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Short title	TH1/TH2 cytokine shift in acute medication-free schizophrenia patients
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Abstract

There is major evidence for the involvement of immunological processes in the pathophysiology of schizophrenia. Especially alterations of T-cell function and activation of the inflammatory response system appear to be linked to schizophrenia. A mild chronic inflammation process has been proposed and repeated findings of altered serum cytokine levels led to the hypothesis of a TH2 shift or cytokine imbalance in schizophrenia. We investigated serum levels of TH1 and TH2 related cytokines in 25 patients suffering an acute schizophrenic episode (all unmedicated, 22 neuroleptica-naïve) at different stages of disorder (18 first episode, FEP; 7 recurrent episode, REP) compared to 25 age and sex matched healthy controls. In patients, we found an increase of the TH2 system cytokines IL-6 (p=0.052) and IL-13 (p=0.039) and a decrease of the TH1 system markers sICAM-1 (p=0.011) and sIL-2R (p=0.063). The effect of a sIL-2R decrease was greater in the FEP subgroup (p=0.01) of patients. We found no group differences in the other investigated immune markers: IL-4, IL-8, TNF-alpha, and Interferon-gamma. Our findings support the notion of a TH1/TH2 imbalance particularly in the acute manifestation phase of schizophrenia. In the long run, this may lead to the identification of cytokine patterns that are applicable as trait or state markers, may be helpful in making or ensuring diagnosis or in monitoring therapy.

Keywords	schizophrenia, cytokines, inflammation, TH1/TH2-shift, first episode, FEP, medication, drug-naïve, neuroleptica-naïve
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Suggested reviewers	Mark Hyman Rapaport, Norbert Müller, Brian Miller, Markus Schwarz, David Goldsmith

Submission Files Included in this PDF

File Name [File Type]

2019-01-09 Cover Letter.docx [Cover Letter]

2019-01-09 Response to reviewers.docx [Response to Reviewers]

2019-01-09 BM SZ FEP REP - Highlights.docx [Highlights]

2019-01-09 BM SZ FEP REP - Highlights Abstract.docx [Abstract]

2019-01-09 Revision BM SZ FEP REP Cyt - manuscript AND suppl mat.docx [Manuscript File]

2019-01-09 BM SZ FEP REP Cytokines matched pairs - conflicts of interest-1.docx [Conflict of Interest]

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To:

Prof. Dr. med. Karl Bechter

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Jena, 2019-01-09

Dear Prof. Karl Bechter,

Thank you very much for considering the manuscript and initiating the review process. We especially would like to thank the reviewers for their very important and insightful comments and recommendations. We revised the manuscript and included additional data and references. We acknowledge that the sample size is small and results must be interpreted carefully. However, we are convinced that our results from the difficult to investigate subgroup of neuroleptica-naïve first episode schizophrenia patients may positively contribute to this field of research. We hope that open questions could be answered and that the revised manuscript will finally match the high standards of your renowned journal. We would be very delighted if you accepted it for publication.

Sincerely,

Berko Milleit

Reviewer 1

This is an important area of research and the authors should be commended on attempting to perform this cross-sectional study. I have the following questions and concerns about the current study:

1. It is rare to have 25 schizophrenic patients that do not have any comorbid psychiatric diagnoses. What were the comorbid psychiatric diagnoses? Also, was the structured interview used to ascertain diagnosis?

Thank you very much for pointing this out. In order to have a sample of acute psychosis schizophrenia patients we had excluded all patients with comorbid alcohol or substance abuse as well as all patients with personality disorder, other psychosis, anxiety disorder and mood disorders. We clarified this by including the procedure in more detail in the text of the manuscript

2.Although the authors contend that they control for smoking, drinking and socioeconomic status, they do not show those data so there is no way to know that this is the case, i.e. how many subjects and controls smoked and what were the values for those who did smoke?

This is also a very important point, thank you very much for asking this question. We included data for smoking, BMI, WBC and highest achieved education in the supplemental material. As can be seen, our patient group was comparable to the general population and had relatively few smokers and a high amount of students, such as our control group.

3.Although there is a table in the supplemental data stating a host of different ranges of assay sensitivity, it is unclear which assay was used for which patient and so it would be nice if that were clarified and also it would be useful to know the interim inter-assay variation for each of the assays that were used.

We included the CV% values in the supplemental material. Only in case of IL-6 and IFN-g data from different tests were pooled to retrieve data for very low concentrations.

4.It would be important for the authors to be a little more concise and rigorous and their description in their cytokines and cytokine receptors and other types of immune mediating receptors. So, for example, cytokine IL-2 receptor is receptor not a cytokine and the sICAM 1 is a receptor not a cytokine. Second, I would be reticent to consider iL-6 to be a TH1 related cytokine but consider it an inflammatory cytokine.

Thank you very much for being clear in this point. We revised the whole manuscript in order to achieve precise language regarding cytokines and other immune-associated molecules.

5. Another challenge is the use of uncorrected data. The authors are looking at multiple different cytokine receptors but are using a p-value of .05. One would anticipate looking at 9 different cytokines or cytokine receptors that one would find some spuriously positive results.   Further, *in the discussion*, the authors tend to conflate results that were significant at a level of > .05 with results that were significant at a level <.05.

Thank you very much for this comment. We discussed this in our group and would like to express our notion that, from our point of view, we did not measure 9 different and independent markers but deliberately selected markers from two, even dependent, systems. Because the results fit into a

plausible pattern, we believe our results are valid. Of course we acknowledge that this is a very small sample. Because it is such a selected subgroup, we think, it could be acceptable not no control for multiple measurements.

6. Another challenge with the paper is the discussion itself. The authors confuse meta-analyses that were done some of which actually looked at first episode versus neuroleptic untreated individuals versus recurrent individuals with single studies and so the discussion is sort of confounded and mixed. Further, there have been a series of studies demonstrating elevations of SlL2 receptor in first episode schizophrenic patients, unmedicated schizophrenic patients as well as medicated schizophrenic patients and so the current citations are rather inadequate.

Again, thank you very much for point this out. We revised the text of this section and included more citations of original studies. We also made it clearer than before that our results on sIL-2R contradict the majority of previous studies. We tried to explain possible differences in the text (different manufacturer of the tests, different preanalytic procedure, different patient selection). Mainly, we think there might be effects of the ongoing disease and medication that leads to finally elevated levels, which might be not the case during first acute episode. Of course, this is highly speculative. Nevertheless, the sIL-2R data from the lab look really robust.

In summary, this is an important area of investigation. The current paper could use some modifications.

We hope that we could address most of your concerns with our modification and hope you will find our paper in the revised form worthwhile for publishing.

Reviewer 2

Milleit D et al.: TH1/TH2 Cytokine shift pronounced in unmedicated first episode as compared to chronic schizophrenia patients.

The authors measured serum levels of TH1 und TH2 related cytokines in 25 patients suffering from an acute schizophrenic episode. 22 patients were neuroleptic-naive. The authors estimated IL-6, IL-13, s ICAM-1 und sIL-2R. Moreover, IL-4, IL-8, TNFa and interferon – g were measured. The authors interpret their findings as TH1/TH2 imbalance.

Allover, this manuscript reflects an interesting topic. The authors are well-known as experts in the field of schizophrenia and immune system. The literature is well chosen.

Thank you very much.

The authors adressed the limitations of the study: The small sample size is one of the shortcomings. On the other hand, the sample of neuroleptic-naive patients is seldom and valuable.

Regarding the statistical analyses, normally a trend to significance is assumed at t<0.01.

Thank you very much for pointing this out. We changed the relevant text.

Exclusion criteria: Acute or inflammatory or autoimmune disease, as well as others. The authors should describe how they measured these criteria.

We clarified this in the text (procedure, medical interviews, temperature, WBC).

The low sICAM1 levels are an intriguing finding. Regarding interferon-g in schizophrenia, the therapeutic use of this molecule in chronic schizophrenia should be mentioned (Grüber et al. J clin. Psychiatry 2014, 75; 1266 – 1267).

Thank you very much for pointing us to this very interesting publication. We gratefully included it in the text.

TH1/TH2 cytokine shift pronounced in unmedicated first episode as compared to chronic schizophrenia patients

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Highlights: In acutely ill antipsychotic-free schizophrenia patients, we found an increase of the TH2 system cytokines IL-6 (p=0.052) and IL-13 (p=0.039) and a decrease of the TH1 system markers sICAM-1 (p=0.011) and sIL-2R (p=0.063). The effect of a sIL-2R decrease was greater in the FEP subgroup (p=0.01) of patients. We found no group differences in the other investigated immune markers: IL-4, IL-8, TNF-alpha, and Interferon-gamma. Our findings support the notion of a TH1/TH2 imbalance particularly in the acute manifestation phase of schizophrenia.

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1 **TH1/TH2 cytokine shift pronounced in unmedicated first episode as compared to chronic**
2 **schizophrenia patients**

3

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8 pathophysiology of schizophrenia. Especially alterations of T-cell function and activation of the
9 inflammatory response system appear to be linked to schizophrenia. A mild chronic inflammation
10 process has been proposed and repeated findings of altered serum cytokine levels led to the
11 hypothesis of a TH2 shift or cytokine imbalance in schizophrenia. We investigated serum levels of
12 TH1 and TH2 related cytokines in 25 patients suffering an acute schizophrenic episode (all
13 unmedicated, 22 neuroleptica-naïve) at different stages of disorder (18 first episode, FEP; 7 recurrent
14 episode, REP) compared to 25 age and sex matched healthy controls.

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17 decrease was greater in the FEP subgroup (p=0.01) of patients. We found no group differences in the
18 other investigated immune markers: IL-4, IL-8, TNF-alpha, and Interferon-gamma.

19 Our findings support the notion of a TH1/TH2 imbalance particularly in the acute manifestation
20 phase of schizophrenia. In the long run, this may lead to the identification of cytokine patterns that
21 are applicable as trait or state markers, may be helpful in making or ensuring diagnosis or in
22 monitoring therapy.

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1. Introduction

Schizophrenia is currently understood as a group of complex debilitating mental disorders with neurodevelopmental and neurodegenerative elements (Keshavan, Nasrallah, & Tandon, 2011; Tandon et al., 2013). Symptoms include disturbances in perception, thinking and emotional responses, but can also include vegetative symptoms and motor symptoms (Tandon, Keshavan, & Nasrallah, 2008; Walther & Strik, 2012). Though schizophrenia varies in course and outcome (Huber, 1997), a typical course of paranoid schizophrenia begins in early adulthood with a prodromal stage with unspecific symptoms, followed by a distinguishable first acute psychotic episode (FEP), a longer recovering period with different outcomes, mostly partial remission, and later relapses in form of recurrent psychotic episodes (REP).

While twin studies suggest a high amount of genetic determination (81%) and smaller but significant common environmental effects (11%) on the liability to develop schizophrenia (Sullivan, Kendler, & Neale, 2003), the exact causes and pathomechanisms of schizophrenia are still unknown. However, there is broad knowledge about the affected systems and structures (Keshavan et al., 2011; Tandon et al., 2008). Known alterations in schizophrenia (Tandon et al., 2008) include structural and functional gray and white brain matter abnormalities, alterations in dopaminergic and glutamatergic pathways and related neural networks, changes in antioxidative defense systems (Yao & Keshavan, 2011), and alterations of immune function (Goldsmith, Rapaport, & Miller, 2016; Khandaker et al., 2015; Norbert Müller, Weidinger, Leitner, & Schwarz, 2015).

Indeed, interactions between symptoms of mental disorders and immunological processes have been described for centuries (Himmerich, Sorge, Kirkby, & Steinberg, 2012), also causing the first biological approaches in terms of therapy in the early 20th century (Wagner-Jauregg, 1936). Therapeutical approaches in modern times include application of immunomodulatory agents such as COX-2 inhibitors (Norbert Müller et al., 2004) or interferon-gamma (Gruber, Bunse, Weidinger, Reichard, & Muller, 2014). A variety of observations in schizophrenia could be integrated once alterations of immune function have been considered as part of the illness, e.g. the observation that the risk to develop schizophrenia increases after a maternal infection during pregnancy (Brown, 2006; Meyer &

Feldon, 2009). More recent studies base the assumption of deregulated immune function in schizophrenia on the finding of altered cytokines, the key signaling molecules of the immune system (Nawa & Takei, 2006). Meanwhile, several alterations of cytokines and other immunological markers in the blood could be replicated in the affected individuals (Goldsmith et al., 2016; Norbert Müller et al., 2015; Potvin et al., 2008). These findings raised expectations in the use of cytokines or patterns thereof as potential biomarkers for the disease or its course (Cox, Chan, & Bahn, 2015; Lai et al., 2016). However, findings in terms of cytokine alterations in schizophrenia are still heterogeneous, in part even contradictory, and thus the idea of using cytokines as biomarkers for schizophrenia also has been a subject to criticism (Koola, 2016; Potvin et al., 2008). One important factor in this discussion is the potential effect of antipsychotic medication on cytokine patterns (Baumeister, Ciufolini, & Mondelli, 2016; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011). While the earliest studies have been conducted in chronic patients on stable antipsychotic medication (for an overview see Potvin et al. (2008)), more recent studies were performed in unmedicated patients (Goldsmith et al., 2016; Upthegrove, Manzanares-Teson, & Barnes, 2014).

In order to better understand the complex interrelation between immune function and the development and outcome of schizophrenia, i.e. its changeability during the course of illness, the investigation of cytokine patterns at different stages of illness seems to be a suitable approach (at-risk phase (Föcking et al., 2016; Smesny et al., 2017); first acute psychotic episode phase (FEP); recurrent episode phase (REP), partial/complete remission or before/after medication phase (Baumeister et al., 2016; Upthegrove et al., 2014) etc.). In the long run, this research aims to identify cytokine patterns that are applicable as trait or state markers and may be helpful in making/ensuring diagnosis or in monitoring therapy (Ritsner & Gottesman, 2009).

In a recently published study (Smesny et al., 2017) we have been investigating a selected number of cytokines and other immune-related soluble receptors in an ultra-high-risk (UHR) population for psychosis and found an influence of omega-3 dietary supplementation on sICAM-1. In the present study we investigated a number of cytokines and other immune-related soluble receptors and mediators in acutely ill schizophrenia patients (n=25). The study population initially consisted of 99 patients and controls. After excluding all patients with assumed co-variates that were thought to have a greater impact on the measured biological markers (see study population/participants in the next section), our remaining selected study population consisted of 18 first episode patients (FEP) and 7 recurrent episode patients (REP), all suffering an acute schizophrenic episode at time of investigation . All selected FEP were neuroleptic naïve, i.e. never treated with any antipsychotic agents. Three patients of the REP group had received prior neuroleptic treatment but were free of neuroleptic medication for at least 7 days at time of investigation. The schizophrenia patients were compared to 25 healthy individuals (HC), matched for age, gender and lifestyle characteristics. Selected cytokines included the well "established" IL-6 as well as uncommon but previously investigated immune markers like sICAM-1 (Schwarz, Riedel, Ackenheil, & Müller, 2000).

We deliberately selected markers of the TH1 and TH2 systems to investigate the repeatedly postulated TH1/TH2 imbalance in acutely ill schizophrenic patients (Avgustin, Wraber, & Tavcar, 2005; Schwarz, Müller, Riedel, & Ackenheil, 2001). TH1 cytokines promote the type 1 cell-mediated immune response especially against microbial pathogens by activating the differentiation of CD4 positive naïve T-cells to the subtype T-helper cells 1 (TH1). TH2 cytokines stimulate the B-lymphocyte maturation and the antigen production (e.g. IgE) as well as the differentiation of naïve T-lymphocytes

128 to the subtype T-helper cells 2 (TH2) after being activated by IL-4 which is secreted by cells of the
129 innate immune system (Potvin et al., 2008).

130 As representatives of the TH1 system we included sIL-2R, IFN-gamma, and sICAM-1. For the TH2
131 system we included IL-4, IL-6, IL-8, and IL-13. These cytokines can also be classified based on their
132 pro-inflammatory (sIL-2R, IL-6, IL-8, TNF-alpha), respective anti-inflammatory (IL-4, IL-13) effects. For
133 further information on each selected cytokine respective immune-related receptor or mediator we
134 compiled additional information in the *supplementary material*.

135 Our hypotheses were (i) that there would be alterations in the signature of the measured immune-
136 related markers in schizophrenia patients, independently from assumed effects of antipsychotic
137 medication. According to previous findings, we expected (ii) a shift towards TH2. Further, (iii) we
138 expected group differences between first and recurrent patient groups.

2. Subjects and Methods

2.1 Study population/participants

141 The study population initially encompassed 99 persons (acute psychosis patients and healthy
142 controls). Application of the below described strict exclusion criteria resulted in a remaining study
143 population of 25 schizophrenia (SZ) patients (mean age ± SD [years]: 28.1 ± 7.5, gender ratio
144 male/female: 12/13) and 25 healthy controls (HC), matched for age and gender (mean age ± SD
145 [years]: 27.4 years ± 7.5, gender ratio male/female: 12/13). Regarding the stage of the disease, two
146 subgroups of patients were distinguished. The first episode patient (FEP) group included 18
147 individuals, the recurrent episode group (REP) 7 individuals. At the time of investigation all FEP were
148 drug-naïve (**table 1**). In the REP group, 4 patients were drug-naïve and 3 patients had received
149 sporadic neuroleptic medication but were medication-free for at least 7 days prior measurement.

150 *All patients* suffered an acute schizophrenic episode according to DSM-IV criteria (American
151 Psychiatric Association, 2000). Diagnoses were made by two independent, board certified
152 psychiatrists (SS, BM). Structured interviews were conducted to affirm diagnosis (Wittchen, Zaudig, &
153 Fydrich, 1997). *Healthy volunteers* were recruited by paper advertisement from hospital stuff
154 (therapists, nurses, trainees) or the general population. It was ensured that the control population
155 was similar to patients in terms of education and lifestyle and, therefore, included students, low-level
156 and mid-level-workers, academics, smokers and non-smokers.

157 *Exclusion criteria: For patients*: acute suicidal ideation and involuntary hospitalization; *For all*
158 *participants:* acute or chronic inflammatory or autoimmune disease (as screened for by standard
159 laboratory tests (white blood cell count, C-reactive protein), measurement of body temperature, and
160 obtaining the medical history of the participant); intake of nonsteroidal or steroidal anti-
161 inflammatory drugs; current alcohol abuse; consumption of illegal drugs within the last 8 weeks; IQ
162 below 70 or brain developmental abnormalities as obtained by standard cranial MRI scan; differential
163 diagnoses of bipolar disorder, borderline personality disorder, antisocial personality disorder,
164 psychotic major depression, delirium . It was ensured that none of the healthy controls had a
165 personal or family history of any mental disorder. All participants gave written informed consent.

166 We excluded 26 patients from the initial sample. 19 patients were excluded because they did not
167 receive the diagnosis of schizophrenia but another diagnosis (mood disorders including
168 schizoaffective disorder and bipolar disorder: 9; other psychosis 3; personality disorders: 3; anxiety

169 disorders: 3; anorexia nervosa: 1). Two patients were excluded because of neuroanatomical
170 malformations obtained by cranial MRI scanning. Two patients were excluded because of heavy
171 drinking. Five patients were excluded because of current drug abuse (all of them used cannabis). Five
172 patients had prior or current neuroleptic medication (2 olanzapine, 1 clozapine, 2 haloperidole).
173 There were some patients who met more than one exclusion criterion (like drug abuse and being on
174 neuroleptic medication).

176 The study design was approved by the Research Ethics Committee of the University Hospital of Jena.

177 **2.2 Blood collection**

178 Fasting morning blood was drawn in sitting position from an antecubital vein into serum separator
179 tubes using a standard Sarstedt® blood collecting system. After clotting, the blood samples were
180 centrifuged (2500 × g, 10 min) to obtain serum, and aliquots (1 mL) were immediately frozen and
181 stored at -72°C until analysis. Biochemical analyses were performed blinded in the laboratory of the
182 Department of Dermatology at the University Hospital Jena. Before analysis, aliquots were thawed at
183 4°C overnight and centrifuged once more (2500 x g, 10 min). Repeated freeze-thaw cycles were
184 avoided as recommended by the manufacturer.

185 **2.3 Determination of cytokines, cytokine receptors and other immune mediating receptors**

186 Serum cytokine levels of sIL-2R, IL-4, IL-6, IL-8, IL-13, sICAM-1, IFN-γ, TNF-α were quantified utilizing
187 commercially available enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems,
188 Vienna, Austria) according to the manufacturers' instructions with double determination of each
189 parameter. To determine very low values of IL-4, IL-6, IFN-γ, and sICAM-1, high sensitivity ELISA
190 (Bender MedSystems, Vienna, Austria) were carried out. Manufacturer catalog numbers and lower
191 detection limits of the used test kits and %CV-values are given in the *supplementary material*.

192 **2.4 Statistical analysis**

193 Statistical analyses were performed using the software package IBM SPSS Statistics 24. Immune
194 marker concentrations were represented as mean and standard deviation.

195 Tests for distribution (Kolmogorov-Smirnov; Shapiro-Wilk) and variance homogeneity (Levene) were
196 performed for cytokine data and resulted in non-normal distribution and heterogeneous variance,
197 hence, non-parametric tests were chosen for all following analyses. We performed the
198 nonparametric Wilcoxon-test for dependent samples (matched pairs) to evaluate group differences
199 in cytokine levels.

200 In the main initial analysis we compared patients (SZ) with healthy controls (HC). Subgroup analyses
201 were performed between FEP or REP and their respective age and gender matched HC group. Group
202 differences were considered significant at $p < 0.05$.

204 3. **Results**

205 **3.1 Patients vs. healthy controls (SZ vs. HC)**

206 Results of the initial group comparison of all patients and all HC are summarized in **table 2**. Results
207 of Wilcoxon-Tests are also provided in **table 2**. An elevation of IL-13 (p=0.039) and a reduction of
208 sICAM-1 (p=0.011) in the entire patients group reached significance level. Elevated IL-6 (p=0.052) and
209 diminished sIL-2R (p=0.63) levels in patients did not exceed the chosen threshold for significance. .

210 **3.2 Subgroup analysis**

211 The comparison of FEP (N=18) with their respective matched controls (N=18, **table 3**) yielded the
212 same result (increased IL-13, p=0.026 and reduction of sICAM-1, p=0.020) as for the overall patient
213 group. Additionally, sIL-2R was significantly diminished (p=0.010) in the FEP group.

214 When comparing REP (N=7) against their respective matched controls (N=7), we found no significant
215 group differences.

216

217   **4. Discussion**

218 We investigated immune markers in acutely ill schizophrenia patients at different phases of illness.
219 We excluded as best as possible the influence of antipsychotic medication by including (in the FEP
220 and REP group) mainly neuroleptic-naïve patients.

221 Our main results are (i) a reduction of sICAM-1 and (ii) an elevation of IL-13 in the entire patient
222 group. Subgroup analysis of FEP revealed (iii) a significant reduction of sIL-2R and (iv). We also found
223 an elevation of IL-6 (p=0.052) which was not significant at the chosen threshold for significance. (v)
224 We did not find significant group differences in the other investigated immune markers (IL-4, IL-8,
225 TNF-alpha, IFN-gamma).

226 Our first finding (i), the decrease of sICAM-1, is in line with previous studies (Kronig et al., 2005;
227 Schwarz et al., 2000). As sICAM-1 represents a signaling molecule required for the activation of TH1
228 helper cells (see also *supplementary material*) and is therefore representing a marker of the cellular
229 immune system, this finding suggests a weakening of the cellular immune system in schizophrenia.
230 This finding also supports the hypothesized relative shift towards the TH2 immune response in
231 schizophrenia.

232 IL-13 is a TH2 associated anti-inflammatory cytokine. Therefore, the finding (ii) of an elevation of IL-
233 13 in patients suggests TH2 activation. Considering the mild inflammation process presumed in
234 schizophrenia (Bechter, 2013; Maxeiner et al., 2014), this finding could reflect a counter-reaction.
235 This assumption is supported by the previous finding of elevated maternal IL-13 being associated
236 with a higher risk of the offspring to develop schizophrenia (Allswede, Buka, Yolken, Torrey, &
237 Cannon, 2016). Though one previous study found a trend towards higher IL-13 (Maxeiner et al.,
238 2014), to our knowledge, serum IL-13 has not been frequently investigated in schizophrenia
239 populations so far.

240 Also our third (iii) finding, reduced sIL-2R in the drug-naïve FEP, may refer to a relative weakening of
241 the TH1 system (Hilkens et al., 1995). This result supports the pattern proposed by our hypotheses.
242 However, many previous studies reported *increased* sIL-2R levels in schizophrenia patients (Akiyama,
243 1999; Rapaport & Lohr, 1994; Rapaport et al., 1994; Sirota, Meiman, Herschko, & Bessler, 2005) as
244 described in detail in a recent meta-analysis, which refers to these studies (Goldsmith et al., 2016).

245 As sIL-2R is very sensitive to drug treatment and other influences (Baumeister et al., 2016; Na, Jung,
246 & Kim, 2014) this discrepancy could be caused by different characteristics of the investigated patient
247 populations. Müller et al. found an increasement of sIL-2R after medication, too (N. Müller, Empl,
248 Riedel, Schwarz, & Ackenheil, 1997). Interestingly, they reported no group differences *before*
249 medication. Also, Bresee et al. found increased sIL-2R levels in schizophrenia on stable medication
250 (Bresee & Rapaport, 2009). They refer to previous studies by Rapaport et al. who investigated sIL-2R
251 in different schizophrenia populations, including unmedicated first episode patients and found
252 consistently elevated levels (Rapaport & Lohr, 1994). We cannot plausibly explain the differences
253 between our results and most previous findings. One difference is that Bresee and Rapaport used
254 commercially available tests from R&D Systems, Inc., USA, while we used tests from Bender
255 MedSystems GmbH, Austria. Other technical differences might be found in the preanalytic
256 procedure. From our point of view and our results it is conceivable that reduced sIL-2R occurs only in
257 the acute FEP phase of schizophrenic illness.

258 Consistent with previous studies (for an overview see Potvin et al. (2008)), we found an elevation of
259 the pro-inflammatory cytokine IL-6, too. However, it was not significant. Since there is evidence that
260 IL-6 is elevated by smoking (Di Nicola et al., 2013), this may be explained by the relatively small
261 amount of smokers in our sample (see also supplemental material).

262 Since in this study all subjects of both patient groups suffered an acute psychotic episode, we assume
263 that the found cytokine alterations are part of the pathology leading to acute exacerbations.

264

265 **5. Limitations of the study**

266 We measured drug-naïve and drug-free schizophrenia patients during a psychotic episode. Due to
267 ethical reasons this naturally leads to a comparably small number of suitable patients during a
268 limited study period. The sample size of 25 vs. 25 therefore has to be seen as a critical factor that
269 affects the sensitivity to detect group differences, and also increases the risk of misinterpretation.
270 This is due to the possibility of random effects which, at this sample size, could affect the results.

271 We were not able in this study to investigate associations between immune marker concentrations
272 and measures of psychopathology, e.g. the severity of delusional ideas of hallucinations. However, all
273 patients were acutely help-seeking in a routine hospital setting. Our interpretation therefore is
274 limited by the fact that we cannot finally exclude, that the reported findings reflect unspecific
275 aspects of acute psychotic episodes, e.g. stress or tension. We need to state that this obviously is a
276 limitation of this research in general, as only a small fraction of studies investigated or reported
277 associations between immune marker findings and symptomatology so far (e.g. only 70 of 1202 or
278 0.6% of all studies identified by PUBMED search on 2018-02-07 with the keywords *schizophrenia* and
279 *cytokine* also contained the keyword *psychopathology*).

280

281 **6. Conclusion**

282 We conclude that our results—significant reduction of TH1 associated sICAM-1 and elevation of TH2
283 associated IL-13support the notion of a TH1/TH2 imbalance in schizophrenia (Schwarz et al., 2001)
284 expressed by a relative cytokine shift in favor of the TH2 system. The result of reduced sIL-2R only in

285 FEP is suggestive that this TH1/TH2 shift is more pronounced in the first acute manifestation phase of
286 illness. We assume that these alterations of immune markers reflect processes inherent to the
287 disease, and that pathomechanisms underlying these alterations may be stronger during the first
288 acute manifestation of the disease.

299 **Authors' contributions**

300 BM had the original idea, selected cytokines based on literature research, screened patients, was
301 responsible for pre-analytics, did statistical analysis and conceptualized and wrote the article. KR
302 screened patients and performed pre-analytics. She also screened literature and prepared the
303 literature database. JH and CM developed laboratory routines and RSPs. JH, KR, CM performed
304 analyses in the laboratory. JH wrote the biochemical methods part and contributed significantly to
305 the overall text and discussion. She also prepared all raw data for statistical analyses. CM did proof-
306 reading and contributed to the text. KL contributed significantly in preparing and processing data and
307 contributed importantly to the final text. UM improved the manuscript significantly and contributed
308 major ideas due to her broad knowledge in this field. PE is the head of the dermatology department
309 and provided the means and structures to perform the measurements. UCH is the director of the
310 laboratory, provided working structures, material, and expertise. SS screened patients, contributed
311 important ideas how to embed the results into a meaningful context, and improved the manuscript.
312 UCH and SS are the senior authors.

313

314 **Possible conflicts of interest**

315 All authors declare that there were no possible conflicts of interest.

316

317 **Tables**

318 **Table 1:** Study population/participants, SD: standard deviation, HC: healthy controls, SZ:
319 schizophrenia patients, FEP: first episode psychosis, REP: recurrent episode psychosis

	HC	SZ

Sex (male/female)	12/13	12/13
Age (in years, mean ± SD)	27.29 ± 7.45	28.18 ± 7.63
FEP/REP		18/7
neuroleptica-free > 7d		25
thereof neuroleptica-naive		22

320 **Table 2:** Results of cytokine analyses (ELISA) Mean and standard deviation (SD), per group in [pg/mL],

321 HC: healthy controls; SZ: patients; N: number of valid data points, p-value of Wilcoxon-test (2-sided),

322 ** statistically significant; * trend

Cytokine	Group	N	Mean	SD	P
IL-2R	HC	25	3.66	1.15	0.063*
	SZ	25	3.00	1.43	
IL-4	HC	21	7.18	20.06	0.398
	SZ	17	24.86	55.56	
IL-6	HC	25	0.52	1.31	0.052*
	SZ	24	2.32	5.88	
IL-8	HC	21	1.15	3.00	0.779
	SZ	17	0.97	1.90	
IL-13	HC	21	8.02	13.69	0.039**
	SZ	18	81.10	142.81	
sICAM-1	HC	25	414.71	82.13	0.011**
	SZ	25	358.31	70.64	
IFN gamma	HC	21	0.17	0.77	0.317
	SZ	17	0.00	0.00	
TNF alpha	HC	21	0.57	1.98	0.273
	SZ	17	2.96	9.75	

323

324   **Table 3:** Subgroup analysis FEP vs HC, p-values of Wilcoxon-test (2-sided); HC: healthy controls, FEP:

325   first episode psychosis, ** statistically significant

Cytokine	p
IL-2R	0.010**
IL-4	0.249
IL-6	0.171
IL-8	0.463
IL-13	0.026**
sICAM-1	0.020**
IFN gamma	0.317
TNF alpha	0.655

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486

487

488

SUPPLEMENTAL MATERIAL

TH1/TH2 cytokine shift pronounced in unmedicated first episode as compared to chronic schizophrenia patients

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Characteristics of the patient group

Patients had a mean BMI of 22.83 (min 17.05, max 29.40; 2 patients had a BMI below 18.5, 4 patients had a BMI above 25). Smoking: 14 patients were non-smokers, 5 smoked up to 10 zigarettes per day and 6 patients smoked more than 10 cigarettes per day. Mean WBC was 6.92 $*10^9$/L. Highest education per category was: "Sonderschule" (special school for people with learning disability, 1 patient), "Hauptschule" (8th grade, 2 patients), "Regelschule" (10th grade, 9 patients), "Abitur" (12th grade, 10 patients, thereof 8 students), academic degree (1), missing data (2).

Investigated cytokines and immune-related receptors and mediators

Cytokines are small (about 25kDa) cell-released proteins that affect the behavior of other cells bearing highly specific receptors for it (Murphy & Weaver, 2017; Resch, 2003). Cytokines can act in an autocrine, paracrine, or endocrine way. Important cytokine groups include interferons (IFN), interleukins (IL), and tumor necrosis factors (TNF). The following list also includes sIL-2R and sICAM-1 which are not cytokines but soluble immune related receptors and mediators.

IL-2/sIL-2R: The effects of IL-2 and its soluble *receptor* sIL-2R *in vivo* are complex and include feedback loops with several cell populations and other cytokines. *In vitro*, IL-2 induces the growth of activated T-cells (Banchereau, Pascual, & O'Garra, 2012). IL-2 controls inflammation by inhibiting the differentiation of pro-inflammatory TH17 cell lines, and interferes also with IL-6 (Banchereau et al., 2012). The soluble *IL-2 receptor* (sIL2-R), which we measured in this study, is not a cytokine but the circulating form of the IL-2 membrane receptor found on lymphoid and some cancer cells. Its presence is thought to be a good marker of T-cell activation. (Witkowska, 2005). However, with the capacity of binding circulating IL-2, it can also prevent the interaction of circulating IL-2 with membrane-bound IL-2 receptors (Potvin et al., 2008) and thus support inflammation. In schizophrenia, serum sIL-2R levels have been reported to be elevated in several studies (Goldsmith et al., 2016; Upthegrove et al., 2014). Already in a very early study, it was demonstrated that sIL-2R increased under influence of antipsychotic medication (Schwarz, Riedel, Gruber, Müller, & Ackenheil, 1998).

IL-4: IL-4 is considered an anti-inflammatory cytokine and the prototypic TH2 cytokine. IL-4, produced by activated T-cells, drives the differentiation of TH2 cells. IL-4 producing TH2 cells play a role in the elimination of extracellular organisms (e.g. helminthes) (Potvin et al., 2008). In schizophrenia, stage-dependent alterations of IL-4 (with alterations only in relapsing patients, but not FEP) (Borovcanin et al., 2012) and a reduction of initially elevated IL-4 levels after antipsychotic treatment (Borovcanin et al., 2013) have been reported. DiNicola et al. (Di Nicola et al., 2013) failed to find a difference between FEP and healthy controls, but reported significantly higher IL-4 levels in unemployed

528   individuals. The authors related this finding to potential effects of environmental factors on cytokine
529   pattern.

530   **IL-6:** IL-6 is considered a pro-inflammatory cytokine. Recent research shows that IL-6 has context-
531   dependent pro- and anti-inflammatory properties as well as hormone-like characteristics (Hunter &
532   Jones, 2015). IL-6 is one of the most frequently studied cytokines in schizophrenia. It was found
533   elevated in most but not all populations of schizophrenia patients (Potvin et al., 2008).

534   **IL-8:** IL-8 was included in this study as a pro-inflammatory cytokine. A few studies have investigated
535   alterations of IL-8 in schizophrenia. Hayes et al. (2014) found increased IL-8 in the cerebrospinal fluid
536   of schizophrenia patients. Zhang et al. (2016) report elevated IL-8 levels in blood serum of stable
537   medicated schizophrenia patients.

538   **IL-13:** IL-13 is considered an anti-inflammatory signature cytokine of the TH2-cytokine family
539   (Allswede et al., 2016; Potvin et al., 2008; Zhu, 2015), and thus was included in this study. Very few
540   studies investigated IL-13 in schizophrenia so far. A trend to higher serum levels in schizophrenia
541   patients (Maxeiner et al., 2014) and a decrease of serum levels after antipsychotic treatment (Pae et
542   al., 2006) were reported.

543   **sICAM-1:** The soluble intercellular adhesion molecule-1 (sICAM-1) is considered a marker for the
544   activation of the cellular immune system, activated by IFNg (Paolieri et al., 1997; Schwarz et al.,
545   2000). It is also suggested that sICAM-1 mediates TH1 cell recruitment (Ogawa et al., 2010). In
546   schizophrenia, decreased serum levels in unmedicated patients and increased levels after
547   antipsychotic treatment have been reported (Kronig et al., 2005; Schwarz et al., 2000; Schwarz et al.,
548   1998).

549   **TNFa:** TNF-alpha is a pro-inflammatory cytokine which activates the TH1 arm of the immune
550   response. In schizophrenia, increased levels have been reported in acutely ill patients while levels
551   have been reported unchanged in treated patients (Goldsmith et al., 2016). Under the influence of
552   clozapine TNF-alpha has been demonstrated to increase in brain tissue (Himmerich et al., 2012;
553   Pollmächer, Hinze-Selch, & Mullington, 1996).

554   **IFNg:** Interferon gamma is an important cytokine with complex immunomodulatory effects and
555   represents the prototypic TH1 cytokine (Potvin et al., 2008). It has been quite often investigated in
556   schizophrenia patient populations, but results are inconsistent with no significant effect size in meta-
557   analysis (Potvin et al., 2008).

Supp. Mat. Table 1			
Manufacturer catalog numbers and lower detection limits of the used test kits (ELISA)			
Assay	**Cat. No**	**lower detection limits**	**Brand**
Human sIL-2R	BMS212/2CE BMS212/2TENCE	0.27 pg/mL	Bender MedSystems
Human IL-4 High Sensitivity	BMS225HS	0.1 pg/mL	Bender MedSystems
Human IL-6	MKL6 1	1.2 pg/mL	Milenia biotec
Human IL-6 High Sensitivity	BMS213HS	0.02 pg/mL	Bender MedSystems
Human IL-8	MKL8 1	3.5 pg/mL	Milenia biotec
Human IL-13	BMS231/3	0.73 pg/mL	Bender MedSystems
Human sICAM-1	BMS201CE BMS201TENCE	2.2 pg/mL	Bender MedSystems
Human IFNg High Sensitivity	BMS228CE BMS228TENCE	0.99 pg/mL	Bender MedSystems
Human IFNg	BMS228HS	0.06 pg/mL	Bender MedSystems
TNF-a	MKTN 1	6 pg/mL	Milenia biotec

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Supp. Mat. Table 2									
Mean CV% for each tested marker									
marker	sICAM1	IL-4	IL-6	IL-6HS	IL-8	IL-13	TNFa	IFNg	IL-2R
mean CV%	5.65	2.08	14.56	6.28	15.13	1.77	8.61	20.57	6.14

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1   **TH1/TH2 cytokine shift pronounced in unmedicated first episode as compared to chronic**
2   **schizophrenia patients**

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15   **Authors' contributions**

16   BM had the original idea, selected cytokines based on literature research, screened patients, was
17   responsible for pre-analytics, did statistical analysis and conceptualized and wrote the article. KR
18   screened patients and performed pre-analytics. She also screened literature and prepared the
19   literature database. JH and CM developed laboratory routines and RSPs. JH, KR, CM performed
20   analyses in the laboratory. JH wrote the biochemical methods part and contributed significantly to
21   the overall text and discussion. She also prepared all raw data for statistical analyses. CM did proof-
22   reading and contributed to the text. KL contributed significantly in preparing and processing data and
23   contributed importantly to the final text. UM improved the manuscript significantly and contributed
24   major ideas due to her broad knowledge in this field. PE is the head of the dermatology department
25   and provided the means and structures to perform the measurements. UCH is the director of the
26   laboratory, provided working structures, material, and expertise. SS screened patients, contributed
27   important ideas how to embed the results into a meaningful context, and improved the manuscript.
28   UCH and SS are the senior authors.

29   **Possible conflicts of interest**

30   All authors declare that there were no possible conflicts of interest.