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Substantial reduction of naïve and regulatory T cells following traumatic stress

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A B S T R A C T

Posttraumatic stress disorder (PTSD) is associated with an enhanced susceptibility to various somatic diseases. However, the exact mechanisms linking traumatic stress to subsequent physical health problems have remained unclear. This study investigated peripheral T lymphocyte differentiation subsets in 19 individuals with war and torture related PTSD compared to 27 non-PTSD controls ($n = 14$ trauma-exposed controls; $n = 13$ non-exposed controls). Peripheral T cell subpopulations were classified by their characteristic expression of the lineage markers CD45RA and CCR7 into: naïve ($CD45RA^+$ $CCR7^+$), central memory (T_{CM}: $CD45RA^-$ $CCR7^+$) and effector memory (T_{EM}: $CD45RA^-$ $CCR7^-$ and T_{EMRA}: $CD45RA^-$ $CCR7^-$) cells. Furthermore, we analyzed regulatory T cells ($CD4^+CD25^+FoxP3^+$) and *ex vivo* proliferation responses of peripheral blood mononuclear cells after stimulation with anti-CD3 monoclonal antibody. Results show that the proportion of naïve $CD8^+$ T lymphocytes was reduced by 32% ($p = 0.01$), whereas the proportions of $CD3^+$ central ($p = 0.02$) and effector ($p = 0.01$) memory T lymphocytes were significantly enhanced (+22% and +34%, respectively) in PTSD patients compared to non-PTSD individuals. To a smaller extent, this effect was also observed in trauma-exposed non-PTSD individuals, indicating a cumulative effect of traumatic stress on T cell distribution. Moreover, PTSD patients displayed a 48% reduction in the proportion of regulatory T cells ($p < 0.001$). Functionally, these alterations were accompanied by a significantly enhanced (+34%) *ex vivo* proliferation of anti-CD3 stimulated T cells ($p = 0.05$). The profoundly altered composition of the peripheral T cell compartment might cause a state of compromised immune responsiveness, which may explain why PTSD patients show an increased susceptibility to infections, and inflammatory and autoimmune diseases.

1. Introduction

Exposure to traumatic stressors such as life-threatening accidents, physical assaults, sexual abuse, or combat experience poses a risk for severe mental disorders, and in particular for the development of posttraumatic stress disorder (PTSD). PTSD is characterized by re-experiencing the traumatic event (in form of intrusive recollections, nightmares or flashbacks), by persistent avoidance of stimuli associated with the trauma and emotional numbing, as well as a constant state of heightened alertness and increased arousal (American Psychiatric Association, 1994). Since the risk for developing PTSD increases with the number of traumatic stressors experienced (Kolassa and Elbert, 2007; Neuner et al., 2004), PTSD is a serious mental health problem in war and conflict regions, where exposure rates are high (Neuner and Elbert, 2007).

In addition to psychiatric morbidity, numerous studies have shown that traumatic stress and especially PTSD are associated with poor self-reported physical health (e.g., heightened rate of infectious diseases), increased health care use and costs, and an elevated risk for multiple comorbid medical disorders such as cardiovascular, respiratory, gastrointestinal, musculoskeletal or inflammatory and autoimmune diseases (Boscarino, 2004; Schnurr and Jankowski, 1999; Walker et al., 2003).

Peripheral T lymphocytes consist of a range of functionally different subpopulations, i.e., naïve, effector and memory T cells, which provide effective protection against a wide range of viruses and other pathogens. Fine regulation of generation, maintenance and function of the peripheral T cell compartment is crucial for an optimal balance between immunity and peripheral tolerance

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(Van Parijs and Abbas, 1998). Dysregulation within the peripheral T cell compartment, e.g., as a consequence of thymic involution and altered T cell activation or homeostasis, is involved in a variety of immunopathologies such as rheumatoid arthritis (Goronzy and Weyand, 2001) and multiple sclerosis (Duszczyszyn et al., 2006; Hug et al., 2003).

Regarding the fundamental role of T cells in infectious diseases and inflammatory or autoimmune disorders, we hypothesized that the enhanced susceptibility to such diseases in PTSD patients could be linked to changes in the composition of the peripheral T cell pool. Indeed, major T cell populations in PTSD patients have been evaluated in several studies, but results obtained so far are contradictory. For instance, it has been reported that PTSD patients exhibit higher numbers of circulating T lymphocytes (Boscarino, 2004; Boscarino and Chang, 1999) whereas other studies reported no differences (Vidovic et al., 2007; Wilson et al., 1999) or even lower T cell numbers (Kawamura et al., 2001). A similar picture emerges with respect to the T helper cell population, with one study reporting an increase (Boscarino and Chang, 1999) of circulating T helper lymphocytes and others showing a decreased proportion (Ironson et al., 1997; Kawamura et al., 2001) or no differences (Altemus et al., 2006; Laudenslager et al., 1998; Vidovic et al., 2007; Wilson et al., 1999). Regarding cytotoxic T cells, the majority of studies found no differences between PTSD patients and controls (Altemus et al., 2006; Laudenslager et al., 1998; Vidovic et al., 2007; Wilson et al., 1999) while two studies reported lower levels (Ironson et al., 1997; Kawamura et al., 2001). In addition, a higher ratio of CD4/CD8 lymphocytes has been suggested in PTSD patients vs. controls (Glover et al., 2005).

Considering the structural diversity among the peripheral T cell pool, we assume that it is inappropriate to compare bulk T cell populations since aberrations may occur in the activation and differentiation states of T cells. Therefore, we decided to provide a detailed characterization of T cell maturation subsets in a sample of PTSD patients, applying a differentiation model of T cells defined by changes in the expression of the lineage markers CD45RA and CCR7. According to this model, naïve T cells ($CD45RA^+$ $CCR7^+$) become activated after antigen stimulation, then differentiate into memory cells, and partly develop into effector cells with a strong cytolytic capability (Hamann et al., 1999; Sallusto et al., 1999). Memory T cells are a heterogeneous population and can be divided into distinct subsets of central memory (T_{CM}) and effector memory cells, respectively, characterized by the presence or absence of the chemokine receptor CCR7 (Sallusto et al., 1999). T_{CM} cells predominantly home to secondary lymphoid organs and lack immediate effector function but rapidly proliferate and gain cytolytic activity upon antigen stimulation. Conversely, the effector memory subset displays immediate effector function, has a low proliferative capacity and migrates to peripheral tissues (Sallusto et al., 2004). The effector memory T cells can be further subdivided into $CD45RA^-$ (T_{EM}) and $CD45RA^+$ (T_{EMRA}) cells, which have been shown to differ in their expansion potential and the expression of perforin (Sallusto et al., 2004).

Peripheral $CD4^+CD25^+$ regulatory T (T_{reg}) cells are crucial for controlling immune responses and maintaining self-tolerance by inhibiting autoreactive T cells (Vignali et al., 2008). The transcription factor FoxP3 (forkhead box P3) has been shown to be essential for the development and suppressive function of peripheral T_{regs} and is used as an intracellular marker for the identification of T_{regs} (Fontenot et al., 2003; Khattri et al., 2003; Ziegler, 2006). Genetic defects in FoxP3 have been shown to cause the severe, systemic autoimmune syndrome IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) in humans (Ochs et al., 2007). Additionally, there is growing evidence that a decrease in number or function of peripheral T_{regs} might contribute to the development of inflammatory and autoimmune diseases, such us multiple sclerosis, asthma, type 1 diabetes, psoriasis, and rheumatoid arthritis (Costantino et al., 2008). Considering the fundamental role of T_{regs} in the regulation of immune responses and the increased prevalence of PTSD to inflammatory or autoimmune disorders (Boscarino, 2004), we further analyzed the frequencies of peripheral T_{regs} in PTSD patients and non-PTSD subjects.

In order to clarify whether changes in the peripheral T cell pool are accompanied by functional alterations such as an altered T cell proliferation capacity, we further investigated the responsiveness of T lymphocytes after T cell receptor (TCR) stimulation with anti-CD3 monoclonal antibody.

In the present study, we present a differentiated characterization of the differentiation state of T lymphocytes in a group of severely traumatized PTSD patients. We demonstrate that PTSD patients exhibit a profoundly altered composition of the peripheral T cell compartment, as indicated by a marked reduction in the proportion of naïve and an increase in $CD45RA^-$ memory T cells, compared to control individuals. Furthermore, this is the first study showing that subjects with PTSD display a substantial reduction in the percentage of peripheral regulatory T cells, which could be a cause of the increased susceptibility to inflammatory and autoimmune diseases in those with PTSD.

2. Method

2.1. Participants

We examined the distribution of blood T lymphocyte subsets in 19 individuals with current PTSD (12 male, 7 female; mean age = 33.6 years, SD = 7.1, range 21–48) according to the DSM-IV (American Psychiatric Association, 1994) and 27 non-PTSD control subjects (9 male, 18 female; mean age = 29.1 years, SD = 8.3, range 19–50). PTSD patients were refugees (4 Africa, 1 Balkan, 14 Middle East and Afghanistan), with chronic (mean symptom duration = 7.2 years, SD = 4.4) and severe (mean sum score in the Clinician Administered PTSD Scale [CAPS] (Blake et al., 1995) = 79.6, SD = 18.6) forms of PTSD due to multiple highly stressful war and torture experiences. On average, patients have lived in Germany for 4.9 years (SD = 3.6). All patients were recruited from the Psychotrauma Research and Outpatient Clinic for Refugees, University of Konstanz, located at the Centre for Psychiatry Reichenau, Germany.

The non-PTSD group was recruited through advertisement and was matched to the patient group with regard to age and region of origin (3 Africa, 11 Balkan, 13 Middle East and Afghanistan). Since this control group varied with respect to the number of traumatic event types experienced (range: 0–9) some of the analyses were repeated with a three group (PTSD, trauma-exposed and non-exposed controls) design. For this purpose we divided the non-PTSD group by median split into a group with substantial exposure to traumatic stressors (4–9 different traumatic event types; $n = 14$) and a control group with no or few traumatic experiences (0–3 traumatic event types; $n = 13$) based on the number of past traumatic event types assessed with the event checklist of the CAPS (Blake et al., 1995).

Subjects were excluded if they reported intake of glucocorticoids, had acute or chronic somatic illnesses, or met criteria for additional mental disorders other than stress-related affective or anxiety disorders. Fourteen PTSD patients and 2 trauma-exposed controls met the DSM-IV criteria for a current major depressive episode. Eight PTSD patients and 2 trauma-exposed controls reported current intake of psychotropic medication (PTSD: 2 hypnotics, 3 anxiolytics, 5 antidepressants and 2 neuroleptics; non-PTSD: 1 hypnotic, 1 antidepressant). Since the pattern of results did not

change if we excluded all medicated participants from the statistical analysis, we only report the original analysis here.

2.2. Clinical interviews

All participants underwent an extensive standardized clinical interview administered by experienced psychologists and trained translators. PTSD symptoms and the number of traumatic event types experienced were assessed with the CAPS (Blake et al., 1995). The vivo checklist of war, detention and torture events (Vivo, 2006), which assesses common traumatic experiences in conflict regions and during torture, allowed for a detailed evaluation of the number of traumatic event types experienced. The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) was used to screen for potential comorbid mental disorders. In addition, the severity of depressive symptoms was assessed with the Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960). After complete description of the study to the subjects, written informed consent was obtained. All procedures were approved by the Ethics Committee of the University of Konstanz.

2.3. Blood sampling

Blood was collected between 10 and 11 a.m. in EDTA-treated tubes for T cell phenotyping and in sodium citrate-treated cell preparation tubes for proliferation assays (BD Vacutainer, Franklin Lakes, NY). In order to control for possible HIV and hepatitis A, B and C infections, an additional blood sample was sent to a diagnostic laboratory for standard hepatitis and HIV tests. All samples were negative for HIV or hepatitis C. Subjects classified with acute or chronic hepatitis A or B ($n = 3$) were excluded from the study. Two patients and one traumatized control showed an infection history for hepatitis B (as indicated by a positive result for hepatitis B core IgG antibody). Since the pattern of results did not change if we excluded them from the statistical analysis, they remained in the sample.

2.4. Lymphocyte phenotyping and T cell proliferation

Whole blood was analyzed for the percentage of total T cells ($CD3^+$), cytotoxic T cells ($CD3^+$ $CD8^+$) and T helper cells ($CD3^+$ $CD4^+$) as well as B cells ($CD45^+$ $CD19^+$), by flow cytometry. T cell maturation subsets were determined according to their expression profile of the surface molecules CD45RA and CCR7.

For quantification of T cell phenotypes, 100 μl whole blood was incubated for 20 min at room temperature with either APC-conjugated anti-CD3 (clone SK7) or a combination of PerCP-conjugated anti-CD3 and APC-conjugated anti-CD8 (clone SK1) or APC-conjugated anti-CD4 (clone RPA-T4), and PE-conjugated anti-CD45RA (clone HI100) and FITC-conjugated anti-CCR7 (clone 150503) monoclonal antibodies (mAbs). For quantification of B-lymphocytes 100 μl blood was stained with PerCP-conjugated anti-CD45 (clone 2D1) and APC-conjugated anti-CD19 (clone HIB19). For quantification of T_{reg} cells, blood samples were stained with PerCP-conjugated anti-CD3, APC-conjugated anti-CD4, FITC-conjugated anti-CD25 (clone M-A251), and intracellular FoxP3 expression was detected using the PE anti-human FoxP3 staining kit (eBioscience, San Diego, CA). Following antibody staining, standard lyse-wash was performed using BD FACS lysing solution; samples were washed twice, and 1×10^5 cells were acquired on a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, CA), and analyzed with FlowJo software (Tree Star, San Carlos, CA). All monoclonal antibodies were purchased from BD PharMingen (San Diego, CA), except CCR7 mAb from R&D Systems (Minneapolis, MN).

Absolute lymphocyte numbers (cells/μl) were measured, using an automated hematology analyzer (XT-2000i, Sysmex, Horgen, Switzerland).

For the proliferation assay, 1×10^5 CFSE-labeled peripheral blood mononuclear cells (PBMCs) were suspended in RPMI medium containing 10% FCS and stimulated for 72 h in 96-well flat-bottom microtiter plates coated with anti-human CD3 mAb (2 μg/ml, clone OKT3, eBioscience), and cell proliferation was measured by flow cytometry in triplicates. The investigator who performed the immunological analyses was blind for the group assignment of the probes.

2.5. Statistical analyses

Group differences in the immunological parameters were analyzed using ANOVAs. The independent variables were either two (PTSD, non-PTSD) or three groups (PTSD, trauma-exposed and non-exposed controls). Statistical significance for the immune measures was assessed by non-parametric permutation tests, using 1000 random permutations of group labels (Good, 2005).

Throughout the text and the tables all data are presented as mean ± standard deviation. In the figures data are displayed as mean + standard errors.

3. Results

3.1. Quantification of naïve and memory T lymphocytes

As shown in Table 1, the PTSD group had experienced a significantly greater number of different traumatic event types than the non-PTSD participants and reported significantly higher CAPS and HAM-D scores.

PTSD patients and control individuals did not differ with respect to absolute numbers of lymphocytes (PTSD: 2028.9 ± 405.7, $n = 18$; non-PTSD: 1936.7 ± 455.9, $n = 20$; $F = 0.43$, $p = 0.52$), or their overall percentage of B-lymphocytes (PTSD: 3.0 ± 1.3% of leukocytes, $n = 18$; non-PTSD: 3.0 ± 1.3% of leukocytes $n = 24$; $F = 0.0$, $p = 0.98$) and $CD3^+$ T lymphocytes (see Table 2). However, as presented in Table 2 and Fig. 1A–D, the percentage of $CD3^+$ T cells of the naïve ($CD45RA^+$ $CCR7^+$) phenotype was reduced in individuals

Table 1
Clinical characteristics of PTSD patients and non-PTSD subjects.

Variables	PTSD		Non-PTSD		$F(1, 44)$	p-Value
	M	SD	M	SD		
Age	33.58	7.16	29.11	8.36	3.57	0.06
CAPS event categories	**6.47**	1.87	**4.00**	2.53	13.13	**0.001**
War and torture event types	**10.68**	5.69	**3.26**	5.10	21.48	**<0.001**
CAPS score	**79.58**	18.63	**11.22**	16.86	168.11	**<0.001**
HAM-D score	**24.37**	7.64	**4.52**	5.21	110.16	**<0.001**

CAPS, Clinician Administered PTSD Scale; HAM-D, Hamilton Depression Rating Scale.
Significant p-values and their correspondent group means are displayed in bold.

Table 2
T cell maturation subsets in PTSD patients vs. non-PTSD individuals.

Variables (%)	PTSD			Non-PTSD			*F*-Value	*p*-Value
	N	*M*	SD	*N*	*M*	SD		
CD3								
Total	18	62.7	10.8	26	66.1	11.4	0.9	0.33
Naïve	19	**30.8**	8.7	26	**38.8**	10.6	7.2	**0.01**
CD45RA$^-$ memory	19	**45.7**	8.7	26	**35.6**	8.1	15.9	**<0.001**
T_{CM}	19	**22.2**	5.1	26	**18.1**	5.6	6.2	**0.02**
T_{EM}	19	**23.5**	7.7	26	**17.5**	6.6	7.9	**0.01**
T_{EMRA}	19	20.6	4.9	26	23.2	11.2	0.9	0.33
CD4								
Total	19	35.2	7.8	26	38.0	7.9	1.4	0.22
Naïve	18	43.9	12.1	25	48.0	11.9	1.2	0.29
CD45RA$^-$ memory	18	52.5	11.8	25	47.2	11.7	2.2	0.16
T_{CM}	18	32.4	6.7	24	29.3	7.5	1.9	0.17
T_{EM}	18	20.1	9.3	24	17.5	9.7	0.7	0.39
T_{EMRA}	18	3.5	3.0	25	4.8	3.7	1.4	0.22
CD8								
Total	18	**19.4**	4.5	23	**23.0**	4.9	5.8	**0.02**
Naïve	18	**25.8**	10.2	23	**38.1**	18.1	6.6	**0.01**
CD45RA$^-$ memory	18	**39.0**	13.2	23	**22.7**	7.4	24.9	**<0.001**
T_{CM}	18	7.5	3.1	23	6.0	4.3	1.5	0.24
T_{EM}	18	**31.5**	12.4	23	**17.2**	7.3	21.6	**<0.001**
T_{EMRA}	18	34.3	14.2	23	38.3	17.3	0.6	0.41

Significant *p*-values and the correspondent group means are displayed in bold.

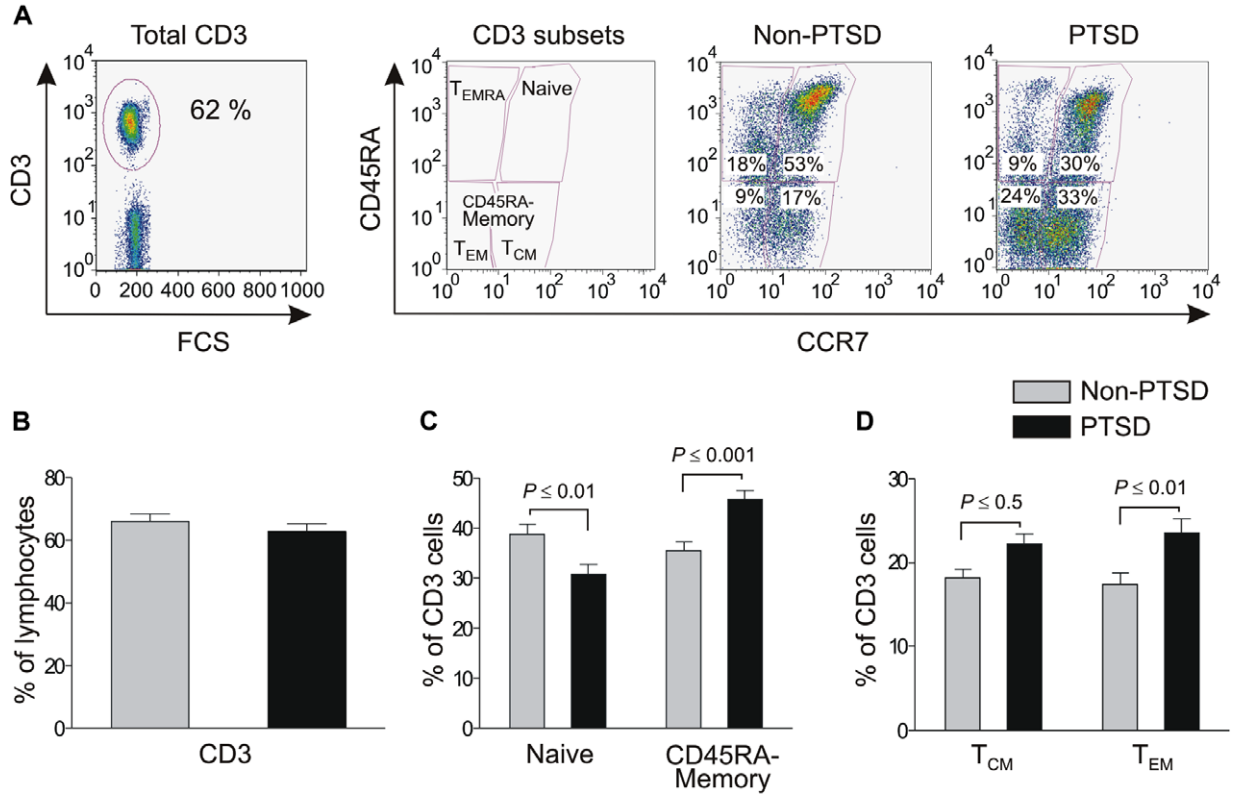

Fig. 1. PTSD patients display an altered peripheral T lymphocyte subset distribution. (A) Representative flow cytometric analysis of the whole T cell ($CD3^+$) population and subset distribution. (B–D) Data are presented as the mean percentages + SEM of (B) total, (C) naive and CD45RA$^-$ memory T cells, or (D) central memory (T_{CM}) and effector memory (T_{EM}) cells from PTSD patients and non-PTSD individuals.

with PTSD compared to non-PTSD subjects, whereas the percentage of CD45RA$^-$ memory phenotype was increased. This was due to an increased frequency of both the T_{CM} (CD45RA$^-$ CCR7$^+$) and T_{EM} (CD45RA$^-$ CCR7$^-$) populations in PTSD individuals. No significant group differences were observed for the $CD3^+$ T_{EMRA} population (CD45RA$^+$ CCR7$^-$).

We further examined whether these alterations occurred in both the cytotoxic ($CD8^+$) and T helper ($CD4^+$) lymphocyte populations. As shown in Table 2, PTSD patients had a significantly lower percentage of $CD8^+$ T lymphocytes compared to control individuals. Further subdivision revealed a massive reduction in the proportion of naïve $CD8^+$ T cells. The percentage of $CD8^+$ T_{EM} cells

was significantly increased in the PTSD group compared to the control group, whereas no differences were observed for the T_{CM} and the T_{EMRA} subsets.

No significant group differences were detected for the percentage of $CD4^+$ T cells and the naïve or memory $CD4^+$ T cell subpopulations (see Table 2).

To clarify whether the above-mentioned alterations are a specific feature of PTSD, or rather constitute a general consequence of trauma exposure, we repeated these analyses after subdividing the non-PTSD group into a group with substantial exposure to traumatic stressors and a control group with no or few traumatic experiences. With respect to the reduction in percentage of naïve T cells and enhancement of memory T cells, the trauma-exposed non-PTSD group displayed an intermediate phenotype positioned between the PTSD group and the non-exposed controls, indicating a cumulative effect of exposure to traumatic stressors on T cell distribution (see Fig. 2).

3.2. Quantification of FoxP3 expressing T cells and proliferation capacity of T cells

Regarding the immunoregulatory function of $CD4^+CD25^+FoxP3^+$ regulatory T cells (T_{regs}) and their role in maintaining self-tolerance (Vignali et al., 2008), we further compared the frequencies of peripheral T_{regs} in PTSD patients and non-PTSD subjects. Strikingly, we found a 48% reduction in the percentage of peripheral T_{regs} in PTSD individuals compared to non-PTSD individuals (PTSD: 1.2 ± 0.6%, $n = 15$; non-PTSD: 2.3 ± 0.9%, $n = 20$; $F = 17.5$, $p < 0.001$, see Fig. 3A and B).

To further investigate the proliferative capacity of T cells we performed a CFSE-based *ex vivo* proliferation assay. As presented in Fig. 3C and D, peripheral blood T lymphocytes of PTSD patients displayed higher *ex vivo* proliferation responses when stimulated with anti-CD3 mAb (PTSD: 46.5 ± 14.8%, $n = 12$; non-PTSD: 34.7 ± 15.3%, $n = 15$; $F = 4.1$, $p = 0.05$).

3.3. Moderating variables

Since our sample consisted of male and female participants as well as of smokers (PTSD: $n = 7$ vs. non-PTSD: $n = 5$) and non-smokers, we repeated all analyses with gender or smoking as additional between-factors, to control for the possible influence of these variables on the immune alterations reported here. For the different immunological variables, no significant main effects of gender and no significant group × gender interactions could be identified. After introducing gender as additional factor, all group differences reported above remained statistically significant, except the *ex vivo* proliferation response ($p = 0.13$). Similarly, we could not identify significant main effects of smoking and no significant group × smoking interactions. After introducing smoking as additional factor, all group differences reported above remained statistically significant, except the overall percentage of $CD8^+$ T lymphocytes ($p = 0.21$), the percentage of $CD3^+$ T_{EM} cells ($p = 0.06$) and the *ex vivo* proliferation response ($p = 0.16$).

4. Discussion

In the present study, we characterize phenotypic changes in T lymphocyte subsets in the peripheral blood of severely traumatized PTSD patients compared to non-PTSD individuals. Our results demonstrate that PTSD patients exhibit a profound reduction in the percentage of $CD3^+$ naïve T lymphocytes, accompanied by an increased proportion of central (T_{CM}) and effector memory (T_{EM}) cells. Interestingly, to a smaller, albeit not statistically significant extent, this effect could also be observed in trauma-exposed non-PTSD individuals, indicating a cumulative effect of exposure to traumatic stressors on T cell distribution. The reduction in the pro-

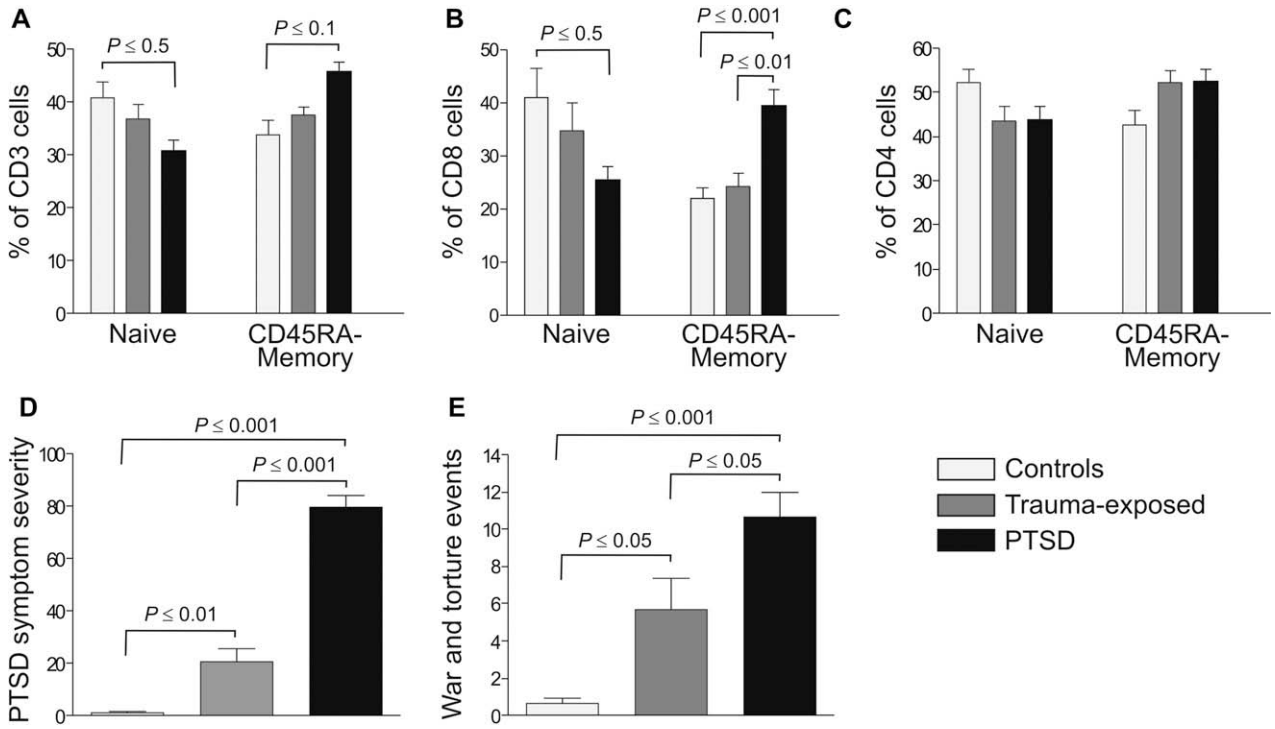

Fig. 2. Cumulative effect of traumatic stress on peripheral T lymphocyte subset distribution. Data are presented as mean percentages + SEM of naive and $CD45RA^-$ memory subsets within the total (A) $CD3^+$, (B) $CD8^+$ and (C) $CD4^+$ population in PTSD patients, as well as trauma-exposed and control individuals. (D) PTSD symptom severity, (E) number of experienced war and torture event types.

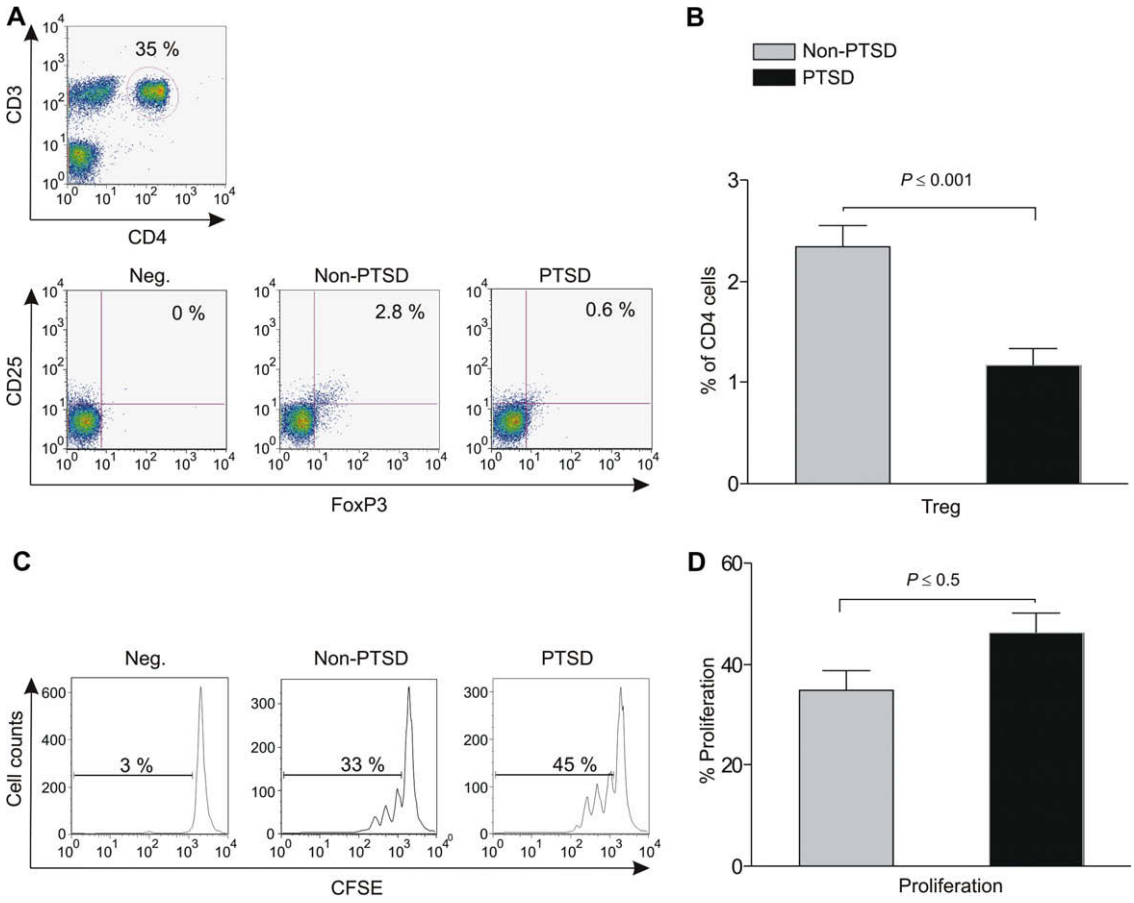

Fig. 3. PTSD patients exhibit a lack of peripheral T_{regs} and increased *ex vivo* T cell proliferation. (A) Representative flow cytometric analysis of $CD4^+CD25^+FoxP3^+$ T_{regs}. (B) Mean percentages + SEM of peripheral T_{regs} in PTSD vs. non-PTSD individuals. The negative control (Neg.) represents gated $CD4^+$ cells without intracellular FoxP3 staining. (C) Representative proliferation profile of PBMCs after *ex vivo* stimulation with anti-CD3 or without stimulation (Neg). (D) Mean percentages + SEM of proliferation response in PTSD vs. non-PTSD individuals.

portion of naïve and the increase of T_{EM} cells were most pronounced within the $CD8^+$ T cell population, whereas $CD4^+$ T cells were not significantly altered. Furthermore, the percentage of regulatory T cells was reduced by 48% in PTSD patients compared to non-PTSD individuals. Functionally, these alterations were accompanied by a significantly enhanced proliferation of anti-CD3 stimulated T cells *ex vivo*. These stress-related alterations of the peripheral T cell compartment might constitute a key factor in the enhanced susceptibility of persons with PTSD to a range of physical diseases.

More specifically, it has been observed that a shrinking repertoire of naïve T cells may correlate with an enhanced susceptibility to infectious diseases. Therefore, we propose that the reduction of the naïve $CD8^+$ T cell pool in PTSD patients could compromise their ability to mount an effective T cell response to various pathogens and thus might be a key factor in the enhanced susceptibility to infectious diseases. This impairment has been confirmed in immunocompromised individuals such as elderly persons where the progressive loss of naïve T lymphocytes is known to be a major reason for the increased risk for age-related diseases (Fagnoni et al., 2000; Shen et al., 1999). Moreover an accumulation of $CD45RA^-$ effector memory cells is characteristic of an aging immune system (Hong et al., 2004) and thus is consistent with other reports showing that psychological stress is associated with immunological aging (Bosch et al., 2009; Epel et al., 2004; Kiecolt-Glaser et al., 2003).

Interestingly, the enhanced proportion of memory cells in PTSD patients only occurred within the $CD45RA^-$ memory pool, i.e., in the T_{EM} and T_{CM} subpopulations, being most prominent in the T_{EM} population. In contrast, the T_{EMRA} population, which re-expresses the CD45RA isoform, did not differ between PTSD patients and control individuals. CD45RA is a high molecular weight isoform of the receptor-type protein tyrosine phosphatase CD45, also known as the common leukocyte antigen, which is required for the regulation of signal transduction pathways involved in T cell activation. $CD45RA^+$ memory cells functionally differ from the $CD45RA^-$ memory pool by their predominantly high lytic potential, their very low expansion potential and their increased sensitivity to apoptosis (Sallusto et al., 2004).

In accordance with our finding of an increased percentage of $CD45RA^-$ memory T cells in the PTSD group, enhanced T cell mediated memory responses to various pathogens, as measured by delayed-type hypersensitivity (DTH) reaction, have been reported in PTSD patients in earlier studies (Altemus et al., 2006; Boscarino, 2004).

The most striking alterations appear in the percentage of peripheral regulatory T cells (T_{regs}), with almost a 50% reduction in PTSD patients compared to non-PTSD individuals. T_{regs} play a pivotal role in maintaining self-tolerance and are essential for the suppression of autoimmune diseases. Deficiency or dysfunction of T_{regs} in humans has been linked to several inflammatory and autoimmune diseases including multiple sclerosis, asthma, type 1 diabetes, psoriasis, and rheumatoid arthritis (Costantino et al., 2008). We therefore propose that the percental reduction of T_{regs} in the blood of individuals with PTSD reported here could be related to the increased risk of PTSD patients for autoimmune diseases in general, and for rheumatoid arthritis, psoriasis, hypothyroidism,

and diabetes in particular (Boscarino, 2004; Kimerling, 2004; Sareen et al., 2005; Weisberg et al., 2002).

In addition, T_{regs} are crucial players in controlling both inflammation and virus-specific T lymphocyte responses. During acute and chronic infections, T_{regs} suppress inflammation to limit immunopathological side effects of inflammation (Mills, 2004). The substantial reduction in the percentage of peripheral T_{regs} in individuals with PTSD could bear the risk of excessive inflammation due to suboptimum control of the immune response. This view is supported by studies reporting enhanced levels of proinflammatory cytokines in PTSD patients (Wessa and Rohleder, 2007).

Assuming that the increased memory population might be accompanied by an altered T cell proliferation capacity, we analyzed the proliferation response of T lymphocytes *ex vivo* after stimulation with anti-CD3 mAb. We found significantly increased proliferation of PBMCs isolated from blood of PTSD patients compared to non-PTSD individuals. It has been shown that memory T cells exhibit a lower activation threshold and a higher proliferative capacity after in vitro stimulation (Sallusto et al., 1999), thus it is possible that the enhanced ratio of memory T cells found in PTSD patients is responsible for the augmentation in T cell proliferation. Recently, it has been proposed that T_{regs} are involved in the suppression of naïve and memory T cell proliferation, thereby altering the quantity of the memory T cell pool (Murakami et al., 2002). Therefore the increased proliferation capability of T lymphocytes in response to T cell receptor (TCR)-triggering could be associated with the reduced proportion of T_{reg} cells since the latter have been shown to inhibit naïve and memory T cell proliferation (Murakami et al., 2002; Piccirillo and Shevach, 2001; Shen et al., 2005).

Whether the observed changes in the distribution of T cell maturation subsets are due to alterations in the thymic output of naïve T cells or peripheral T cell turnover needs to be established in future studies.

Neuroendorine profiles of individuals with PTSD show anomalies, characterized by elevated norepinephrine levels (Southwick et al., 1999). With respect to cortisol, result are more heterogeneous with studies reporting lower (Yehuda, 2001), normal (Eckart et al., 2009), or even higher (Lindauer et al., 2006) levels in individuals with PTSD. Since lymphocytes express both glucocorticoid receptors and functional adrenergic receptors (McEwen et al., 1997; Nance and Sanders, 2007), it might be speculated that an altered neuroendocrine profile could have led to the observed changes in the immune outcomes in PTSD. Future studies should attempt to answer the question of whether T cells subsets differ in their sensitivity to stress hormones, possibly by differentially expressing adrenergic or glucocorticoid receptors.

Given the considerable prevalence of traumatic stress, and in particular the high prevalence of PTSD in populations affected by conflict, terror and combat (Neuner and Elbert, 2007; Neuner et al., 2004), the results of this study are of high societal and economic relevance for health care. A considerable body of clinical investigations has revealed that a variety of therapeutic interventions may effectively reduce trauma-related mental suffering (National Collaborating Centre for Mental Health, 2005). In a recent study, we demonstrated that successful treatment – in this case by means of Narrative Exposure Therapy – also significantly reduced cough, diarrhoea, and fever (Neuner et al., 2008). This leads us to suggest that successful psychotherapeutic intervention may improve immune function, possibly through alterations of the T cell compartment. Given the importance of these associations for a broad range of trauma-affected individuals from victims of violence and abuse to peacekeeping forces and rescue workers, more attention should be given to the potential for improving physical, in addition to mental health, through trauma treatment.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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