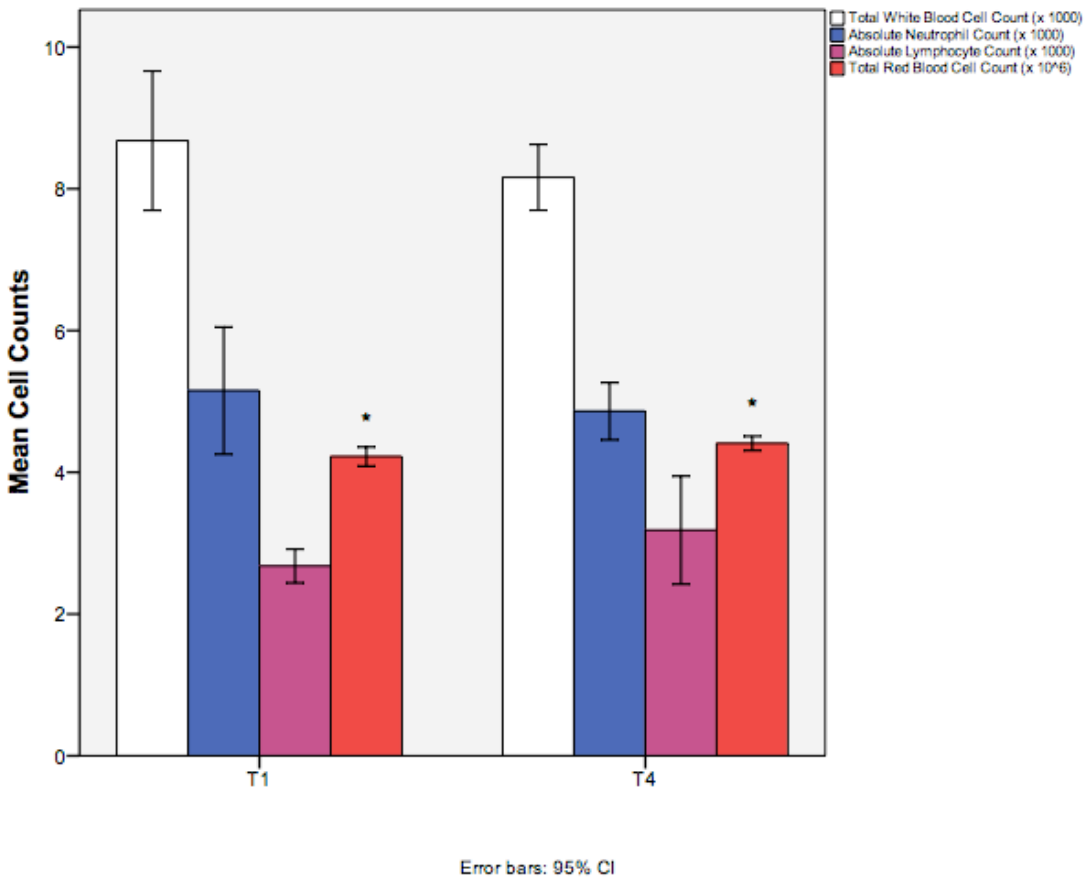
Table 5 – Immune system statistics for T1 and T4

	Time 1			Time 4			
	N	Mean	SD	N	Mean	SD	P
Complete Blood Count with Differential							
Total White Blood Cell Count (x 10^3)	153	8.4	2.8	126	8.2	2.6	0.578
Absolute Neutrophil Count (x 10^3)	150	57.7	11.9	125	57.7	11.8	1.000
Neutrophil Percent	153	5.0	2.6	125	4.9	2.3	0.869
Absolute Lymphocyte Count (x 10^3)	150	32.5	10.7	125	31.9	11.3	0.628
Lymphocyte Percent	153	2.5	0.9	125	3.2	4.2	0.096
Total Red Blood Cell Count (x 10^6)	46	4.2	0.5	123	4.4	0.6	0.045
Hemoglobin Count (g/dL)	46	12.5	1.8	123	13.2	1.5	0.022
Hemocrit Count	46	37.1%	0.049	123	38.9%	0.044	0.020
Platelet Count (x 10^3)	46	323.0	91.0	123	304.0	80.5	0.190
Lymphocyte Subset Count							
CD3 Percent	153	76.16%	0.086	122	76.37%	0.062	0.825
CD3 Absolute Count	153	1890.01	677.2	121	1757.88	653.2	0.105
CD3,CD4 Percent	153	47.58%	0.11	122	48.38%	0.096	0.541
CD3,CD4 Absolute Count	150	1200.11	456.8	121	1118.4	443.5	0.139
CD3,CD8 Percent	153	26.06%	0.088	122	25.64%	0.078	0.685
CD3,CD8 Absolute Count	152	649.61	338.9	121	613.64	348.7	0.391
CD19 Percent	153	14.84%	0.062	121	14.94%	0.076	0.906
CD19 Absolute Count	150	389.75	217.6	121	353.74	216.4	0.176
CD56,CD16 Percent	153	6.61%	0.035	121	7.60%	0.041	0.038
CD56,CD16 Absolute Count	150	168.03	92.59	121	179.51	111.8	0.356
CD4:CD8 Ratio	150	2.509	5.554	121	2.871	6.6	0.626
NK Activation Assay							
NK Background Activity	116	50.35%	0.21	94	16.00%	0.13	<0.001
NK Activity (PHA)	121	71.42%	0.18	95	52.06%	0.19	<0.001
NK Activity (HSP60)	121	51.84%	0.23	95	38.20%	0.17	<0.001

Table 6 – Pearson correlations of immune data

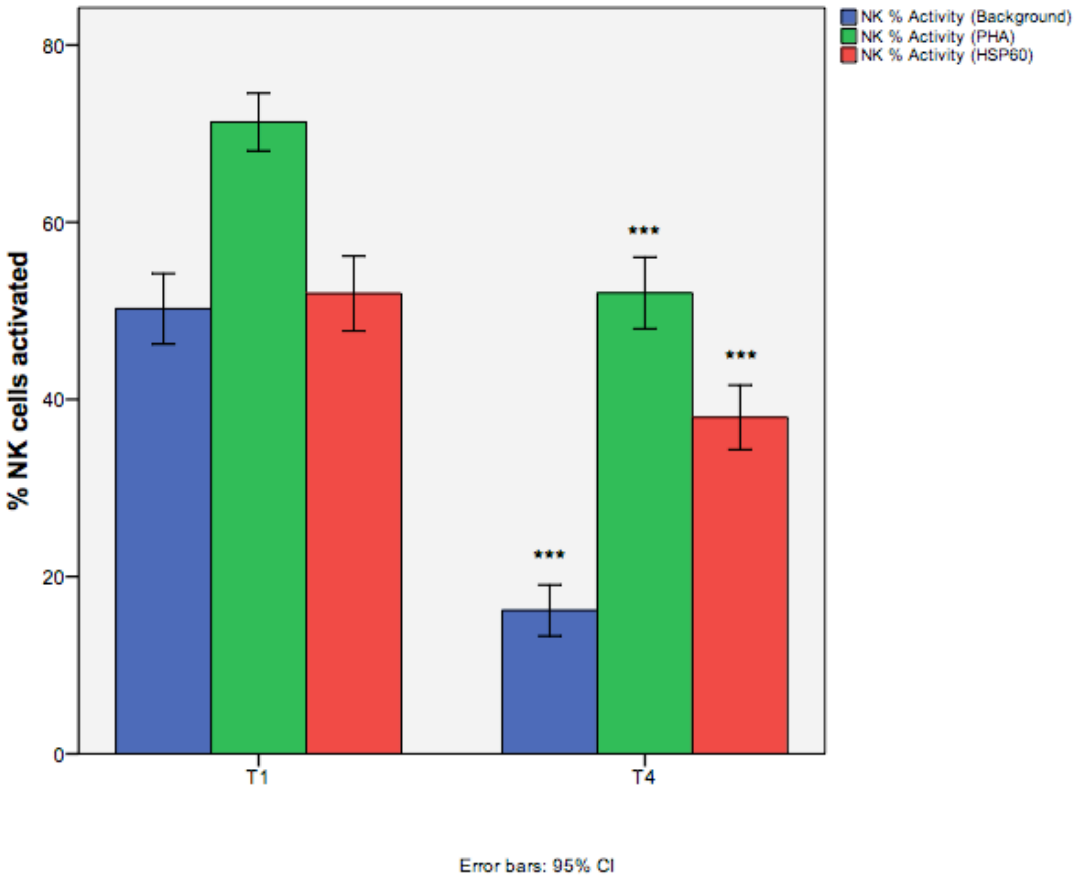
		Total Red Blood Cell Count	Hemoglobin Count	Hemocrit Count
Total Red Blood Cell Count	Pearson Correlation		0.707**	0.790**
	Sig. (2-tailed)		<0.001	<0.001
	N		169	169
Hemoglobin Count	Pearson Correlation	0.707**		0.981**
	Sig. (2-tailed)	<0.001		<0.001
	N	169		169
Hemocrit Count	Pearson Correlation	0.790**	0.981**	
	Sig. (2-tailed)	<0.001	<0.001	
	N	169	169	

**. Correlation is significant at the 0.01 level (2-tailed).



*** p < 0.001
**p < 0.01
*p < 0.05

Figure 4 – Immune measures of T1 vs. T4



*** p < 0.001
**p < 0.01
*p < 0.05

Figure 5 – Natural killer cell activity T1 versus T4

Table 7 – Descriptive statistics of immune status grouped by PTSD diagnosis

	Not at clinical level for PTSD (PSS)			At clinical level for PTSD (PSS)			
	N	Mean	SD	N	Mean	SD	P
Complete Blood Count with Differential							
Total White Blood Cell Count (x 10^3)	59	8.15	2.7	218	8.40	2.8	0.530
Neutrophil Percent	59	57.85	11.4	214	57.70	12.0	0.932
Absolute Neutrophil Count (x 10^3)	59	4.87	2.2	217	4.96	2.5	0.794
Lymphocyte Percent	59	30.96	11.0	214	32.58	10.9	0.314
Absolute Lymphocyte Count (x 10^3)	59	2.98	4.0	217	2.78	2.6	0.635
Total Red Blood Cell Count (x 10^6)	51	4.40	0.5	117	4.34	0.6	0.460
Hemoglobin Count (g/dL)	51	13.11	1.5	117	12.91	1.7	0.464
Hemocrit Count	51	38.78%	0.046	117	38.26%	0.047	0.502
Platelet Count	51	307.43	71.4	117	309.95	89.1	0.858
Lymphocyte Subset Count							
CD3 Percent	58	73.66	8.4	215	76.96	7.3	0.008
CD3 Absolute Count	58	1708.4	697.7	214	1867.7	660.8	0.109
CD3,CD4 Percent	58	47.37	7.0	215	48.08	11.7	0.660
CD3,CD4 Absolute Count	58	1095.6	438.0	211	1183.9	456.7	0.189
CD3,CD8 Percent	58	24.16	8.3	215	26.33	8.4	0.082
CD3,CD8 Absolute Count	58	562.24	316.8	213	653.88	349.2	0.072
CD19 Percent	57	17.33	9.6	215	14.22	5.8	0.022
CD19 Absolute Count	57	407.37	275.5	212	364.58	199.9	0.276
CD56,CD16 Percent	57	8.51	4.7	215	6.67	3.5	0.008
CD56,CD16 Absolute Count	57	184.11	107.1	212	170.58	100.5	0.375
CD4:CD8 Ratio	58	3.80	9.5	211	2.37	4.7	0.269
NK Activation Assay							
NK % Activity (Background)	45	23.16	25.71	163	38.35	24.35	<0.001
NK % Activity (PHA)	47	56.22	22.72	168	64.86	20.26	0.013
NK % Activity (HSP60)	47	42.68	21.00	167	46.83	22.13	0.252

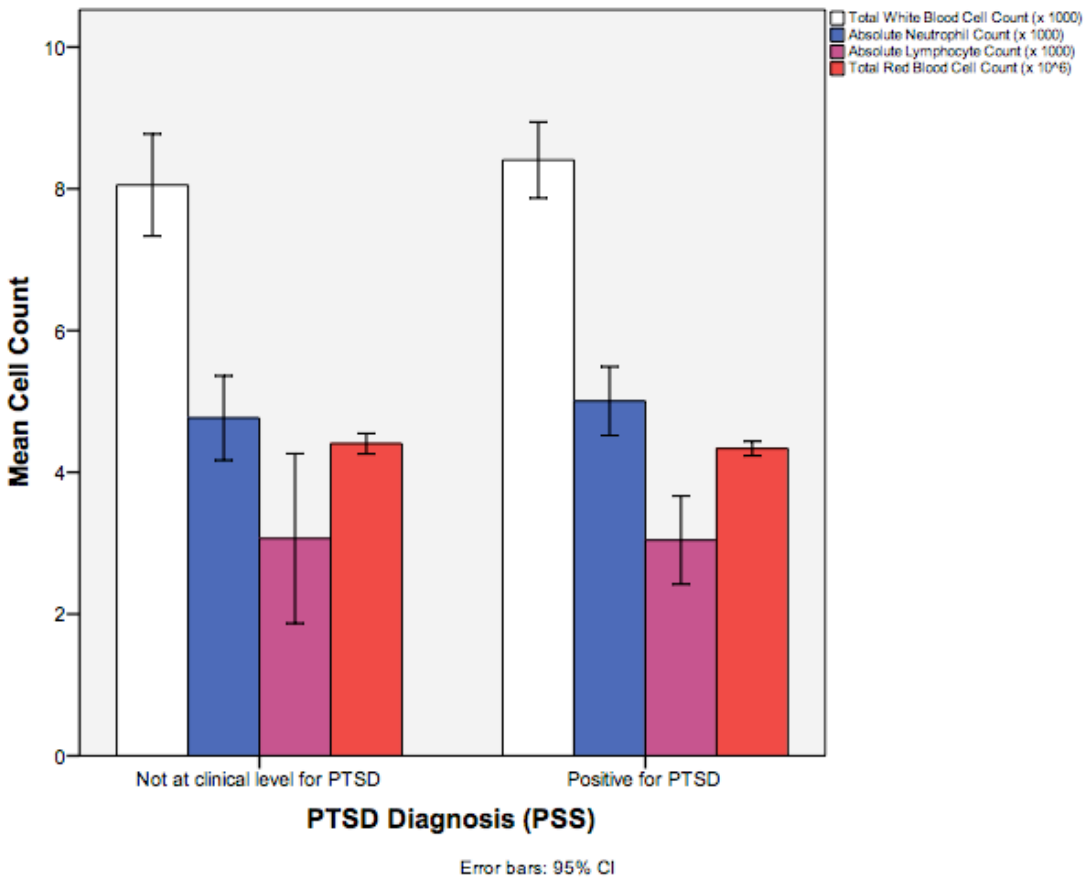
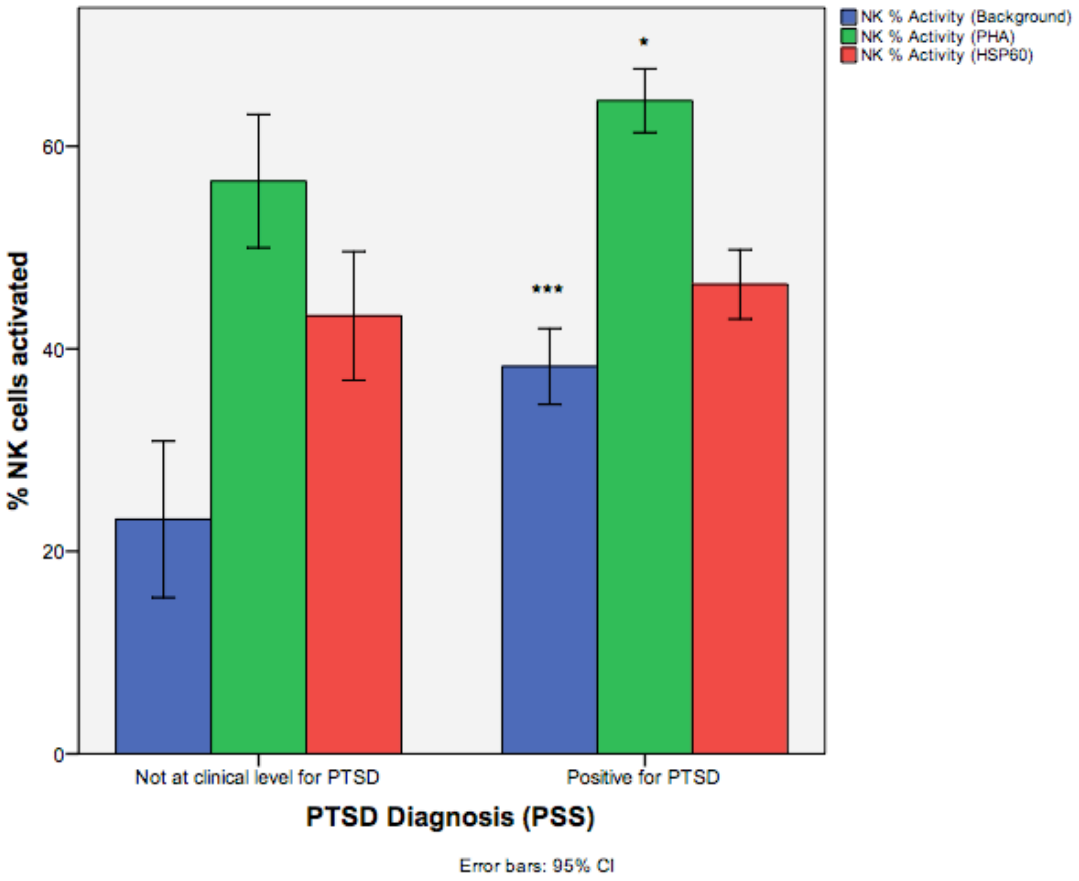
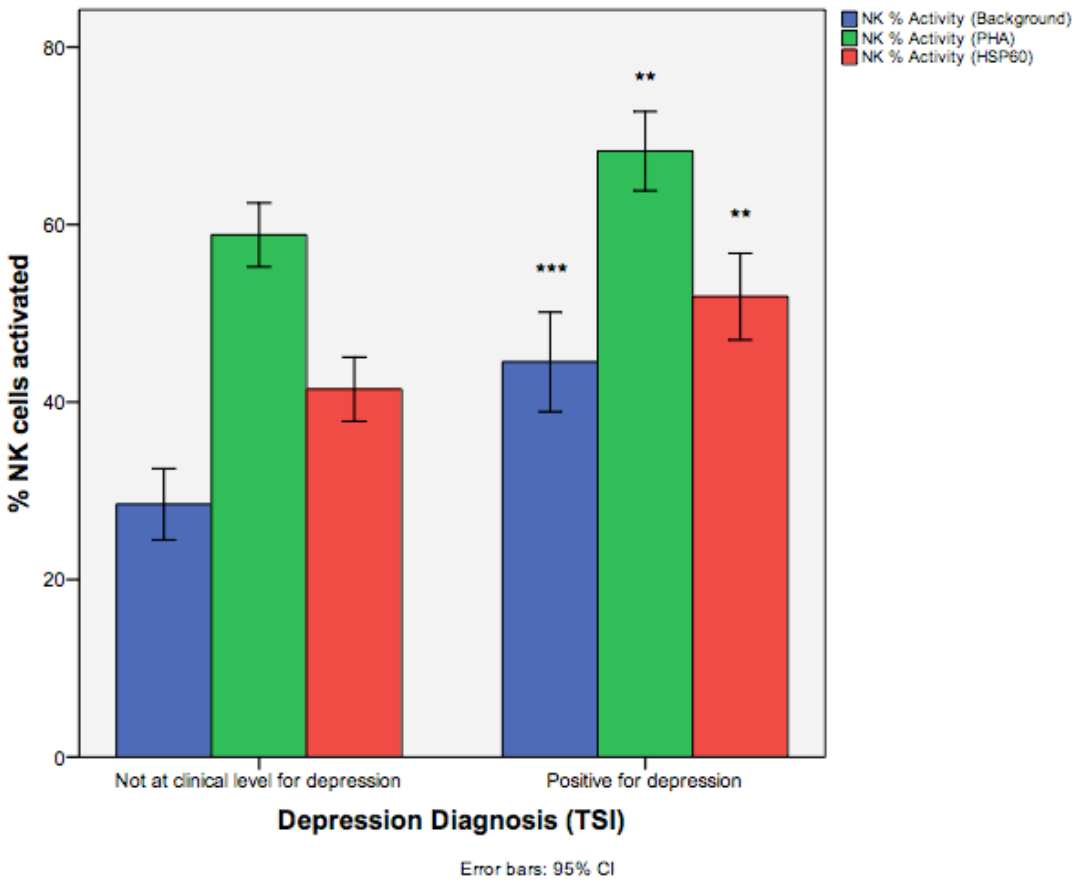


Figure 6 – WBC differential vs. PTSD diagnosis



*** p < 0.001
**p < 0.01
*p < 0.05

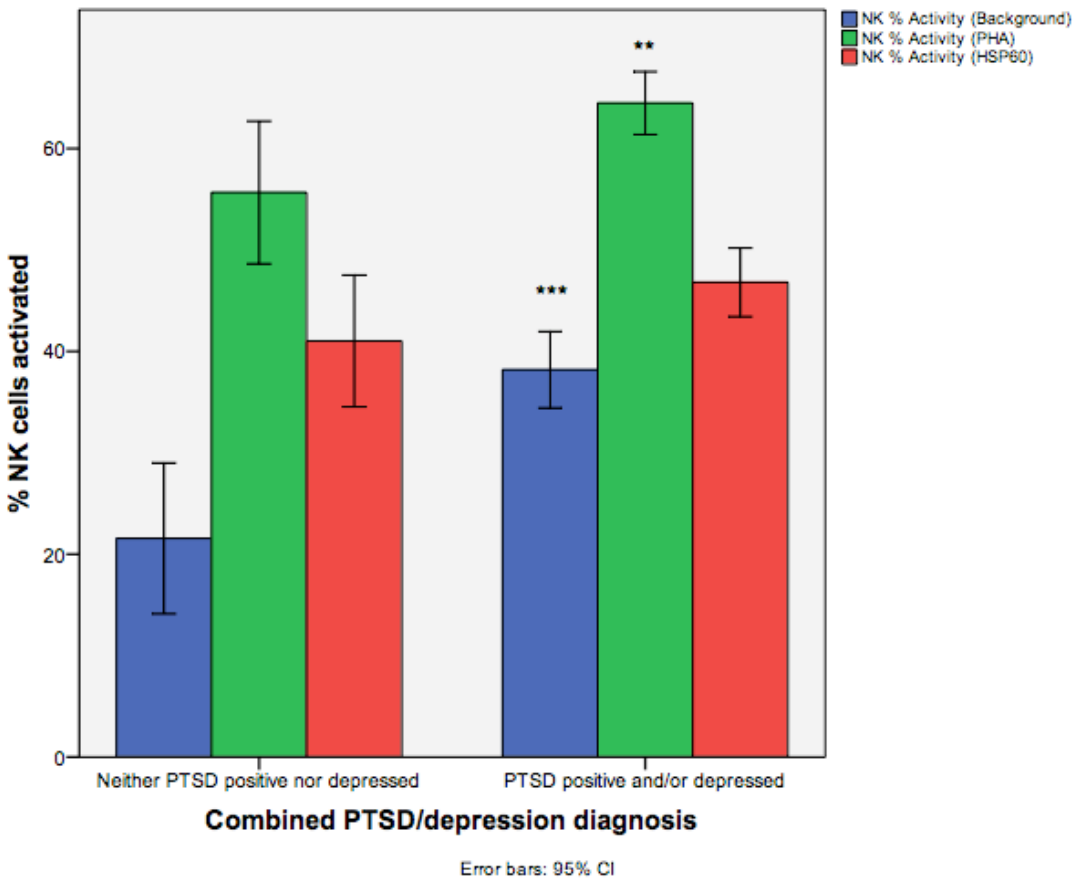
Figure 7 – NK activity vs. PTSD diagnosis



	Subclinical for Depression			At clinical level for Depression			
	N	Mean	SD	N	Mean	SD	P
NK Activation Assay							
NK % Activity (Background)	125	28.7%	0.2	85	44.2%	0.3	<0.001
NK % Activity (PHA)	128	59.5%	0.2	88	67.9%	0.2	0.004
NK % Activity (HSP60)	129	42.1%	0.2	87	51.4%	0.2	0.002

*** p < 0.001
**p < 0.01
*p < 0.05

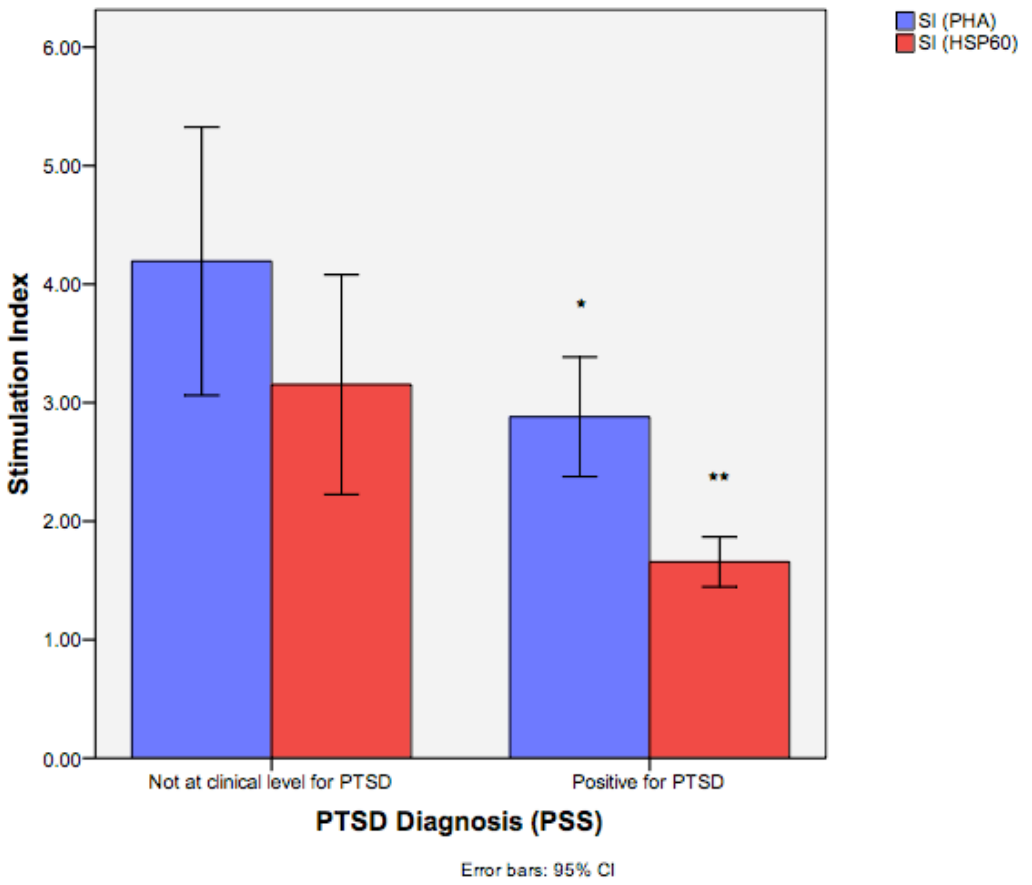
Figure 8 – NK activity and depression diagnosis



	Neither PTSD positive nor depressed			PTSD positive and/or depressed			
	N	Mean	SD	N	Mean	SD	p
NK % Activity (Background)	40	21.6%	23.3%	168	38.3%	24.9%	<0.001
NK % Activity (PHA)	42	55.3%	22.9%	173	64.8%	20.2%	0.008
NK % Activity (HSP60)	42	40.5%	20.1%	172	47.2%	22.2%	0.073

*** p < 0.001
**p < 0.01
*p < 0.05

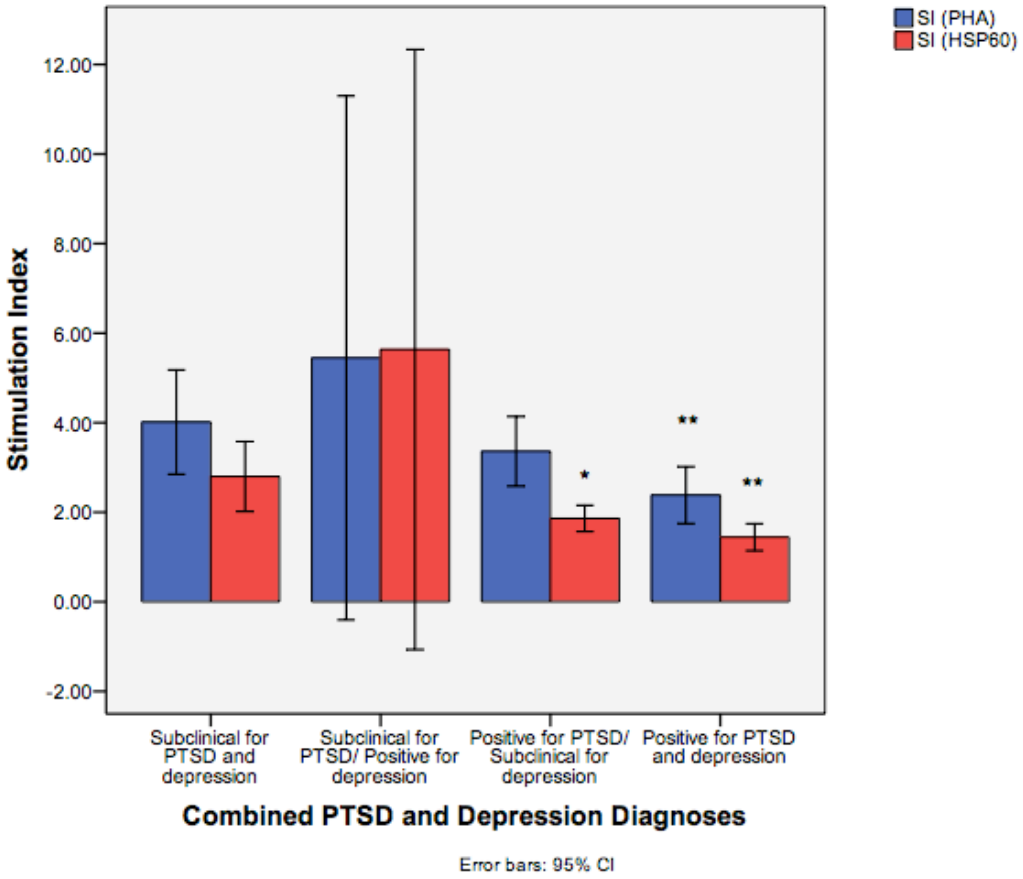
Figure 9 – NK activity and combined PTSD/depression diagnosis



	Not at clinical level for PTSD			Positive for PTSD			
	N	Mean	SD	N	Mean	SD	p
SI (PHA)	40	4.19	3.54	161	2.90	3.21	0.026
SI (HSP60)	40	3.15	2.90	160	1.65	1.34	0.003

*** p < 0.001
**p < 0.01
*p < 0.05

Figure 10 – Stimulation index and PTSD diagnosis



	N	Mean	SD	p[a]
SI (PHA)				
Subclinical for PTSD and depression	35	4.0145	3.38985	
Subclinical for PTSD/ Positive for depression	5	5.4462	4.71154	0.404
Positive for PTSD/ Subclinical for depression	82	3.3818	3.50227	0.368
Positive for PTSD and depression	79	2.3916	2.80066	0.009
SI (HSP60)				
Subclinical for PTSD and depression	35	2.7982	2.2741	
Subclinical for PTSD/ Positive for depression	5	5.6343	5.39644	0.307
Positive for PTSD/ Subclinical for depression	82	1.8513	1.32182	0.026
Positive for PTSD and depression	78	1.4431	1.33515	0.002

a. Independent samples t test comparing the subclinical PTSD/depression group with the others.

*** p < 0.001
**p < 0.01
*p < 0.05

Figure 11 – Stimulation index grouped by PTSD and depression diagnosis

CHAPTER V

DISCUSSION

Results of the NK activation assay agree with results of similar studies that reported a negative correlation between PTSD and NK in victims of partner violence in so much as the degree of activation in response to a mitogen was decreased. The increase in NK cell activity in response to a typical cellular stress molecule (HSP60), measured by SI, was significantly less in participants suffering from PTSD than in those without PTSD. The SI controls for background levels of activity by dividing activity of cells induced with either PHA or HSP60 by the background level of activation. The closer to a value of 1 the less change in activation is occurring. As seen on Figure 10 the SI was significantly decreased in women who suffered from PTSD as compared to those who did not (p for SI_{HSP60} was 0.003, p for SI_{PHA} was 0.023). This drop in reactivity to both a typical cellular stress signal and a polyclonal mitogen could put PTSD positive women at risk for a number of diseases, including tumor development and viral infection.

Also, while the results of the lymphocyte subset counts showed significant increases in the percentage of T cells similar to the findings published by Ironson et al (1997) and Woods et al (2005), this study found a decrease in the percentages of lymphocytes that were B or NK cells. Increases in T lymphocytes

may be explained through a drop in diurnal cortisol levels associated with PTSD diagnosis (Anisman et al, 2001; Kanter et al, 2001; Woods et al, 2003; Woods, 2005) as well as by increased levels of pro-immune cytokines (Gill et al, 2008). Cortisol has been shown to suppress immune cell function and numbers (Mavoungou et al, 2004) and therefore a decrease in the hormone may permit for increased numbers of T lymphocytes.

When looking at the percent of NK cells considered activated our PTSD positive subjects exhibited significantly high levels of background activation when compared to women at the subclinical level for PTSD ($p < 0.001$), something not reported previously in the literature. Also, the raw percentage of NK cells activated by HSP60 was not significantly different between PTSD positive and negative groups. What may be occurring in women who are clinically PTSD positive is that the NK cells are simply over stimulated in vivo and unable to be activated significantly more ex vivo. Again this may have to do with cortisol levels in PTSD positive women. Mavoungou et al (2004) found that natural cytoxicity receptors (NCR) on the surface of NK cells are down regulated by cortisol. Also, cytokines such as tumor necrosis factor (TNF) and interleukin-6, which are activation factors for lymphocytes including NK cells, have been shown to occur at higher levels in patients with PTSD (Gill et al, 2008), which may account for increased basal activation.

A problem that arose while analyzing the data was that the different scores for the subtypes of IPV were all highly correlated with one another (all $p < 0.001$) as shown in Table 8. This meant that linking a specific score to a specific

immune outcome would not be possible with this data set. Also, separating the effects of depression, PTSD, or a combination of the two was not possible due to high correlations between the two and low sample sizes for those diagnosed with only one of the disorders (Table 9). The SI_{PHA} was significantly correlated with PSS PTSD score ($p = 0.014$) and TSI depression score ($p = 0.005$) (Table 10). Also, the SI_{HSP60} was very strongly correlated with the total PTSD severity score ($p < 0.001$) and depression ($p = 0.006$). Therefore, any separate effects of PTSD and depression on stimulation of NK cells were not observable in this dataset.

Table 8 - Pearson correlations between different IPV scales

		Threat of Violence	Physical Violence	Sexual Violence	ISA-NP	Homicide Risk (DA)
Threat of Violence	Pearson Correlation		.881***	.552***	.775***	.697***
	p		<0.001	<0.001	<0.001	<0.001
	N		289	289	289	288
Physical Violence	Pearson Correlation	.881***		.598***	.683***	.625***
	p	<0.001		<0.001	<0.001	<0.001
	N	289		289	289	288
Sexual Violence	Pearson Correlation	.552***	.598***		.478***	.458***
	p	<0.001	<0.001		<0.001	<0.001
	N	289	289		289	288
Index of Spouse Abuse (NP)	Pearson Correlation	.775***	.683***	.478***		.707***
	p	<0.001	<0.001	<0.001		<0.001
	N	289	289	289		288
Homicide Risk (DA)	Pearson Correlation	.697***	.625***	.458***	.707***	
	p	<0.001	<0.001	<0.001	<0.001	
	N	288	288	288	288	

***. Correlation is significant at the 0.001 level (2-tailed).

Table 9 - Pearson correlations between PTSD and depression

		Total PTSD Severity Score (PSS)	Depression (TSI)
Total PTSD Severity Score (PSS)	Pearson Correlation		.689***
	p		<0.001
	N		289
Depression (TSI)	Pearson Correlation	.689***	
	p	<0.001	
	N	289	

***. Correlation is significant at the 0.001 level (2-tailed).

Table 10 - Pearson correlations of PTSD/depression and NK stimulation indexes

		Total PTSD Severity Score (PSS)	Depression (TSI)
SI_{PHA}	Pearson Correlation	-.173*	-.198**
	p	0.014	0.005
	N	202	202
SI_{HSP60}	Pearson Correlation	-.365***	-.195**
	p	<0.001	0.006
	N	201	201

***. Correlation is significant at the 0.001 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CHAPTER VI

CONCLUSION

PTSD positive women in this study exhibited significantly different immune system statuses than those who showed subclinical PTSD symptom scores. These differences included a significantly higher percentage of T cells (CD3+ lymphocytes), which caused lower percentages of B and NK cells (CD19+ and CD56+ lymphocytes, respectively) in the lymphocyte subset counts despite no significant change in the absolute numbers of these cells. Additionally, the activities of their NK cells were much different from women who were not PTSD positive. Significantly increased baseline NK activity may account for the decreased SI seen in response to the mitogens.

Protection of the body by the immune system requires a careful balance of both the number and the activation potential of immune cells. When a shift in either of these parameters occurs the defenses of an individual from a variety of pathogens can be compromised, causing an increase in the likelihood of illness and disease. Identifying situations that may lead to these shifts is the key in enacting effective preventive care. The very broad goal of this study was to identify if IPV and the PTSD or depression associated with it were linked in anyway with immune system health risks. Therefore this thesis falls into the

emerging medical fields of neuro- and psycho-immunology, which seek to discover the mechanisms through which the brain talks and listens to the immune system and how the process might vary depending on the specific environmental situations and psychological states (Maier et al, 1994; Phillips & Evans 1995; Boscarino & Chang, 1999; Maier, 2003). Contributing to the understanding of these associations requires a wide array of scientific backgrounds and thus necessitates an integrated approach.

Thus, the next step for this study is to bring together the results reported in this thesis and the findings of collaborators to take these correlations of health statuses to risk factors and determine the causal relationships that bind them. Advancement in this specific study promises not only to give researchers insight into these connections between mind and body but also a chance to develop more complete and effective approaches to treating victims of spousal and intimate partner abuse. By building on each the strengths of seemingly distinct fields like immunology, endocrinology, psychology and sociology we can move past simple observation and gain true understanding, advancing all the sciences involved.

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APPENDIX

HUMAN SUBJECTS APPROVAL

Date: January 30, 2009

To: Stephanie Woods
College of Nursing
The University of Akron
Akron, Ohio 44325-3701

From: Sharon McWhorter, IRB Administrator

Re: IRB Number 20040408-8 *"PRSD, Cortisol, Immune Function with Battering over Time"*

Thank you for submitting your Application for Continuing Review of Research Involving Human Subjects for the referenced project. Your protocol represents minimal risk to subjects and has been approved under Expedited Category #9.

Approval Date:	January 29, 2009
Expiration Date:	February 5, 2010
Continuation Application Due:	January 22, 2010

In addition, the following is/are approved:

☐ Waiver of documentation of consent
☐ Waiver or alteration of consent
☐ Research involving children
☐ Research involving prisoners

Please adhere to the following IRB policies:

- IRB approval is given for not more than 12 months. If your project will be active for longer than one year, it is your responsibility to submit a continuation application prior to the expiration date. We request submission two weeks prior to expiration to insure sufficient time for review.
- A copy of the approved consent form must be submitted with any continuation application.
- If you plan to make any changes to the approved protocol you must submit a continuation application for change and it must be approved by the IRB before being implemented.
- Any adverse reactions/incidents must be reported immediately to the IRB.
- If this research is being conducted for a master's thesis or doctoral dissertation, you must file a copy of this letter with the thesis or dissertation.
- When your project terminates you must submit a Final Report Form in order to close your IRB file.

Additional information and all IRB forms can be accessed on the IRB web site at:
http://www.uakron.edu/research/orssp/compliance/IRBHome.php

☐ Approved consent form/s enclosed

Cc: Stephanie Woods - IRB Chair