Eur Arch Psychiatry Clin Neurosci (2010) 260:509–518
DOI 10.1007/s00406-010-0098-x

ORIGINAL PAPER

Acute schizophrenia is accompanied by reduced T cell and increased B cell immunity

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Received: 1 August 2009 / Accepted: 8 January 2010 / Published online: 27 January 2010

Abstract Previous studies of lymphocyte distribution in schizophrenia have yielded inconsistent results, as summarized in the present study. Based on our own original data, potential confounds that might explain these variations are analyzed and discussed. Blood samples from 26 patients with acute paranoid schizophrenia were investigated in comparison with 32 matched healthy controls by flow cytometry (CD3, CD4, CD8, CD19, and CD56 phenotyping). A subgroup of drug-free patients was followed up after 6 weeks of treatment. Cotinine levels and the free cortisol index (FCI) were provided in order to control for medication, smoking, and stress. Cotinine levels correlated with natural killer (NK) cell counts ($CD3^-/CD56^+$: $r = -0.383$, $P = 0.003$) while the FCI was related to B cell numbers ($CD19^+$: $r = 0.390$, $P = 0.003$). Considering these covariates, a lower level of T helper cells ($P = 0.010$), a reduced CD4/CD8 ratio ($P = 0.029$), and elevated B cells ($P = 0.008$) were found during acute psychosis. After 6 weeks of medication, an inverse pattern was observed in initially drug-free patients: total T cell ($P = 0.005$), T helper ($P = 0.003$), and T suppressor/cytotoxic cells ($P = 0.005$) increased, while B cell counts declined ($P = 0.049$). In conclusion, acute paranoid schizophrenia may be accompanied by a reduced T cell defense and a shift towards B cell immunity, which normalizes in response to treatment. In addition to disease stage or subtype and medication, cigarette smoking and stress are important co-factors.

Keywords Schizophrenia · Flow cytometry · Lymphocytes · Medication · Smoking · Stress · Review

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Introduction

Apart from hypotheses of impaired neurodevelopment and neurogenesis [6, 27], the complexity of schizophrenia has blossomed into countless theories attempting to explain the pathophysiology of the disorder. Among these etiological explanations, several immune-related hypotheses have been proposed implying infectious as well as autoimmune-related mechanisms [7, 12, 37, 49, 50, 56]. Mediators of the immune system, especially cytokines, are known to be in close communication with the central nervous system via the hypothalamo-pituitary–adrenal axis (HPA), which in turn activates the release of neurotransmitters and hormones [36]. There is indeed evidence for schizophrenia-related alterations in cytokine expression, as recently

reviewed by Potvin et al. [38], but cellular components of the immune response may be likewise affected. Therefore, several studies have focused on studying the numerical distribution of lymphocyte subsets in schizophrenia by flow cytometry [2, 14, 18, 30–33, 39, 42–45, 47]. However, the results were quite inconsistent, as summarized in the "Discussion" and Table 1.

Part of this variation might be explained by different methodological approaches (e.g., the analysis of Ficoll-separated peripheral blood mononuclear cells versus whole-blood assays, or the usage of different surface markers for phenotyping) and the inclusion of subjects with different schizophrenia subtypes or disease stages. However, studies should also account for additional confounding factors such as medication, cigarette smoking, cannabis abuse, or stress. Several lines of evidence suggest that antipsychotic drug treatment has an impact on the immune system. Sperner-Unterweger et al. [47] and Maino et al. [30] observed a normalization of all measured alterations in lymphocyte distribution after successful treatment of psychotic episodes. The effect of antipsychotic medication on cytokine levels in schizophrenia has been reviewed by Drzyzga et al. [11]. The effect of smoking appears to be important as well because nicotine abuse among schizophrenia patients is approximately three times that of the general population, reaching a prevalence of about 80% [24]. Cigarette smoking has suppressive effects on the immune system [20, 23]. Nicotine, the major active chemical in cigarettes, has been shown to impair the antigen-driven T cell response and inhibit proliferation in rodents as well as humans [13, 15, 16, 25]. Stress-associated changes in immunity are another possible explanation for changes in lymphocyte distribution in acutely ill schizophrenic patients. Two large meta-analyses revealed an association of naturally occurring chronic stressors with an overall leukocytosis, which manifests as a relative neutrophilia with reduced numbers of T helper, T cytotoxic/suppressor cells, natural killer (NK) cells and B cells [19, 59]. In addition, diminished antibody production has been observed in chronic stress [52].

Different methods to assess the confounding effects of nicotine intake and stress in clinical studies have been described. Self-reported information about smoking (e.g., number of cigarettes per day) or stress [documented by visual analog scales (VAS)] is often imprecise and subject to bias, with accuracy varying according to circumstances. The trier inventory for the assessment of chronic stress (TICS) may be helpful for assessing chronic lifetime stressors in more detail [46]. However, neither the TICS nor other stress inventories have been validated yet for use in schizophrenic subjects. A biochemical assessment gives a much clearer indication of the effects of tobacco intake and stress. Previous studies indicate that cotinine levels serve as a useful biomarker of tobacco exposure [5]. Cotinine is a metabolite of nicotine and has an in vivo half life of approximately 20 h. Nicotine from food products is not expected to impact assay sensitivity or to be clinically relevant. A blood cotinine concentration of 10 ng mL^{-1} is employed as a cutoff for non-smokers versus smokers. Other non-invasive alternatives are collection of urine, saliva, or hair (with suggested cutoffs of 200, 5, and 0.3 ng mL^{-1}, respectively). A rough assessment of HPA activity can be performed by collecting single blood or saliva samples for analysis of total or free cortisol in the early morning hours, and the resulting hormone value is interpreted as an index of unstimulated HPA activity [55]. In blood samples, the free cortisol index (FCI) is a reliable surrogate marker for free cortisol, which is the biologically active component and has an in vivo half life of 60–90 min [28]. It is calculated by dividing the serum total cortisol [nmol/L] by the cortisol-binding globulin (CBG) level [mg/L]. Although easy to assess, single basal cortisol measures are reported to have a rather low intraindividual stability; and peak levels of plasma cortisol, and ACTH after direct stimulation [by corticotropin releasing hormone (CRH) application] are more reliable for the assessment of the HPA axis [9, 22]. However, CRH tests are quite expensive and may be difficult to perform in acutely psychotic patients, because the procedure is more time consuming than a single blood or saliva sampling. Another stress component, the acute stress response with sympathetic activation of the adrenal medulla, can be assessed by the measurement of catecholamines and metanephrines in urine. This can be done by collecting urine for 24 h or by expressing the measures as ratios to urine creatinine concentration [57]. However, several types of drugs and food can alter urine catecholamine levels, e.g., theophylline, erythromycin, lithium, salicylates, coffee, tea, bananas, chocolate, citrus fruits, and vanilla. Moreover, it is sometimes difficult to perform 24 h urine collection, or to get urine from acutely psychotic patients at a time point which is close to the blood collection.

Our study aimed to examine T and B cell immunity while controlling for important confounding factors: (1) Only acutely ill patients with paranoid subtype schizophrenia were included. (2) Medication effects were assessed by including a subgroup of drug-free cases that was followed for 6 weeks of drug treatment. Furthermore, cumulative drug dosage was included as a covariate in the statistical analysis. (3) Subjects with substance abuse (e.g., alcohol or cannabis) were excluded. (4) Nicotine consumption was assessed using cotinine blood levels, which were included as a covariate in the statistical analysis. (5) To control for prevailing hypothalamo-pituitary–adrenal stress axis activation, we chose the FCI as a simple but quite reliable and objective estimate.

Table 1 Flow cytometric studies on the numerical distribution of lymphocyte subsets in the peripheral blood of patients with schizophrenia (in chronological order)

Author(s) [results were controlled for …]	Sz sample size (drug-free/medicated)	Sz disease phase (acute/stable)	C sample size	Marker(s)	Lymphocytes subsets	Results *P*-value(s)	Effect size (*d*)
McAllister et al. [33] [none of the potential confounds]	0/34	?/? (34)	33	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	nsd	
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
				CD19	B cells	nsd	
				$CD19^+/CD5^+$	B1a subset of B cells	Increased in 30% of Sz patients ($P < 0.05$)	1.65
Henneberg et al. [18] [drug-effects]	26/4	30/0	30	CD11	T cells	Increased in acute Sz versus C ($P < 0.05$)	0.62
				$CD11^+/CD4^+$	T helper cells	Increased in acute Sz versus C ($P < 0.05$)	0.57
				$CD11^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
Ganguli and Rabin [14] [drug-effects, substance abuse]	?/? (116)	?/? (116)	166	$CD19^+$	B cells	nsd	
				$CD19^+/CD5^+$	B1a subset of B cells	Decreased in Sz versus C ($P < 0.01$)	−0.47
Sasaki et al. [44] [drug-effects]	3 (drug-naïve)/11	14/0	20	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	nsd	
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
				$CD3^+/CD25^+$	Activated T cells	Increased after 4 weeks of treatment in Sz versus C ($P < 0.01$)	1.20
				$CD3-/CD56^+$	NK cells	Increased in acute Sz versus C ($P < 0.01$)	1.61
				$CD20^+$	B cells	nsd	
Schattner et al. [45] [drug-effects]	23 (at least 2 weeks)/0	11/12	16	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	nsd	
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
Rothermundt et al. [42] [drug-effects]	4/40	44/0	44	$CD3^+$	T cells	Decreased in acute Sz (compared to reference range)	#
				$CD3^+$/HLA-DR^+	Activated T cells	Within reference range	
				$CD19^+$	B cells	Increased in acute Sz (compared to reference range)	#
Printz et al. [39] [none of the potential confounds]	0/29	0/29	30	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	nsd	
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
				$CD20^+$	B cells	nsd	
				$CD20^+/CD5^+$	B1a subset of B cells	Increased in stabile Sz versus C (trend, $P < 0.09$)	0.56

Table 1 continued

Author(s) [results were controlled for …]	Sz sample size (drug-free/ medicated)	Sz disease phase (acute/stable)	C sample size	Marker(s)	Lymphocytes subsets	Results *P*-value(s)	Effect size (*d*)
Sperner-Unterweger et al. [47] [drug-effects, substance abuse]	21 (drug-naïve)/35	56/0 (21 first-episode)	16	$CD3^+$	T cells	Increased in drug-free first-episode Sz versus C ($P < 0.05$)	0.69
				$CD3^+/CD4^+$	T helper cells	Increased in drug-free first-episode Sz versus C ($P < 0.05$)	0.66
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
				$CD3^-/CD16^+$	NK cells	Decreased in Sz relapse versus C ($P < 0.02$)	−0.79
						All significant changes normalized during therapy	
Mazzarello et al. [32] [drug-effects, substance abuse]	0/24	0/24	5	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	Increased $CD4^+/CD8^+$ ratio in stabile Sz versus C ($P < 0.03$)	#
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	Decreased in stabile Sz versus C ($P < 0.01$)	#
				?	NK cells	nsd	
				?	B cells	nsd	
Rudolf et al. [43] [none of the potential confounds]	1/30	?/? (31)	31	$CD3^+$	T cells	nsd	
				$CD3^+/CD25^+$	Activated T cells	nsd	
				$CD3^+/HLA\text{-}DR^+$	Activated T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	nsd	
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
				$CD3-/CD56^+$	NK cells	nsd	
				$CD19^+$	B cells	nsd	
Maino et al. [30] [drug-effects]	40 (at least 3 months)/0	40/0	20	$CD3^+$	T cells	Decreased in acute Sz versus C ($P < 0.03$)	−0.61
				$CD19^+$	B cells	Increased in acute Sz versus C ($P < 0.02$)	0.48
						All significant changes normalized during therapy	
Baskak et al. [2] [drug-effects, substance abuse]	14 (at least 6 months)/0	14/0	14	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	nsd	
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd in whole group, decreased in 11 drug-naïve acute Sz versus C ($P < 0.02$)	−0.83
Maxeiner et al. [31] [none of the potential confounds]	?/? (12)	?/? (12)	No controls	$CD4^+$	T helper cells	nsd	
				$CD8^+$	T suppressor/cytotoxic cells	nsd	

Table 1 continued

Author(s) [results were controlled for …]	Sz sample size (drug-free/ medicated)	Sz disease phase (acute/stable)	C sample size	Marker(s)	Lymphocytes subsets	Results *P*-value(s)	Effect size (*d*)
Steiner et al. (the present study) [drug-effects, smoking, substance abuse, stress]	11 (at least 6 weeks)/15	26/0	32	$CD3^+$	T cells	nsd	
				$CD3^+/CD4^+$	T helper cells	Decreased in acute Sz versus C ($P < 0.05$)	−0.74
				$CD3^+/CD8^+$	T suppressor/cytotoxic cells	nsd	
				$CD3^-/CD56^+$	NK cells	nsd	
				$CD19^+$	B cells	Increased in acute Sz versus C ($P < 0.01$)	0.87

Sz Schizophrenia, *C* control subjects, *CD* cluster of differentiation, *NK* natural killer, *nsd* no significant differences in cell distribution

Cohen's $d \geq 0.2$ is indicative of a small effect, ≥ 0.5 a medium and ≥ 0.8 a large effect size

Calculation of effect size was not possible, because necessary data are not shown in the *manuscript*

Materials and methods

Blood samples

Serum and heparinized peripheral blood were obtained from 26 patients with an acute episode of paranoid schizophrenia and 32 age- and sex-matched healthy control subjects (students and staff from the University of Magdeburg). Demographic data are summarized in Table 2. Eleven patients were unmedicated for at least 6 weeks before admission, and 15 had already been taking atypical antipsychotics (amisulpride, aripiprazole, clozapine, olanzapine, quetiapine, risperidone, or ziprasidone) for 26 ± 21 days, but still suffered from acute psychosis. Only benzodiazepines were allowed as additional psychotropic medication (for 6 days before T0). All patients fulfilled the DSM-IV criteria for paranoid schizophrenia, as diagnosed by two independent psychiatrists (JS, HB) using all available information from structured clinical interviews for DSM-IV (SCID) and clinical records [1, 53].

Serum and heparinized peripheral blood were collected between 9:00 and 11:00 a.m. The blood specimens from schizophrenic patients were taken on admission to the hospital (T0); a second sample was obtained from initially drug-free patients after 6 weeks of antipsychotic treatment (T6). In all subjects a complete differential blood cell count with differential, high-sensitivity C-reactive protein (CRP), and liver enzymes were measured. In addition, a urine drug screen was performed. For statistical purposes, the dose of antipsychotic drugs was converted into cumulative chlorpromazine-equivalent doses (Σ CPZe) [40, 54]. Psychopathology was monitored using the positive and negative syndrome scale (PANSS) for schizophrenia [26].

Blood samples were obtained in accordance with German laws and the guidelines of the local institutional review board. Patients and control subjects gave written informed consent. Subjects with a previous medical history of allergy, (auto)immune disease, cancer, chronic terminal disease, coronary heart disease, diabetes/metabolic disorder, hypertension, infections, neurological illness, substance abuse, or severe trauma were excluded. Elevated CRP levels, treatment with corticosteroids or immunosuppressive and immunomodulating agents were also exclusion criteria. Only control cases without a past or family history of neuropsychiatric disorders were included.

Flow cytometry

Phenotypic analyses were performed by multicolor flow cytometry. Ficoll-Hypaque-separated cell samples were surface-stained utilizing directly labeled monoclonal antibodies with the following specificities: CD3 peridinin chlorophyll protein, CD4 allophycocyanin, CD8 phycoerythrin, CD19

Table 2 a Demographic data and T0 measures of comparison subjects and patients with paranoid schizophrenia, b the subgroup of initially drug-free patients was followed up after 6 weeks of treatment (T0 and T6)

(a) Diagnosis	Comparison group T0 ($n = 32$)	Schizophrenia group T0 ($n = 26$)
Age (year)	34.4 ± 10.8	34.7 ± 11.3
Duration of disease (year)	–	8 ± 9
Males/females (*n*)	20/12	17/9
Smokers/non-smokers (*n*)	14/18	16/10
Measures	T0	T0
PANSS total score	–	84.8 ± 11.2
PANSS positive score	–	20.1 ± 4.9
PANSS negative score	–	22.1 ± 6.5
PANSS general score	–	42.7 ± 5.6
T cells: $CD3^+$ (cells/μL)	1,396 ± 532	1,211 ± 503
T helper cells: $CD3^+/CD4^+$ (cells/μL)	973 ± 396	710 ± 295
T suppressor/cytotoxic cells: $CD3^+/CD8^+$ (cells/μL)	410 ± 177	332 ± 199
NK cells: $CD3^-/CD56^+$ (cells/μL)	396 ± 170	540 ± 221
B cells: $CD19^+$ (cells/μL)	195 ± 109	300 ± 238
(b) Diagnosis	Schizophrenia group, drug-free T0 ($n = 11$)	Schizophrenia group T6 ($n = 11$)
Age (year)	35.1 ± 13.2	
Duration of disease (year)	8 ± 10	
Males/females (*n*)	7/4	
Smokers/non-smokers (*n*)	6/5	
Measures	T0	T6
PANSS total score	90.8 ± 10.8	64.2 ± 21.0
PANSS positive score	24.3 ± 5.4	13.6 ± 4.2
PANSS negative score	21.7 ± 5.6	16.5 ± 8.2
PANSS general score	44.8 ± 6.0	34.0 ± 9.1
T cells: $CD3^+$ (cells/μL)	1,063 ± 612	1,425 ± 608
T helper cells: $CD3^+/CD4^+$ (cells/μL)	621 ± 358	956 ± 392
T suppressor/cytotoxic cells: $CD3^+/CD8^+$ (cells/μL)	280 ± 254	492 ± 259
NK cells: $CD3^-/CD56^+$ (cells/μL)	461 ± 261	496 ± 314
B cells: $CD19^+$ (cells/μL)	364 ± 257	218 ± 121

Values are given as mean ± standard deviation

phycoerythrin, CD56 allophycocyanin (Becton–Dickinson, Heidelberg, Germany). The procedure was performed in 96-well U-bottom plates by incubating 3×10^5 cells $well^{-1}$ with the appropriate antibodies for 20 min at 4°C. Antibodies were used at concentrations as recommended by the manufacturer. Nonspecific binding was eliminated by mixing the samples with a 1:5 solution of commercial human IgG (Octagam, Octapharma, Langenfeld, Germany). Samples were washed three times in phosphate-buffered saline/0.1% bovine serum albumin at $300 \times g$ for 3 min by exchanging 200 μL buffer between each of the centrifuging steps. After the last removal of supernatants, cells were resuspended in 50 μL 4% paraformaldehyde solution. The plates were incubated at room temperature in the dark for 10 min. Finally, the cells were washed three times as described earlier and subjected to flow cytometry. At least 10,000 cells per sample were analyzed using a dual-laser cytometer (FACS Calibur, Becton–Dickinson, Heidelberg, Germany) with Cell Quest Pro 3.0 or Summit 4.3 (Dako, Hamburg, Germany) software.

Biochemical analysis

Cotinine was quantified by direct enzyme-linked absorbent assay (Calbiotech, Spring Valley, CA, USA) following the manufacturer's protocol. The inter- and intra-assay coefficient of variation was <15%. In brief, 10 μL of serum and cotinine standard were added in duplicate to each well and incubated with 100 μL cotinine enzyme conjugate for 1 h at room temperature. Wells were washed with distilled water before addition of 100 μL tetramethylbenzidine (TMB) substrate solution. This was incubated for 30 min at room temperature before the reaction was stopped with stop solution. Plates were read at dual wavelengths of 450 and 650 nm on a plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of cortisol was performed with the Immulite-System (DPC Diagnostic Products Corporation, Los Angeles, CA, USA), a fully automatic random access chemiluminescence enhanced enzyme immunoassay system. CBG was determined by a commercial radioimmunoassay (ZenTech S.A., Angleur, Belgium).

Statistical analysis

Statistical analyses were performed with the SPSS 15.0 package (Statistical Product and Service Solutions, Chicago, USA). Parametric tests were applied due to a normal distribution of the data, as indicated by Kolmogorov–Smirnov-tests. Cumulative drug dosage (Σ CPZe), cotinine levels, and the FCI were included as covariates in the following analysis of variance (ANCOVA). We explored changes in T and B cell immunity at T0. The Holm–Bonferroni correction was used for multiple comparisons. Based on these results, we expected a normalization of (1) diminished T helper cell counts and (2) elevated B cell counts during reconvalescence (T6). A repeated measures ANCOVA was conducted for the comparison of T0 and T6 measures. *T*-tests were applied, if no influence of the covariates was observed in the ANCOVA (*P* of covariates >0.1). All statistical tests were two-tailed, with significance defined as $P < 0.05$.

For a systematic meta-analysis, effect sizes were calculated according to Cohen [8]. Cohen's *d* is defined as the difference between two means divided by the pooled standard deviation for those means.

Results

Results at T0

No significant differences were observed between the diagnostic groups regarding age ($T(1,56) = 0.109$, $P = 0.914$), gender ($\chi^2(1,58) = 0.052$, $P = 0.820$), and smoking habits ($\chi^2(1,58) = 1.818$, $P = 0.178$).

The numerical distribution of lymphocyte subsets at T0 was not related to age, sex, duration of disease, or severity of clinical symptoms (PANSS). However, cotinine levels correlated with NK cell numbers ($CD3^-/CD56^+$: $r = -0.383$, $P = 0.003$), and the FCI was related to B cell counts ($CD19^+$: $r = 0.390$, $P = 0.003$). ANCOVA also revealed an influence of smoking on NK cell numbers (cotinine: $F(1,56) = -4.262$, $P = 0.044$), while B cell numbers were associated with stress level (FCI: $F(1,56) = 7.799$, $P = 0.007$). No further influence of the covariates Σ CPZe, cotinine, and FCI was observed.

The numerical distribution of total T cells and T suppressor/cytotoxic cells was not significantly diagnosis-dependent on admission ($CD3^+$: $F(1,53) = -3.388$, $P = 0.071$; $CD3^+/CD8^+$: $F(1,53) = -1.002$, $P = 0.321$), but T helper cell counts were lower in the schizophrenia group ($CD3^+/CD4^+$: $F(1,53) = -7.172$, $P = 0.010$, Cohen's $d = -0.74$; Fig. 1a). Moreover, the CD4/CD8 ratio was significantly decreased (ratio in controls: 1.45 ± 0.56; ratio in schizophrenic patients: 1.03 ± 0.60; $F(1,53) = -5.054$, $P = 0.029$). The number of NK cells was not significantly different on admission ($CD3^-/CD56^+$: $F(1,53) = 3.277$, $P = 0.076$), but B cells were significantly increased ($CD19^+$: $F(1,53) = 7.597$, $P = 0.008$, Cohen's $d = 0.87$; Fig. 1a).

These changes in lymphocyte distribution during acute psychosis were confirmed by analyzing the subgroup of drug-free patients (reduced T helper cell counts: $F(1,38) = -4.959$, $P = 0.032$; increased B cell counts in the schizophrenia group: $F(1,38) = 3.956$, $P = 0.045$). ANCOVA revealed an influence of smoking on NK cell numbers (cotinine: $F(1,38) = -4.513$, $P = 0.040$), while B cell counts were associated with the stress level (FCI: $F(1,38) = 9.114$, $P = 0.005$).

Changes between T0 and T6

The numerical distribution of lymphocyte subsets at T6 was not significantly related to the improvement of clinical symptoms (PANSS), nor was any correlation observed with cotinine or FCI. Similarly, ANCOVA revealed no influence of these covariates.

Psychopathology improved significantly from T0 to T6 (PANSS total: $T(1,25) = 3.946$, $P = 0.001$; PANSS positive: $T(1,25) = 3.842$, $P = 0.001$; PANSS negative: $T(1,25) = 2.965$, $P = 0.007$; PANSS general: $T(1,25) = 3.169$, $P = 0.004$; Table 2). Several changes were observed over the time course of patients who were untreated at T0: Total T cell, T helper and T suppressor/cytotoxic cell counts were increased ($CD3^+$, $T(1,10) = 3.567$, $P = 0.005$; $CD3^+/CD4^+$, $T(1,10) = 3.821$, $P = 0.003$; $CD3^+/CD8^+$,

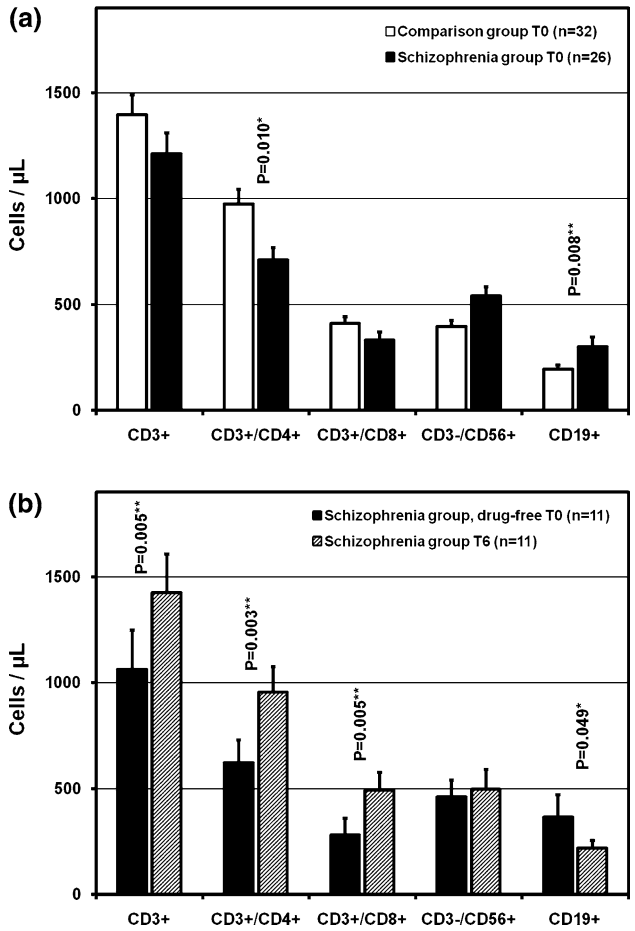

Fig. 1 **a** Differences in numerical distribution of lymphocyte subsets in healthy subjects and individuals with acute paranoid schizophrenia at T0. **b** The subgroup of initially drug-free patients was followed up after 6 weeks of treatment (T0 and T6). *Annotation:* Data are given as mean ± SEM (Standard error of mean), $*P < 0.05$, $**P < 0.01$

$T(1,10) = 3.610$, $P = 0.005$; Fig. 1b), while B cell counts decreased ($CD19^+$, $T(1,10) = -2.238$, $P = 0.049$; Fig. 1b). The CD4/CD8 ratio was not significantly altered.

Discussion

The aim of this study was to investigate the distribution of lymphocyte subsets in acute paranoid schizophrenia, controlling for a set of important confounding factors, namely medication, cigarette smoking, and hypothalamo-pituitary–adrenal stress axis activation. In summary, lower T helper cell numbers were evident during acute psychosis when controlling for the aforementioned confounding factors. They were accompanied by a reduced CD4/CD8 ratio and a psychosis-related increase in B cell counts. Acutely ill medicated schizophrenia cases (T0) showed T- and B-cell counts which were intermediate between those of unmedicated schizophrenia cases and controls (data not shown). This finding may speak in favor of a normalizing effect of treatment. Indeed, after 6 weeks of antipsychotic treatment (T6), a normalized pattern was observed in initially drug-free patients: total T cell as well as T helper and T suppressor/cytotoxic cell numbers were increased while B cell counts decreased. Nicotine consumption correlated negatively with NK cell counts, while stress was related to elevated B cell numbers.

As mentioned in the introduction, results of previous flow cytometric studies have been quite heterogeneous (see Table 1). Some studies are in accordance with our results and describe reduced T cell numbers [2, 30, 32, 42]. Nevertheless, other groups reported unchanged T cell numbers [31, 33, 39, 43–45], and some investigators observed even higher T cell counts in the peripheral blood of acutely ill schizophrenic patients [18, 47].

The present evidence of increased total B cell numbers in acutely ill schizophrenic patients supports the reports of Maino et al. [30] and Rothermundt et al. [42]. Other studies, which describe unchanged or lower total B cell counts in schizophrenia, had investigated patients on medication in stable disease phases [14, 32, 33, 39, 43], similar to our results after 6 weeks of successful antipsychotic treatment. This indicates that in previous studies the inclusion of patients in different disease stages and medication status contributed to the inconsistent results. Furthermore, including subjects with different schizophrenia subtypes and using different surface markers for lymphocyte phenotyping have probably contributed to the heterogeneity of the previous findings (Table 1). Over and above the aforementioned influences, neuroleptic medication may have interfered with the results, e.g., via lymphocytic D2, D3, D4 and D5, muscarinic, and nicotinic acetylcholine, 5-HT1A, 5-HT2, or 5-HT5A serotonin receptors [17]. Interaction of dopamine with D2 and D3 dopamine receptors is known to activate the adhesion of T cells via β1-integrin, promoting the extravasation of blast-like T cells into the central nervous system [21, 29, 51]. This mechanism can be suppressed by neuroleptic drugs, which seem to contribute to a normalization of schizophrenia-related changes in peripheral blood T and B cell distribution [3, 29, 30].

Our results support the findings of Riedel et al. [41], who observed a reduced type-IV (T cell dependent) delayed skin hypersensitivity reaction after intracutaneous application of antigens in drug-free schizophrenic patients. Moreover, Craddock et al. [10] showed lower proliferative responses in T cells in both unmedicated and minimally medicated schizophrenia patients as compared with controls. Analogous to HIV infections and in line with the infectious theory of schizophrenia, a reduced CD4/CD8 ratio, as we found it in acutely ill schizophrenic patients,

may promote the reactivation of Herpes virus and Toxoplasma gondii infections [56]. Furthermore, there is evidence for a shift from the cellular toward the humoral immune system in schizophrenia, arising from studies that document an increased prevalence of antiviral antibodies and autoantibodies in schizophrenia, e.g., against CMV or cholinergic muscarinic receptors [4, 48, 56]. The increased B cell numbers during acute psychosis in the present and previous studies [30, 42] distinctly support a link between acute schizophrenia and increased B cell immune activity.

Apart from disease stage/subtype and medication, confounding factors such as cigarette smoking stress are probably partially responsible for the heterogeneity of previous results: We observed an influence of nicotine consumption on NK cell numbers. This is in line with previous reports of alterations in NK cell number and activity due to cigarette smoking [34, 35, 58]. With respect to the effects of stress, our study yielded a positive correlation between the current stress level (as assessed by the FCI) and B cell counts. In contrast, publications on chronic stress found reduced numbers of T helper cells, T cytotoxic/suppressor cells, NK cells, and B cells along with a diminished antibody production [19, 52, 59].

The present study has certain limitations that need to be taken into account: (1) Even though we observed strong effects in the patient group, the findings should be reproduced in larger clinical samples, particularly focusing on drug-naïve schizophrenia cases. (2) The specificity of the observed immunological changes has to be evaluated by future investigations, including cases with other neuropsychiatric disorders.

In conclusion, acute paranoid schizophrenia may be accompanied by a reduced T cell defense and a shift towards B cell immunity. These changes normalize in response to treatment. Apart from disease stage/subtype and medication, confounding factors such as cigarette smoking, cannabis abuse, and stress have to be considered because they probably account for a major part of the heterogeneity of previous flow cytometric studies on lymphocyte distribution in schizophrenia.

Acknowledgments This work was supported in part by grants of the Saxony-Anhalt Ministry of Research (Grant No. XN3594O/0405M, N2-OGU) and Stanley Medical Research Foundation (Grant No. 07R-1832) to BB and JS. We thank Hendrik Bielau (HB) for his diagnostic re-assessment of schizophrenia cases and are grateful to Henrik Dobrowolny for his skillful assistance in statistical analysis.

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