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Inflammatory Effects and Immune System Correlates of Rape

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The purpose of this study was to evaluate the feasibility of measuring stress hormones and immunity following rape. The long-term goal is to evaluate the predictive value of stress-immune-inflammatory responses to later health outcomes. Fifteen women reporting rape were compared with 16 control participants. Serum stress hormones, proinflammatory cytokines, acute phase proteins, functional assays, and lymphocyte subsets were measured in blood samples. Women reporting rape had higher cytotoxic cells, lower B lymphocyte counts, higher proinflammatory biomarkers, and decreased lymphocyte proliferation compared to the control group. This finding suggests that rape produces activation of the innate immunity and suppression of some aspects of adaptive immunity. If these immune changes persist, they may contribute to the pathophysiology of long-term health sequelae by provoking chronic inflammation and decreased cellular immunocompetence.

Keywords: rape; trauma; sexual assault; immunity; inflammation

Rape is not an uncommon event, as evidenced by Department of Justice statistics. In a national survey of 8,000 Americans, 17.6% of women and 3% of men reported having been raped; 76% of women raped and/or physically assaulted after age 18 were assaulted by a personal acquaintance (Tjaden & Thoennes, 1998). Being African American, single, a student, or of low socioeconomic status are all risk factors for women reporting rape. Only about 8% of those who are raped report the event to the authorities (Rodgers & Gruener, 1997). The aftermath of rape can be devastating. Half of rape victims suffer vaginal and perineal trauma (Geist, 1988), and sexually transmitted diseases occur in up to 30% (Koss & Heslet, 1992). The long-term effects of rape are both mental and physical, but incidences of various sequelae are not accurately known, because many women do not report the crime nor do they seek help and counseling. At least one-third of rape victims develop posttraumatic stress disorder (PTSD), panic attacks, depression, and physical health problems. Physical health effects of rape have largely been discovered

through survey research and are greater when the woman has been sexually assaulted by an intimate partner rather than a stranger (Plichta & Falik, 2001). Female victims report lower perceived health status, more somatic symptoms, and more negative health behaviors, more headaches, chronic pain syndromes, gynecological disorders, premenstrual syndrome, gastrointestinal disorders, morbid obesity, and substance abuse (Friedman & Schnurr, 1995; Koss & Heslet, 1992; Lampe et al., 2000). In a study of 826 elderly women (median age 75 years), 12.7% reported prior sexual assault which was associated with higher incidences of arthritis and breast cancer. Multiple episodes of assault carried a two- to threefold increased risk of these diseases (Stein & Barret-Connor, 2000). Campbell's (2002) meta-analysis of studies of outcomes of women reporting intimate partner violence showed the list of illness symptoms to be lengthy, including those listed above along with hypertension, influenza or colds, fainting, and seizures.

There is an association of physical illness and PTSD in trauma victims. An analysis of chronic sufferers of PTSD found an increased risk of autoimmune diseases in a national sample of 2,490 Vietnam War veterans. Autoimmune disorders included rheumatoid arthritis, psoriasis, insulin-dependent diabetes, and thyroid disease. The research found that veterans with comorbid PTSD were more likely to have higher T-cell counts, hyperreactive immune responses on delayed cutaneous hypersensitivity tests, higher immunoglobulin-M levels, and decreased dehydroepiandrosterone levels. The author suggests that these biological differences are consistent with many inflammatory diseases and syndromes, such as cardiovascular and autoimmune diseases (Boscarino, 2004). Women are more likely than men to develop PTSD (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995), and both men and women have higher utilization of medical care, more intense physical symptoms, and poorer health functioning than non-PTSD subjects recruited from primary care practice sites (Gillock, Zayfert, Hegel, & Ferguson, 2005). Children and adolescents with PTSD also have an increased incidence of physical illnesses such a chronic fatigue syndrome (Seng, Graham-Bermann, Clark, McCarthy, & Ronis, 2005).

Although these many illnesses are disparate, their diversity may be explained through psychoneuroimmunology. A unifying mechanism mediating the stress-illness response involves macrophages and the innate immune and inflammatory response system (IRS). Stressors, through neuroendocrine signaling, and production of oxidative stress and tissue damage, appear to provoke the IRS, involving macrophages, neutrophils, liver cells, fat cells, and endothelium (Black, 2003). Acute stress is associated with activation of the sympathetic nervous system and release of epinephrine and norepinephrine. Adrenergic receptors on multiple target cells are activated, responses that lead to upregulated defensive reactions.

THE NORMAL IMMUNE RESPONSE

The body's defenses against stress, trauma, and microbes are orchestrated through innate and specific immune responses (reviewed in Segerstrom & Miller, 2004). In the innate response, cells are drawn to the site of tissue trauma and are actively phagocytic, removing debris, pathogens, and injured cells. This activity produces inflammation. Macrophages and other cells are signaled to release a group of messenger molecules, the proinflammatory cytokines (interleukin-1 beta [IL-1β], interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF-α]. These cytokines act on many sites, including the brain and the liver. The brain responds by elaborating fever, sickness-type behaviors (fatigue, loss of appetite, weakness, etc.) and activating the stress response (Kelley et al., 2003). The liver responds by releasing a group

of acute phase proteins, which are measurable in the blood and serve as an index of inflammation. C-reactive protein (CRP) is one of these factors. This innate inflammatory response is rapid and important, and protects during the time interval between injury, tissue damage, or other stressors and later activation of the specific immune response. Because inflammation can damage normal tissues if it is prolonged or excessive, the body normally regulates it through anti-inflammatory cytokines such as interleukin-10 (IL-10) or through cortisol (Suzuki et al., 2002). The role of this anti-inflammatory action is probably to constrain the inflammatory process from running out of control and causing excessive oxidative and necrotic damage (Munck, Guyre, & Holbrock, 1984; Sapolsky, Romero, & Munck, 2000).

The specific immune response is activated by antigens presented to it by macrophages and other cells. Antigens are non-self proteins, such as from microorganisms, foreign cells, or cancer cells, which are recognized and attacked by the specific immune system. Unlike the innate response, the specific immune response requires recognition of the antigen and identification of it as non-self. The two basic divisions of the specific immune system are humoral and cellular. Humoral immunity is protective against many bacterial and parasitic infections, while cellular immunity acts against intracellular pathogens and virally infected cells. Antibodies (immunoglobulins) are the basis of humoral immunity, while cellular immunity involves the activation of cytotoxic (cell-killing) lymphocytes. A group of helper cells, T helper (Th) lymphocytes, are necessary for both cellular and humoral immunity. T helper cells (Th1 and Th2) are polarized in terms of the cytokines they secrete when activated. Th1 signature cytokines are interleukin-2 (IL-2) and interferon-gamma (IFN-γ). These cytokines help cellular immune processes and also feed back to stimulate innate immune responses. Th1 cytokines also activate natural killer (NK) cells, which are cytotoxic cells of the innate immune system. The Th2 (or type-2) cytokines are required for humoral immunity. Th2 cytokines include IL-10 and IL-4.

In health there is a balance between the Th1 and Th2 arms of the immune system. When one system becomes activated it depresses the activity of the other system. Stress is known to suppress Th1 cells, leading to a shift toward Th2, largely through the action of glucocorticoids. Stress, through release of proinflammatory cytokines, also elicits alterations in brain chemistry such as serotonergic pathways, which can ultimately lead to depressive illness (Anisman & Merali, 2003).

These interacting immune responses exist to protect and defend health, but if mediators are not regulated or hormones are present in inappropriate amounts, the host can be damaged. This wear and tear is referred to as "allostatic load," and it leads to disorders of impaired immunity, cardiovascular pathology, obesity, osteoporosis, depression, anxiety, abnormal neurological function, and PTSD (McEwen, 2003). The stress of rape may be so acutely severe and its long-term effects potentially so catastrophic that allostatic load in some victims may become too great for normal healthy function. The inflammatory response system may dominate and deplete the woman's adaptive resources and provoke a pathophysiological state leading to adverse health outcomes. This is illustrated in the conceptual model for this study in Figure 1.

Although some studies have delineated the nature of the hormonal response to rape, almost no studies have been conducted that examine the immune system effects of rape. Therefore, the selection of variables to be investigated in this exploratory study was based on immune measures known to be affected by acute stress. A meta-analysis of over 300 studies on the effects of stress and immunity (Segerstrom & Miller, 2004), guided the selection of variables. These authors suggest that the early stress response is protective and allows for a very fast acting nonspecific response that accelerates wound repair and prevents infection.
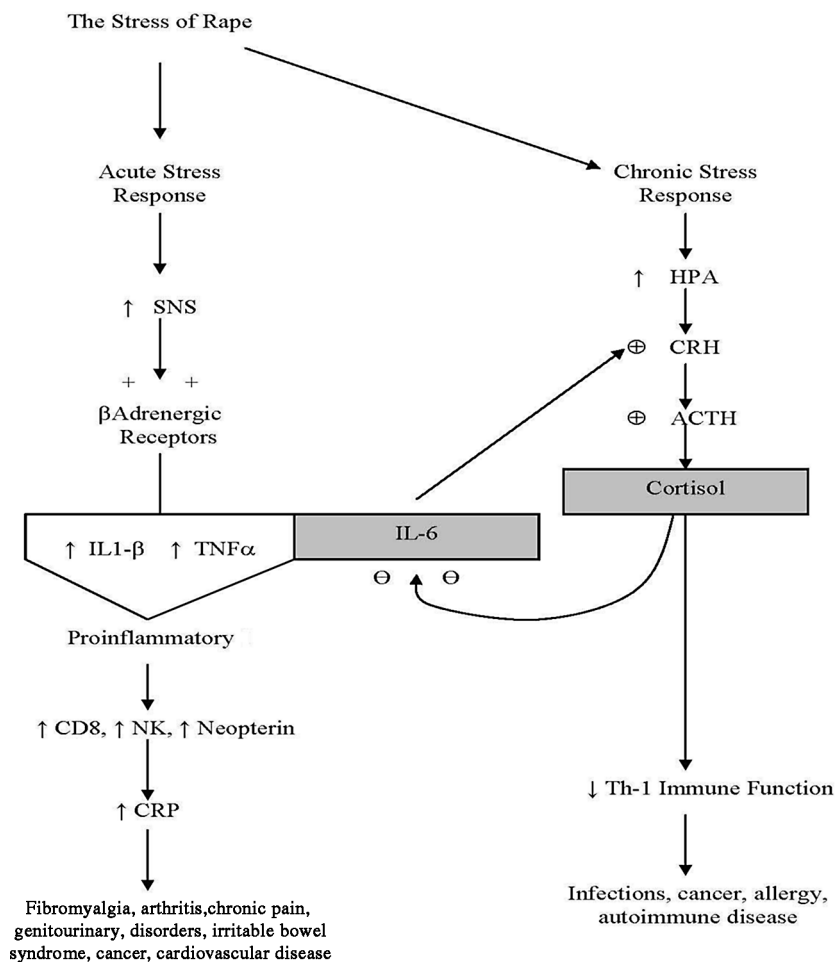
The Stress of Rape

Acute Stress Response → ↑ SNS → + ↓ + βAdrenergic Receptors

Chronic Stress Response → ↑ HPA → ⊕ CRH → ⊕ ACTH → Cortisol

↑ IL1-β ↑ TNFα | IL-6

Proinflammatory

↑ CD8, ↑ NK, ↑ Neopterin

↑ CRP

Fibromyalgia, arthritis, chronic pain, genitourinary, disorders, irritable bowel syndrome, cancer, cardiovascular disease

↓ Th-1 Immune Function

Infections, cancer, allergy, autoimmune disease

Figure 1. Conceptual model for the role of inflammation and immunity in later health outcomes of rape. IL-6 produced in acute stress response is proinflammatory and negatively regulated by cortisol. If inflammation is unchecked or cellular immunity is depressed, then many disparate illnesses may result.

The products of this innate immune activation (IL-1, IL-6, TNF-α) stimulate the hypothalamic-pituitary-adrenal (HPA) axis, with subsequent cortisol release from the adrenal cortex. Cortisol can then down-regulate the innate response while the slower-acting specific immune response continues. Under conditions of acute, but *brief,* stress, the meta-analysis of the literature suggests that Th1 cytokines are reduced and Th2 cytokines are increased, with IFN-γ production decreasing and IL-10 increasing. Lymphocyte proliferation is also found to be decreased in many stress studies. These effects are believed to be due to redistribution of cells and suppression, possibly by stress hormones, of the Th1 axis.

The most frequently used experimental paradigm for acute stress in humans has been academic examination stress. However, the stress produced by rape is orders of magnitude greater than academic examination stress, and rape is initially an acute stressor but continues to produce a prolonged and severe stress response in most women. Stressful event sequences, such as might be associated with the trauma of rape, in the Segerstrom and

Miller meta-analysis were found to be associated with decreased cortisol, increased NK cytotoxicity, and decreased CD4 and CD8 percentages.

This article describes an exploratory pilot study to determine the feasibility of measuring inflammatory, immune, and stress hormone variables at the time of a first rape event in female victims. These data were compared to healthy, nonstressed control women's data. A major limitation was that women reporting rape were examined whenever they arrived at the Sexual Assault Crisis Center, so circadian influences could not be controlled.

The general hypotheses for this study were derived from research on stress, trauma, and immunity. It was hypothesized that the innate inflammatory response would be activated through stress hormones and sympathetic nervous system activation in women reporting rape and that this activation would persist for many hours after the trauma of rape. It was also hypothesized that there would be relationships among stress hormones and immune variables, with cortisol levels being associated with suppression of cellular immunity.

METHOD

Participants

After approval from the University Institutional Review Board and the Sexual Assault Crisis Center, Sexual Assault Nurse Examiners (SANE) recruited subjects who were reporting a first-time rape and had come to the Center for help. An exclusionary criterion was a current situation of interpersonal violence. The time frame following rape was 24 to 72 hours. All but two participants were examined in the afternoon and evening (between 1:00 p.m. and midnight), but the range was from 10:00 a.m. to midnight. Subjects were asked if they wished to enroll in a study of the immune effects of rape and were assured of confidentiality. No participants refused. Fifteen participants agreed to be in the study and were able to provide adequate volumes of blood for all sera analyses. Venipunctures on four women reporting rape did not produce adequate heparinized blood to perform all of the flow cytometry, in vitro cultures, and lymphocyte proliferation assays, so there are some missing data for some subjects for some of these assays. (We did not wish to add to these women's burdens by performing an additional venipuncture.) Sixteen healthy control women, self-reporting low levels of stress, free of medication, and in the follicular phase of their menstrual cycles, except for one postmenopausal woman, were included as controls. These women provided morning blood samples between the hours of 8:00 and 11:00 a.m. These normal control participants were women that our laboratory has studied in order to determine normal population levels of the various endocrine and immune variables under investigation in several of our studies. The women included as controls were randomly selected based on ages and menstrual cycle phase from our data base of control subjects.

Variables

This was an early exploratory and feasibility study so minimal data were collected on women reporting rape. Exclusion criteria were an ongoing current situation of interpersonal violence and age under 18 years. Age, race, time of assault, and types of assault (vaginal, oral, anal, or mixed), and the SANE nurse's checklist of the patient's behavior at the time of examination were data collected from participants after informed consent was obtained. Although many women were excluded from the study based on their age, all women who were offered participation agreed to be in the study. The women being examined who were eligible for inclusion believed that their participation might help women in similar situations in the future.

Behavior

Behavior was coded based on nurses' observation and summed as either present (1) or absent (0), and numerical scales for uncontrolled (range 0–5) and controlled (range 0–4) behavior were constructed. Examples of uncontrolled behavior include yelling, sobbing, fidgeting, agitation, and loudness. Examples of controlled behavior include staring, listlessness, quietness, and trembling.

Blood Collection

After the SANE examination was completed, which usually took 1–2 hours, the routine venipuncture was performed and an extra 10 ml of blood were drawn into a serum separator tube and two heparinized collection tubes. The blood samples were either brought immediately to the lab, or, if the data were collected very late at night, the tubes were refrigerated and the heparinized tubes had serum-free RPMI-1640 media with 50 µg/ml gentamicin added to them to assure viability of the cells, and these samples ($n = 5$ of the 15) were brought to the lab the next morning. All samples were processed within 12 hours. Cell viability was not significantly affected by refrigerated storage of blood in media overnight as determined by exclusion of trypan blue.

Control subjects came to the lab in the morning hours (8:00 to 11:00 a.m.) for venipuncture, and their blood samples were processed as described above. Date of last menstrual period was recorded, and only women in the follicular stage (days 13–16) were used in these analyses.

The analysis consisted of assays that evaluated the function of the lymphocytes (proliferation, cytokine secretion), assays that measured amounts of cytokines and hormones present in the blood, and counts of different lymphocyte subsets.

Cytokine Production Assays In Vitro

The heparinized blood was diluted 1:5 with RPMI-1640 media supplemented with 1% glutamine and 50 µg/ml gentamicin, with no added fetal calf serum. One-ml aliquots were added to wells to which 5 µg/ml E.coli lipopolysaccharide (LPS) (Sigma) and 5 µg/ml phytohemagglutinin (PHA) (ICN) were added. PHA and LPS are polyclonal mitogens that stimulate lymphocytes and monocytes to release cytokines. The cultures were incubated for 66 hours, as were the lymphocyte proliferation cultures. Time curves were done by our lab to determine the optimal length of incubation of the whole blood cultures; the cultures produced maximal cytokines at 36 hours, but we saw no loss of cytokines at 66 hours. Unstimulated cultures were run, and the cytokine production from unstimulated cultures was subtracted from the stimulated cultures. The cultures were spun at 1,500 rpm for 5 minutes, and the supernatants were aliquoted and frozen at −80°C until analysis.

Flow Cytometry

The remainder of the heparinized blood was prepared for flow cytometry or lymphocyte proliferation. One hundred µl of blood was pipetted into plastic capped tubes and incubated with CD3, CD4, CD8, CD19, and CD56 antibodies as well as antibody controls and blanks. The fluorochrome conjugated antibodies (Becton Dickenson) were either labeled with fluorescein isothiocyanate or phycoerythrin. The cells were prepared using immunolyse kits (Coulter) and fixed for analysis in a Beckman Coulter Epics XL flow cytometer, which can analyze up to four fluorochromes with a single argon air-cooled laser. The data were expressed as percentages, and the CD4/CD8 was a ratio.

Lymphocyte Proliferation

For lymphocyte proliferation studies, the heparinized blood was pipetted onto 4 ml of Ficoll in 12-ml tubes, and the Ficoll prep was then centrifuged at 22° C at 1,200 rpm for 30 minutes. The white cell layer was removed and washed twice in RPMI-1640 with 5% fetal calf serum, 50µg/ml gentamicin, and 1% glutamine at 1,200 rpm for 10 minutes. The cells were then resuspended and counted by trypan blue exclusion using a hemocytometer. The samples collected at night and stored in media did not show a significant increase in number of nonviable cells when measured by trypan blue exclusion the next morning. Cells were suspended in media and pipetted into wells in a 96-well plate at a concentration of 100,000 viable cells per well. Media and mitogens were added for a final volume of 200 µl per well. Controls and stimulated assays were done in triplicate. PHA was added to wells in two concentrations (1 µg/ml and 5 µg/ml). The plates were incubated in a 37° C humidified incubator with 5% CO_2 for 66 hours.

At 66 hours these cultures were pulsed with 0.5 µCi of tritiated thymidine per well for an additional 6 hours. The cells were then harvested on glass filter paper, and planchets were cut and placed in biodegradable liquid scintillation cocktail. The vials were counted in a liquid scintillation counter (Packard), background counts subtracted, and the data expressed as stimulation index (SI) (stimulated CPM/unstimulated CPM).

Hormones and Cytokines

Analyses were done in batches within one month of collection on frozen sera samples. Cortisol and ACTH were measured by ELISA (DRG, Germany) (lower limit of detection 2.5 ng/ml). CRP was measured using an ultrasensitive ELISA from DRG. Neopterin was measured with kits obtained from ALPCO (New Hampshire). Cytokine ELISAs were performed on samples after appropriate dilution using kits from e-Bioscience (San Diego, California). The inter- and intra-assay coefficients of variation were 10% or less for these ELISAs. The plates were read at the appropriate wavelengths on a Scantron plate reader, and the data were further analyzed using the GraphPad Prism Program.

Statistical Analyses

IL-6, IFN-γ, IL-10,and CRP were log 10 transformed to achieve a normal distribution and *t* tests were computed to compare rape victims to control subjects. Since this was a small data set, in all cases except for serum IL-6 (two extreme high outliers in women reporting rape), and two very high outliers in CRP (one from each group), all data were retained. Pearson product moment correlation coefficients were performed to determine relationships between hormones, behavior, and immune variables.

RESULTS

Demographics

The mean age of the rape victims was 30 years (range 18 to 51 years), and the mean age of the control group was 24.4 years (range 21 to 51 years). All control participants were White, while three women reporting rape were African American and the remainder White. The majority were seen within 24 hours of the rape. All but one was vaginally raped, and six were also penetrated orally and rectally. None were in current domestic abuse and violent situations. All but two women were examined in the afternoon and evening (range 10:00 A.M.

to midnight). Four of the 15 women reporting rape had experienced assault or interpersonal violence in the past, although none had experienced these during the previous year.

Behaviors

The behaviors reported by the SANE nurses indicated that victims' behavior dichotomized to controlled or uncontrolled. The mean score on the Controlled scale was 3.14 and on the Uncontrolled scale 1.14. The rape victims were more likely to exhibit controlled behavior.

Lymphocyte Percentages

The percentages of surface cell markers of lymphocyte populations measured by flow cytometry indicated that CD8 levels (cytotoxic cells) were significantly higher in women reporting rape (10%) compared to the control group (6.1%) ($t = 3.24$, $df = 24$, $p < .008$), and CD19 percentages were significantly lower in women reporting rape (6.1%) compared to the control group (19.8%) ($t = 4.04$, $df = 25$, $p < .001$). Serum cortisol levels were positively correlated with the CD8 count ($r = .61$, $p < .047$).

Lymphocyte Proliferation

Figure 2 depicts the proliferation of lymphocytes stimulated with PHA. Lymphocytes from the rape victims had a decreased stimulation index in PHA stimulated cultures at 5 µg/ml ($SI = 18.8$) compared to controls ($SI = 56.3$) ($t = 1.7$, $df = 22$, $p < .03$).

Whole Blood in Vitro Assays

In vitro production of mitogen stimulated IFN-γ and IL-10 production differed, with higher IFN-γ (232 ng/ml) in women reporting rape compared to the control participants (74.3 ng/ml) ($t = 2.87$, $df = 27$, $p < .008$), and IL-10 production was also higher (7.3 ng/ml) than
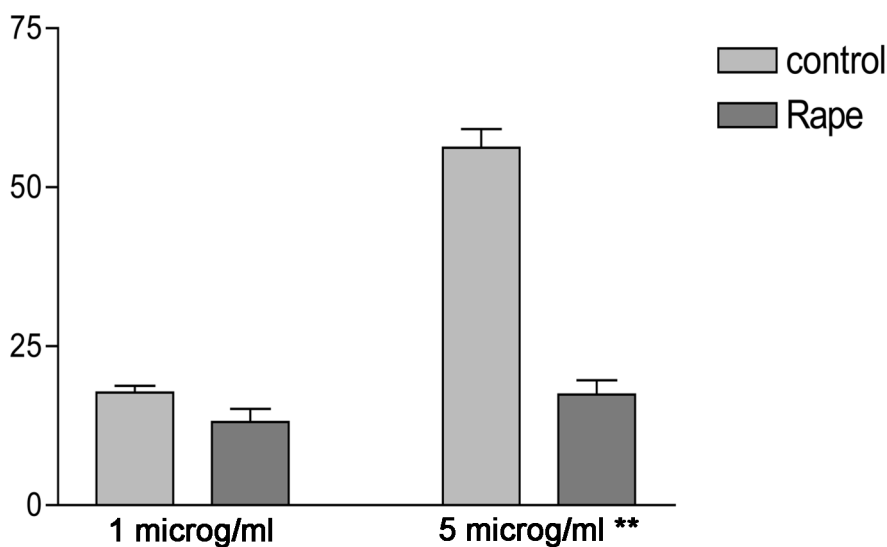

Figure 2. Lymphocyte proliferation (stimulation index) with PHA. PHA at 5 µg/ml added to mononuclear cell cultures produced a higher stimulation index in control subjects compared to women reporting rape. Error bars are standard error of mean.[**]$p < .01$.

in the control group (2.9 ng/ml) ($t = 3.15$, $df = 26$, $p < .004$). However, the Th1/Th2 ratio, computed as the ratio of IFN-γ/IL-10 produced in culture, was significantly lower in women reporting rape (32.3) compared to the control subjects (90.2) ($t = 2.14$, $df = 26$, $p < .041$).

Serum Cytokines, Inflammatory Proteins, and Hormones

There were statistically significant elevated levels of serum IFN-γ ($t = 7.02$, $df = 27$, $p < .001$), CRP ($t = 3.8$, $df = 28$, $p < .001$), IL-10 ($t = 3.04$, $df = 26$, $p < .006$), and IL-6 ($t = 4.47$ $df = 26$, $p < .001$) in women reporting rape compared to the control group (see Figure 3). TNF-α was not measurable in the serum, and neopterin was not different in the two groups (see Table 1). ACTH was higher in the women reporting rape ($t = 3.47$, $df = 28$, $p < .003$), but cortisol was not significantly different (see Table 1).

Correlations

Behavioral control was correlated with serum cortisol ($r = .84$, $p < .009$), while uncontrolled behavior was associated with CRP ($r = .71$, $p < .004$). Controlled behavior was also correlated with lower CD4 counts ($r = -.75$, $p < .01$), reduced CD4/CD8 ratios ($r = -.84$, $p < .001$), and lower proliferation indices with PHA ($r = -.63$, $p < .001$) suggesting T cell suppression. There were no associations between penetrations and any of the biological variables. Although most women had some tissue trauma, the extent of such was not determined.

DISCUSSION

The data suggest that women reporting rape who present to a Sexual Assault Crisis Center are in a state of acute stress that is manifested by behavior and immune/inflammatory
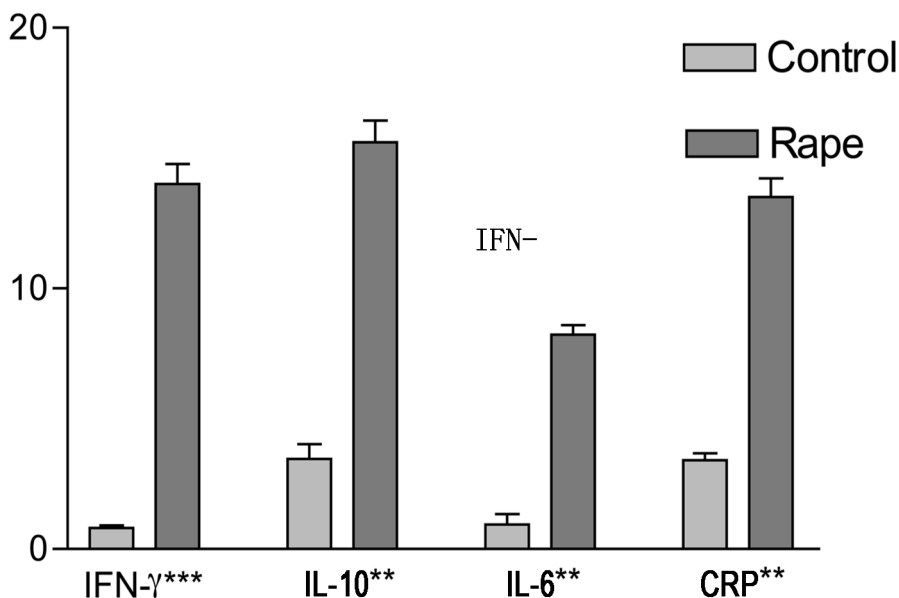

Figure 3. Cytokines and CRP differences. C-reactive protein and cytokines are compared between women reporting rape and control subjects. Cytokines are in pg/ml; CRP in mg/L. Error bars are standard error of mean. $^{**}p < .01$. $^{***}p < .001$.

TABLE 1. Concentrations of Hormones, Inflammatory Proteins, and Cytokines (Means and Standard Error of Measurement)

Serum Concentrations	Control Subjects	Women Reporting Rape
Cortisol (ng/ml) *ns*	117.9 ± 15.6	113.8 ± 20.1
ACTH (pg/ml)[***]	2.76 ± .93	5.62 ± .8
CRP (mg/L)[***]	2.6 ± .87	13.5 ± 2.9
IL-6 (pg/ml)[***]	0.95 ± 1.5	7.24 ± 0.65
IL-10 (pg/ml)[**]	3.45 ± 2.3	15.56 ± 3.26
IFN-γ (pg/ml)[***]	.81 ± .40	14.03 ± 3.01
Neopterin (nM/L) *ns*	6.54 ± .44	6.58 ± .21

[**]$p < .01.$ [***]$p < .001.$

differences when compared to healthy control subjects. A recent meta-analysis of stress and immunity which analyzed over 300 studies suggests that the response we observed is typical of a person experiencing extreme acute stress (Segerstrom & Miller, 2004). Our data suggests that the acute inflammatory response is dominant in the first 24–72 hours following the rape, but there are features of T cell suppression at that time as well (reduced T cell proliferation, decreased Th1/Th2 in vitro ratio).

We collected very little data on these women in this exploratory work as we wished to not intrude on them during this sensitive time. The data we did collect showed that these women evidenced activation and upregulation of the innate inflammatory response system, suppression of aspects of the specific immune response, a range of cortisol and ACTH levels, and generally either a controlled or uncontrolled pattern of behavior during the SANE nurse's examination.

When compared to normal levels in healthy control subjects, several inflammatory markers and proinflammatory cytokines were elevated in women reporting rape. A limitation of the study was the difference in time of day of data collection. IL-6 is known to have a biphasic circadian rhythm (Vgontzas et al., 2005), with two nadirs at 8:00 A.M. and 9:00 P.M. and two zeniths at 7:00 P.M. and 5:00 A.M., with the stronger peak at 5:00 A.M. This pattern is disturbed by sleep deprivation, so the differences between the control group and the women reporting rape in IL-6 in part may be related to the time of day of data collection as well as the likelihood that the women's sleep was disturbed following rape. However, the mean concentration measured in women reporting rape was higher than the normal evening IL-6 concentration reported in the literature (Bauer et al., 1994). Most remarkable was the elevation in CRP, a factor that is not circadian dependent, which was three times the level found in the control subjects. Elevated levels of serum IL-10 and IFN-γ were also noted in the rape victims. The source of the proinflammatory cytokines in serum is from a variety of cells, including lymphocytes, macrophages, NK cells, fat cells, and endothelium (Black, 2003). A possible mechanism for these increased levels is related to the effects of sympathetic nervous system activation, which stimulates beta adrenergic receptors on cells, causing them to secrete cytokines, which produce a proinflammatory state. The proinflammatory cytokines act on the HPA to stimulate cortisol release and on the liver to stimulate production and release of acute phase proteins such as CRP (see Figure 1). CRP activates the immune system to recognize and destroy microbial invaders. Considering the stress as well as physical trauma and injury suffered by the rape victims in our study, this proinflammatory upregulation is not surprising. The extent of traumatic injury to the women in the study was not measured, but certainly would be an influence on the extent of inflammation following the rape event. Rape is often accompanied by an actual or perceived threat to life,

so our data may reflect immune responses related to the magnitude of the stressor of rape. In addition, the introduction of semen and microorganism and the presence of overt or micro-trauma to mucosal tissues may have upregulated inflammatory responses. While unlikely, ejaculate itself may play an immune/inflammatory role, as spermatic fluid contains LPS binding protein receptor, which when activated can activate inflammation (Malm et al., 2005). On the other hand, seminal plasma generally is considered immunosuppressive so as to protect sperm cells within the female reproductive tract, with millions of sperm not destined for fertilization removed by an intense post-coital leukocytic and proinflammatory local response in the cervix and uterus that is initiated by prostaglandins in seminal fluid (Denison, Grant, Calder, & Kelly, 1999). Systemic immune responses in the immediate postcoital period during consensual sexual intercourse have not been studied.

There are multiple sources of serum IL-10. It may be that IL-10 is the response to an inflammatory signal, acting to dampen the excessive potential inflammation caused by the response to rape. ACTH and cortisol levels were not correlated with any of the cytokines in women reporting rape, although cortisol was marginally correlated with neopterin levels ($r = .45$, $p < .08$). As Yehuda, Resnick, Schmeidler, Yang, and Pitman (1998) have reported, we also observed clearly hypocortisolemic women in the sample, with the lowest levels of cortisol being 32 ng/ml in one woman in an afternoon collection. On the other hand, the data presented in Table 1 indicate that the mean cortisol level that was representative of late afternoon and evening collection in the rape victims was essentially the same mean value as the normal control subjects, who had blood collected in the morning hours. This finding, along with the elevated ACTH in rape victims, may suggest that the women reporting rape had high cortisol and ACTH levels for the time of day they were collected, suggesting activation of the HPA.

As seen in other stress research, the CD3+ CD8+ cell population was increased in the circulation of rape victims (Segerstrom & Miller, 2004; Zorrilla et al., 2001), and the count was correlated with serum cortisol levels. These cytotoxic cells, as well as NK cells (CD56), are thought to be redistributed in acute stress due to the effects of the sympathetic nervous system. We did not observe a difference in NK cells, but we did see a significant decrease in CD19+ cells, which are activated B lymphocytes, in rape victims. B cells are less available to the vascular compartment than T cells, and, thus, while they do display β-adrenergic receptors, they may not be affected by relatively mild acute stress, such as an academic examination, because they are less accessible. While a drop in circulating per-centages of CD19 cells in acute stress has not been a typical finding, such a decrease was found to be associated with panic disorder (Schleifer, Keller, & Bartlett, 2002). Potentially, the prolonged state of sympathetic activation that may be present in a rape victim may ulti-mately result in suppression of both T and B cells, as well as NK cells. Rape is a stressor of great magnitude. Rape is often accompanied by an actual or perceived threat to life, and so our data may reflect immune responses related to the magnitude of the stressor of rape.

There appears to be a general T cell suppression in terms of proliferation responses to PHA in the women reporting rape. A reduction in proliferation response is seen frequently in acute stress research. The Th1/Th2 ratio in whole blood cultures from women reporting rape was lower than it was in the control group. It is possible that the mechanism for this effect is through the action of stress hormones, which preferentially inhibit the Th-1 axis (Visser et al., 1998).

Of interest are the possible relationships of behavior at the time of assessment. CRP was related to higher uncontrolled behavior (crying, shouting, agitation), while cortisol was positively correlated with controlled behavior (staring, quietness, trembling). Within the very small sample studied, these relationships may provide clues to the women's pathophysiology. Women displaying uncontrolled behaviors may be highly anxious and

showing excessive sympathetic activity, while the women who display controlled behavior may be internalizing their stress and showing more depressive symptoms. The neuroendocrine response at the time of trauma is known to be predictive of later psychological problems (depression, PTSD) (McFarlane, Atchison, & Yehuda, 1997; Yehuda et al., 1998). Correlations between behaviors and the biological variables are intriguing but must be interpreted very cautiously with this small data set.

This study was exploratory and had significant limitations. The circadian influence on cortisol, ACTH, and IL-6 was not controlled. Women reporting rape were examined by the SANE nurses when they came to the clinic, which was usually in the afternoon or evening. The possibility of the blood draw and the SANE examination being additionally stressful needs to be considered, although for most women the SANE approach reduces their feelings of anxiety and stress. The number of women examined was small. The data collected on women reporting rape were minimal. The degree of traumatic tissue damage and semen exposure may play a role in the inflammatory response, and these data were not collected. The presence of specific inflammatory or autoimmune diseases was not assessed in the women reporting rape. The mean ages of the samples were not equivalent, nor were the menstrual cycle stages of the women reporting rape known.

The study reported here examines a window in time, which may or may not predict future events. Additional work is required to determine whether the changes reported here are pathophysiological or predictive of later outcomes in any way. Providers of help to traumatized victims should be aware of the possibilities that both biological and behavioral responses of victims may set the stage for later disturbances in endocrine, inflammatory, and immune factors.

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