CLINICS

RAPID COMMUNICATION

Psychosocial factors and T lymphocyte counts in Brazilian peacekeepers

Angela M. Monteiro da Silva,[I] Francisco A. B. Speranza,[II] Solange Kiyoko Ishii,[II] Raphael Hirata Jr,[III] Ana Luíza Mattos-Guaraldi,[III] Lucimar Gonçalves Milagres[III]*

[I] Exército Brasileiro, Forte Duque de Caxias, Centro de Estudos de Pessoal, Rio de Janeiro/RJ, Brasil. [II] Instituto de Biologia do Exército (IBEx), Rio de Janeiro/RJ, Brasil. [III] Universidade do Estado do Rio de Janeiro (UERJ), Faculdade de Ciências Médicas, Disciplina de Microbiologia, Imunologia e Parasitologia, Rio de Janeiro/RJ, Brasil.

OBJECTIVE: To investigate the associations between psychosocial factors and peripheral blood CD4 and CD8 T lymphocyte numbers in Brazilian peacekeepers.

METHODS: Venous blood was collected from 759 peacekeepers who had just returned from a peace mission in Haiti. Among the 759 soldiers, 642 individuals completed the psychosocial measures. CD4 and CD8 T lymphocyte counts were measured by flow cytometry using a commercially available kit. Psychosocial factors, including military peace force stressors, clinical stress, anxiety and depression, were recorded. As a reference for T lymphocyte numbers, we measured T lymphocyte counts in 75 blood donors from the *Instituto de Biologia do Exército*, Rio de Janeiro.

RESULTS: The median numbers of CD4 and CD8 T lymphocytes in the blood donors were 819 cells/μl and 496 cells/μl, respectively, with a CD4:CD8 ratio of 1.6. Significantly ($p<0.05$) lower CD4 T cell counts (759 cells/μl) were recorded for peacekeepers, with similar CD8 levels (548 cells/μl) and smaller CD4:CD8 ratios (1.3, $p<0.001$) compared to blood donors. These differences were due to a group of 14 military personnel with CD4 and CD8 medians of 308 and 266 cells/μl, respectively. Only one (7.1%) of these 14 individuals was diagnosed with clinical stress compared with 13.5% of the individuals with normal levels of CD4 T lymphocytes. One individual out of 628 (0.16%) had a Lipp's Stress Symptom Inventory score of 3, indicating near exhaustion.

CONCLUSION: The prevalence of psychological disorders was low and there were no associations with CD4 or CD8 T cell numbers.

KEYWORDS: T Cell Count; Psychosocial Factors; Peacekeepers.

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E-mail: milagreslucimar@gmail.com, lucimar@uerj.br

*corresponding author

INTRODUCTION

Since the end of the Cold War, multinational peacekeeping operations have dramatically increased. United Nations peacekeeping operations were created to support the implementation of cease-fires or more comprehensive peace agreements. Currently, peace operations involve increasingly complex tasks, from protecting civilians to rebuilding institutions in post-conflict states (1).

In the United Nations, traditional Peacekeeping Mission militaries may be placed in non-combatant roles in ''war zones.'' Such situations involve various stressors with possible short- and long-term effects on troop health (2). The change from peacekeeping operations to more stressful conditions favors potentially stressful events for involved military personnel due to increased exposure to dangers (3,4). Examples of typical peace mission stressors include being subjected to harassment without responding, witnessing atrocities, coming into contact with dead bodies and mortal remains, risking death and injury (5), witnessing the misery of local populations and experiencing other events capable of inducing cultural shock.

The use of force when necessary has become more frequent and more mandates of Peace Operations have been undertaken under chapter VII of the United Nations Charter. Article 42 of the Charter enables the United Nations Council to use force to maintain or restore international peace and security if the non-military measures that will be or that have been proven are considered inadequate. As the United Nations does not have any armed forces at its disposal, the Council uses

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Article 42 to authorize the use of force by a peacekeeping operation, by multinational forces or through interventions by regional organizations (6).

Since 2004, Brazil has sent contingents of more than a 1000 military personnel to the United Nations Stabilization Mission in Haiti (MINUSTAH) every six months and has been responsible for the military command of this operation (7). Contingent III-MINUSTAH (this study) was deployed under Chapter VII and the Brazilian peace troops were fighting against Haitian gangs.

Soldiers who participate in peacekeeping operations in which there are unexpected acts of violence and instability are at a higher risk for developing posttraumatic stress disorder (PTSD) and other disturbances related to exposure to extreme or severe stress (4).

In addition to psychiatric morbidity, traumatic stress and especially PTSD are associated with poor self-reported physical health (e.g., heightened rate of infectious diseases) and an elevated risk for multiple comorbid medical disorders (8,9). Until laboratory biomarker tests for PTSD and other disorders are developed and validated, mental health professionals must rely upon a patient's clinical history and mental status assessment to correctly diagnose a disorder and provide treatment (10).

Chronic psychosocial stress favors the development of generalized immune dysfunction (8,11). Peripheral T lymphocytes comprise a range of functionally different subpopulations, i.e., naïve, effector and memory T cells (12). The major T cell populations have been evaluated in PTSD patients in several studies, but the results obtained thus far are contradictory (8,11).

We investigated the association between a number of psychosocial factors (military peace force stressors, clinical stress, anxiety and depression) and blood T lymphocyte ($CD3^+CD4^+$ and $CD3^+CD8^+$ cells) numbers of a Brazilian peace troop (Contingent III-MINUSTAH) that had been in Haiti for the previous six months.

MATERIALS AND METHODS

Study participants

Study group. The military group comprised 759 peacekeepers of Brazil's third MINUSTAH (United Nations Stabilization Mission in Haiti) contingent. They had just finished their peacekeeping duties and returned from Haiti. In the operational theater, most of them (73%) had an operational role, were directly involved in military operations and performed tasks such as carrying out patrols, guarding sensitive sites, maintaining crowd control and operating checkpoints. The other (27%) had support or engineering roles and performed administrative, health or engineering tasks. All of the participants were male, aged 19-51 years and had been in Haiti for a period of approximately six months.

Blood samples were collected during medical exams conducted during demobilization procedures between November and December of 2005. Blood samples were immediately processed and analyzed by flow cytometry, as described below. After blood sample collection, the military personnel completed psychosocial measures.

Among the 759 total soldiers, 117 who either did not complete the psychosocial measures or did not complete them properly were excluded. Therefore, a final total of 642 participants (84.6% of the initial sample of 759) completed the psychosocial measures.

Reference group for T lymphocyte numbers

As a reference for the T lymphocyte numbers in our population, blood donors were recruited from the *Instituto de Biologia do Exército* (IBEx), Rio de Janeiro, between July of 2006 and May of 2008. The participants, 23 females and 52 males, were aged 18-64 years, of the 75 donors, 36 individuals were military personnel (9 female, 27 male), whereas 39 individuals were civilians (14 female, 25 male) and were relatives or friends of military personnel. Selection criteria were based on procedures that included retrospective serological testing and a prospective written survey designed to disqualify individuals associated with a range of known risk categories.

Psychosocial instruments

The following psychosocial instruments were employed in this study: Military Peace Force Stressors Inventory (MPFSI), Lipp's Stress Symptom Inventory (13), Beck's Depression (BDI) and Anxiety (BAI) Inventories and a social-demographic questionnaire. All of these instruments are described below.

The MPFSI was developed at the Centre for Personnel studies (CEP, Rio de Janeiro) (14) for use in the psychological demobilization of Brazilian troops. This inventory identifies the most intense and frequent stressors of peacekeeping contingents on mission. The MPFSI contains 46 items assessed by a 6-point scale that varies from 0 (not stressful) to 5 (extremely stressful), giving a theoretical score ranging from 0 to 230 (15).

The Lipp's Stress Symptom Inventory indicates whether a person is experiencing clinical stress (13). In affirmative cases, a stress stage is indicated, as follows: alarm reaction (score 1), resistance phase (score 2), near exhaustion (score 3) or exhaustion (score 4). A score of zero indicated individuals with no clinical stress.

The BDI is a self-reported depression measure that is largely used in clinical and scientific research (16) to assess the severity of the affective, cognitive, motivational, vegetative and psychomotor components of depression. The total score can range from 0 to 63.

The BAI is one of the most commonly used self-evaluation anxiety measures. The BAI presents 21 anxiety symptom-related items (17) and the total score can theoretically range from 0 to 63.

Flow cytometric analysis

$CD3^+/CD4^+$ and $CD3^+/CD8^+$ T lymphocyte counts were measured in conformity with the National Program for Sexually Transmitted Diseases and AIDS of the Brazilian Health Ministry. Peripheral blood lymphocyte phenotypes were assessed by two-color flow cytometry analysis using a commercially available kit with fluorochrome-labeled monoclonal antibodies (BD FACSCount™ CD4 reagent system; Becton, Dickinson and Company, San Jose, CA, USA). Briefly, whole blood was added to prealiquoted reagent tubes containing fluorochrome-labeled antibodies that specifically bind to CD3+, CD4+ and CD8+ lymphocyte surface antigens. The cells were then fixed and analyzed using the FACSCount instrument. In addition to monoclonal antibodies, the reagent tubes also contain fluorochrome-labeled reference beads that act as both a

fluorescent standard to locate the lymphocytes and a quantitation standard. A paired control-reagent tube set containing four bead levels (zero, low, medium and high) was also run on each test day to verify the instrument accuracy and linearity. Control and calibrating reference beads analyzed by the internal software (versions 1.1 and 1.2) to enumerate the absolute cell counts, giving the absolute $CD3^+$, $CD4^+$ and $CD8^+$ cell counts, the percentages of $CD4^+$ and $CD8^+$ cells of the total $CD3^+$ cells, and the $CD4^+/CD8^+$ cell ratio. All analyses were automatically performed within the FACSCount and results were printed from the instrument. Absolute cell counts (cell/μl) were recorded (18).

Statistical analysis

The significance levels of the differences between groups were examined using the independent samples t test, Mann-Whitney U test (unpaired samples) or Wilcoxon matched-pair test (paired samples). These analyses were performed with GraphPad-Prism software, version 4.02. $P<0.05$ was considered statistically significant. Correlation coefficients between the T lymphocyte counts and psychological parameters were evaluated by Pearson's (parametric data) or Spearman's (nonparametric data) tests.

Ethics

The procedures used in this study were approved by the Ethical Committee of *Hospital Universitário Pedro Ernesto*, Rio de Janeiro State University (HUPE/UERJ), registration number 1548- CEP/HUPE of October 30th, 2006. Written informed consent was obtained from all volunteers during the interviews.

RESULTS AND DISCUSSION

Distribution of lymphocyte subsets according to the age and sex of blood donors

No significant differences in the median or mean values of the CD4, CD8, or CD3 cell counts or the CD4:CD8 ratio were found between the civilian and military or the male and female populations. For the subsequent analysis of lymphocyte counts, we considered civil and military population as one group.

As shown in Table 1, the CD4 levels (759 cells/μl) and the CD4:CD8 ratio (1.3) of the peacekeepers were significantly lower compared with those of the blood donors (819 cells/μl and 1:6, respectively). In contrast, similar results were obtained for the CD8 counts in both populations (548 cells/μl *versus* 603 cells/μl for blood donors and peacemakers, respectively).

Table 2 - Median and mean (95% CI) of T lymphocyte counts (cells/μl) in the peripheral blood of peacekeepers (n = 14) with reduced CD4 counts after the operational mission in Haiti.

Parameter	Median	Mean (95% CI)
$CD3^+$	666	641 (554-728)
$CD3^+CD4^+$	308	300 (269-331)
$CD3^+CD8^+$	266	288 (224-352)
CD4/CD8 ratio	1.2	1.2 (0.9-1.4)
Age (range)	24	25.7 (21-32)

However, a group of peacemakers (n = 14) had CD4 numbers below 352 cells/μl (median of 308 cells/μl, Table 2) and reduction in CD8 cell numbers (median of 266 cells/μl, Table 2). Plasma was collected from these 14 individuals during demobilization and was assayed for HIV antibodies and HIV-1 RNA using a qualitative RNA PCR assay (19). Both tests showed negative results for all individuals and clinical and laboratory exams indicative of acute infectious illness were also negative (data not shown). Six months later, 4 of those 14 individuals had their blood samples recollected and reanalyzed. At that time, their T lymphocyte counts presented normal values (data not shown), suggesting a transitory change in T lymphocytes in the peripheral blood of peacemakers.

PTSD patients can exhibit higher numbers of circulating T lymphocytes (8,20), whereas other studies reported no differences (21) or even lower T cell numbers (11). PTSD patients can also exhibit marked reductions in the proportions of naïve T cells and peripheral regulatory T cells. In contrast, increased memory T cell frequencies have been reported. All of these alterations could be a cause of the increased inflammatory and autoimmune disease susceptibility (11). One of our goals in future studies will be to more specifically analyze the T lymphocyte phenotype composition in our population before deployment and during demobilization.

Psychosocial factors of the Brazilian Military Peace Force

Table 3 shows the psychosocial parameter intensities for peacekeepers. No significant differences in the psychosocial factors (BDI, BAI and MPFSI) were observed between peacekeepers with normal and low CD4 levels. Table 4 presents the Lipp's clinical stress scores. Most peacekeepers with normal CD4 counts were categorized as having no stress (84.7%). One individual out of 628 (0.16%) had a score of 3, indicating near exhaustion. Among the 14 peacekeepers with low CD4 counts, no individuals had Lipp's

Table 1 - T lymphocyte counts (cells/μl) of blood donors (n = 75) and the military group returning from Haiti (n = 759).

Parameter	Blood donors		Peacekeepers	
	Median	Mean (95% CI)	Median	Mean (95% CI)
CD3	1439	1488 (1372-1603)	1419	1469 (1435-1502)
CD4	819	883 (803-963)	759*	789 (770-807)
CD8	496	550 (498-602)	548	603 (584-622)
CD4/CD8 ratio	1.6	1.8 (1.6-2.0)	1.3**	1.5 (1.4-1.5)
Age (range)	32	33.5 (18-64)	24	27 (19-51)

*$p<0.05$.
**$p<0.001$.

Table 3 - Psychosocial parameters and CD4 levels of the Brazilian Military Peace Force (n = 642), Contingent III.

Stressor parameters	Individuals with normal CD4 levels		Individuals with low CD4 levels	
(normal range)	Mean (n)	Median	Mean (n)	Median
BDI (0 – 63)	3.2 (518)	2	2.5 (12)	2.5
BAI (0 – 63)	3.5 (555)	1	1.3 (10)	0.5
MPFSI (0 – 230)	58.8 (340)	50	62.6 (12)	62.5

Table 4 - Clinical stress score distribution between peacekeepers with normal CD4 levels (n = 628) and low CD4 counts (n = 14).

Lipp's Clinical Stress Scores	Individuals with normal CD4 levels, n (%)	Individuals with low CD4 levels, n (%)
0 (no stress)	532 (84.7)	13 (92.9)
1 (alarm stage)	10 (1.6)	0
2 (resistance stage)	85 (13.5)	1 (7.1)
3 (near exhaustion)	1 (0.16)	0
4 (exhaustion)	0	0

scores of 3 or 4 and only one (7.1%) individual had a score of 2. Therefore, no association between Lipp's clinical stress levels and CD4 numbers was found. No significant differences were found in any of the psychosocial parameters analyzed in peacemakers with operational or engineering roles (data not shown). In addition, no significant correlations were found between CD4 numbers and military peace force stressors or depression and anxiety (data not shown). However, notably, the group of 14 individuals with low CD4 T cell numbers represented a small fraction (1.84%) of the sample (n = 759).

Individuals who are exposed to an event that threatens serious injury/death or is perceived as such respond in different ways. Most will suffer minimal (seconds), brief (hours) or short-term (days/weeks) abnormalities, but a small number will suffer from significant psychopathology over longer-term (months) or chronic (lifetime) time frames. A number of understudied yet important topics exist in the field, including factors that impact resilience and vulnerability (22).

In the present study, Brazilian peacekeepers were generally able to maintain a state of equilibrium in the face of challenging circumstances. Our data did not reveal any association between psychological parameters and CD4 T cell numbers. Although difficult, future studies will require accessing, assaying and following populations at risk for exposure to trauma before any exposure occurs (ideally, pre-deployment soldiers). This identification and the use of a biomarker could eventually improve the diagnosis and prevention of clinical stress.

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■ AUTHOR CONTRIBUTIONS

Silva AM and Speranza FA were responsible for the design of the study and for the technical execution. Ishii SK and Hirata Jr R were important for the technical execution. Mattos-Guaraldi AL helped to design the study and provided financial support. Milagres LG participated in the study design, data analysis and manuscript writing.

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